



МЛАДЕЖКО НАУЧНО ДРУЖЕСТВО "АСКЛЕПИЙ"
YOUTH SCIENTIFIC ASSOCIATION "ASCLEPIUS"

НАУКА И МЛАДОСТ КОНФЕРЕНЦИЯ

SCIENCE AND YOUTH CONFERENCE

2023

НАУЧНИ ДОКЛАДИ
SCIENTIFIC REPORTS

Пловдив, април
Plovdiv, april

21-23, 2023



Медицински университет – Пловдив
Младежко научно дружество
„Асклепий“



Medical University of Plovdiv
Youth Scientific Association
“Asclepius”

Наука и младост
Конференция

Science and Youth
Conference

2023

Научни доклади

Scientific reports

Пловдив

Plovdiv

2023

Editor-in-Chief

Professor Blagoi Marinov, MD, PhD

Executive Editor

Chief assist. prof. Dimitar Penkov, MPharm, PhD

University Publishing Centre

Medical University of Plovdiv

15A, Vassil Aprilov Blvd., 4002 Plovdiv

www.publisher.mu-plovdiv.bg

ISSN 1314-9229 (print)

ISSN 2683-0922 (online)

Contents

I. Social Medicine	7
Alternative Dispute Resolution in Healthcare – Historical Overview <i>K. Dimitrov, T. Miteva-Katrandzhieva</i>	7
Application of the Pilates Method for Prevention and Control of Postural Disorders and Spinal Curvatures in Adolescents <i>G. Koleva, S. Davidova, S. Durmushalieva, P. Kasnakova</i>	13
Association between Shift Work and Cardio-metabolic Diseases: a Literature Review <i>M. Todorova, S. Harizanova, D. Bakova</i>	18
A 23-year-old Man with a Rash after Returning from Italy <i>A. H. Syed, R. Komitova, S. Krumova, P. Argirova, O. Boykinova</i>	24
II. Biomedical session	28
Oxygen Uptake Efficiency Slope (OUES) as a Useful Indicator for Assessment of the Functional Capacity at Submaximal Effort During Cardio-Pulmonary Exercise Testing <i>A. Byandova, Z. Taralov, P. Dimov</i>	28
Review of the Proposed Pathophysiological Mechanisms for Postural Orthostatic Tachycardia Syndrome as Part of Long-Covid Syndrome <i>P. Georgiev, K. Avramov, Z. Taralov</i>	32
Insomnia – the False Face of Two of the Most Common Sleep Disorders – 2 Case Studies <i>D. Stoyanova, K. Avramov, T. Georgiev, A. Draganova, K. Terziyski</i>	39
A Rare Case of Hepatosplenic Fungal Infection in Previously Healthy Child <i>D. Najar, A. Uchkunov, L. Chochkova-Bukova</i>	44
Application of User-Friendly 3D Printing for Customized Products in Various Medical Fields <i>B. Balabanov, I. Gerdzhikova, T. Grancharova</i>	48
Infantile Amnesia – Empirical Study and Possible Reasons (Review) <i>B. Stoencheva, K. Stoyanova, D. Stoyanov</i>	52
Atypical Formation of the Median Nerve in Fossa Axillaris. Case Report <i>Z. Todorova, V. Zaikova</i>	57
The Significance of FOT (Forced Oscillation Technique) in the Diagnosis of Small Airways Dysfunction <i>Z. Zigova, T. Damyanova, M. Georgiev, P. Stoimenova</i>	60
Alopecia Areata Association with Thyroid Autoimmunity and Dysfunction: Current Review of Literature <i>L. Todorova, S. Petrov</i>	65
Insomnia with Short Sleep Duration and the Impact on the Cardiovascular System – Current Knowledge and Future Perspectives <i>G. Udeyana, T. Georgiev, K. Avramov, A. Draganova, K. Terziyski</i>	70

Physiological Demands and Pathophysiological Problems in Ultramarathon Running – a Literature Review _____	77
<i>N. Mandadzhiev, K. Koleva, S. Mandadzhieva</i>	

Signs of Early and Established Rheumatoid Arthritis and Their Treatment Outcomes _____	87
<i>M. Chterevev, A. F. Abdi, V. Krishna, P. Todorov</i>	

III. Pharmacy and Pharmacotherapy Session _____ 94

Ropinirol-based Delivery Systems for Nose-to-brain Administration – Review Article _____	94
<i>Y. Tchoukova, B. Pilicheva</i>	

“Green Synthesis” of Silver and Iron Nanoparticles – Preparation, Characterization and Photothermal Potential _____	99
<i>N. Kostadinov, V. Velchev, T. Grancharova, S. Simeonova, P. Zagorchev</i>	

Novel Beta-lactam Antibiotics in the Treatment of Resistant Infections _____	103
<i>G. Jery, A. Prasad, H. Zlatanova</i>	

Pectin-based Drug Formulations for Gastrointestinal Targeted Delivery _____	109
<i>P. Simeonov, P. Lukova, B. Pilicheva</i>	

Recent Advances in the Formulation of Polysaccharide-based Microparticles Using Jet Break-up and Microfluidic Techniques _____	116
<i>A. Tsormpatzian, P. Lukova, D. Penkov, P. Katsarov</i>	

Computational Methods for <i>in silico</i> Prediction of Chemical Toxicity _____	122
<i>M. Milusheva, V. Gledacheva, S. Nikolova, S. Khattak</i>	

Simultaneous Determination of Piracetam and Cinnarizine in Capsule Dosage form by Reversed Phase High Performance Liquid Chromatography _____	127
<i>D. Stoilov, L. Peikova, B. Tsvetkova</i>	

IV. Therapeutic Session _____ 131

Continuous Positive Airway Pressure for Improvement of Respiratory Function in Infantile Spinal Muscular Atrophy: a Case Report _____	131
<i>A. Prasad, G. Jery, K. Gaberova</i>	

Acute Q Fever Pneumonia: a Case Report. _____	134
<i>D. Milanova-Ilieva, N. Dimitrova, K. Ketev</i>	

Acute Spondylodiscitis as a Debut of Infective Endocarditis _____	137
<i>I. Kuchmov, I. Manolov, B. Dobрева-Yatseva</i>	

Reactivation of Hepatitis B after COVID-19 Infection – Case Presentation _____	141
<i>S. Chilova, G. Raicheva, V. Popov, V. Trendafilova, I. Nenova</i>	

Cardiovascular Ochronosis – a Case Report _____	144
<i>E. Marinova, S. Kitov, Z. Ivanov, M.-F. Kitova</i>	

Optical Coherence Tomography in Patients with Mild Cognitive Impairment: Is Retinal Thickness an Early Predictable Marker for Neurodegeneration? _____	148
<i>V. Ivancheva, D. Marinova, A. Lyubenov</i>	

Differential Diagnosis of Endocrine Hypertension – a Case Report _____	152
<i>E. Chobankova, D. Stoyanova, B. Nonchev</i>	

V. Dental Medicine Session _____ 155

Er:YAG Laser Debonding of Porcelain Veneers – a Pilot SEM Study _____	155
<i>M. Tsanova-Stamatova, G. Tomov</i>	

Management of Epulis during Pregnancy by the Dental Practitioner – Literature Review _	160
<i>V. Dimitrov, D. Kitov, R. Tsareva, K. Vladov, K. Yamakov</i>	

Assessment of Different Techniques for Adhesive Cementation of All-Ceramic Systems _	166
<i>E. Ramadanova, E. Vasileva</i>	

Comparison of the Opacity of Conventional and Resin Modified Glass Ionomer Cements	170
<i>D. Ganeshapooban, S. Shahid, V. Stefanova, V. Todorova</i>	

Management of a Patient with Multiple Carious Lesions – a Case Report _____	175
<i>M. H. Sadiq, S. Nicheva</i>	

Oral Manifestations in a Patient with Bulimia – a Case Report _____	179
<i>M. Hadzhieva, I. Totova, G. Tomov</i>	

Prevalence of Black Stain and Associated Risk Factors in Children _____	182
<i>K. Tabutova, M. Shindova</i>	

VI. Surgical Session _____ 187

Effect of Operative Approaches on Functional Outcomes in Distal Humerus Fractures ____	187
<i>S. Karamitev, V. Stavrev</i>	

Tumor of the Heart – a Clinical Case _____	191
<i>A. Vehbi, S. Kitov, M.-F. Kitova, G. Markov</i>	

Management Options for Cervical Intraepithelial Neoplasia _____	194
<i>G. Yamakova-Vladova, K. Vladov, P. Uchikov, R. Terjumanov, K. Yamakov</i>	

A Rare Presentation of Orbital Dermoid Cyst in Adulthood: a Case Report _____	197
<i>P. Penchev, P.-P. Petrov, I. Ivanov, B. Iliev, Y. Enchev</i>	

“Happy” New Year or Why You Should Stay away from Pyrotechnics: a Case Report of Firework-related Injury of Both Eyes _____	200
<i>S. Aptikadir, Y. Idriz, N. Stoyanova, V. Mitkova-Hristova, N. Sivkova</i>	

Arteriovenous Malformation Associated with Intracerebral Haematoma and Subarachnoid Hemorrhage: a Case Report _____	204
<i>I. Ivanov, I. Kehayov, P.-P. Petrov, P. Penchev</i>	

Flesh-eating Disease – a Case Report _____	207
<i>V. Hadzhiminev, G. E. Markov, A. Sarpanov, K. Kalacheva, A. S. Ivanov</i>	

I. Social Medicine

Alternative Dispute Resolution in Healthcare – Historical Overview

Kostadin Dimitrov, Tsonka Miteva-Katrandzhieva

Department of Social Medicine and Public Health, Faculty of Public Health,
Medical University of Plovdiv

Background: Alternative dispute resolution (ADR) is widely used in various fields to resolve disputes without involving the traditional tort system. With the surge of litigations against healthcare workers, ADR gains popularity as a cheaper, faster and more effective method than court. **Objectives:** We aimed to conduct a historical overview of ADR and its inculcation into healthcare. **Materials and methods:** A systematic search was conducted in the electronic databases of PubMed and Google Scholar. **Results and discussion:** ADR refers to techniques used to resolve conflicts without the involvement of the Judicial System. There are two well-known forms of ADR – Mediation and Arbitration. ADR has been applied for conflict resolution in various fields. Amongst the countries with greatest impact on validation of ADR as an effective alternative to litigation is the USA. In the 1980's ADR gained popularity with the spread of the implementation of ADR at state level. Due to the surge of lawsuits for medical malpractice in the USA that resulted in burden on the Juridical System a reform took place. The reform went through three stages – Medical Screening Panels, Binding Arbitration and Mediation. NHS introduced mediation in medical negligence in the United Kingdom in 2016 after receiving a positive result in pilot program launched in the year 2014. **Conclusion:** Our article highlights the historical development of ADR and its introduction to the healthcare field. It is proven to be a cheaper, faster and more effective method of medical dispute resolution than litigation.

Keywords: *alternative dispute resolution methods, history of alternative dispute resolution methods, alternative dispute resolution method in healthcare, mediation*

Introduction

Healthcare is a complex and dynamic environment in which conflicts and disputes of different nature can arise between the patient (and/or their family) and medical personnel. Traditionally, in these cases, one of the parties in the conflict files a lawsuit. The nature of the judicial process is adversarial; it is ineffective in terms of time and costs, and it is not able to meet the expectations of consumers in every case. Due to the shortcomings of the traditional judicial system, healthcare increasingly focuses on methods for resolving disputes between doctors and patients outside the framework of the judicial system. Alternative dispute resolution method is a term that encompasses a wide range of approaches to resolving conflicts in a non-confrontational manner, without involving the judicial system (1) (2); i.e. using approaches

that are an alternative to full-scale judicial proceedings.

Objectives

The aim of this study was a historical overview of the Alternative Dispute Resolution approaches and their implementation in healthcare.

Materials and methods

A review of the available scientific literature was conducted through a systematic search in the electronic databases of PubMed and Google Scholar.

Results and discussion

Types of alternative dispute resolution methods

The methods of conflict resolution without involving the judicial system are divided

into two major groups – formal and informal. The informal methods are more flexible and are carried out in an informal environment. (3)

➤ Negotiation

This method involves a meeting between the parties to the dispute, during which the parties' views are presented and information is exchanged between them, with the ultimate goal of reaching a consensus. With this method, the parties to the dispute engage in direct communication, exchange proposals and requests, until a consensus decision is reached for both parties. The informal nature of this method serves as a buffer for smoothing out misunderstandings between those involved in the dispute. (3) (1)

➤ Screening Panel

This is a method of alternative dispute resolution specifically designed for healthcare. (4) The composition of the screening panel may vary, but usually consists of a doctor and a lawyer. Their task is to establish the validity of the claim before it reaches the court and to encourage the resolution of the dispute. (5)

➤ Mediation

Mediation is one of the most important and successful methods of alternative dispute resolution. It is a voluntary, flexible and confidential process in which a third party (mediator) assists the parties to the dispute in reaching a consensus, without the intervention of the court. (6) The mediator's main role is to assist the parties in clarifying the conflict, creating a safe environment of trust for discussing issues that may be emotionally and psychologically difficult, and guiding the conversation towards reaching a consensus. Unlike a judge or jury, the mediator cannot make the final decision or take sides in the dispute. (7)

➤ Arbitration

This method represents a confidential and privately organized process in which a third party chosen by the parties to the dispute or by a consensus procedure for selection participates. The arbitrator hears the parties to the dispute and makes a decision on which one is right. By its very nature, arbitration can proceed as a simplified court case, or specific rules agreed upon by the parties can be applied. (8) In arbitration, the parties may be represented by lawyers, and the arbitrator may be an expert in the field of the dispute. (2) Arbitration is a formal form of alternative dispute resolution, the aim of which is to be cheaper and faster than a standard court case

Prerequisites for active use of alternative methods for conflict resolution in healthcare

There are several prerequisites for the reform of the traditional judicial system as the main method for resolving conflicts between doctors and patients. The main ones are the shortcomings of this system and the incomplete satisfaction of the parties involved. Due to the imperfections of the judicial system, evidence and witnesses, the judicial decision is not always fair. (9) Another significant drawback of the judicial system is the high administrative costs, which are due to the need to prove guilt in every individual case and determine compensation amounts based on unclear criteria. Direct and indirect legal costs in healthcare in the United States amount to over \$55 billion annually. (10) Part of the indirect losses to the healthcare system are reflected in the practice of defensive medicine by doctors. By its nature, it represents a deviation from good medical practice due to doctors' fear of being sued. 59% of the doctors included in a study on the

practice of defensive medicine among specialties at increased risk of litigation state that they have behavior of over-treatment, which means that they conduct tests and consultations that they do not consider necessary. 39% of the doctors included in the study report that they avoid providing medical care to high-risk patients. (11) One major drawback of the traditional judicial system is the long period of time it takes to reach a decision. According to a study conducted by the United States General Accounting Office on cases against doctors, the average time from filing a lawsuit to resolution is 14 months (GAO 1984). Another study by Jury Verdict Research found that the average duration of medical malpractice cases in 2000 was 45 months (12).

Another reason for the need for alternative dispute resolution methods in medicine is the dissatisfaction of consumers. In most cases, court cases do not answer the most important questions for patients: What exactly happened? What are the consequences of the error for their health? Why did the error occur? How will the error be rectified? How will the error be prevented from happening again in the future? (13) The main reason for the majority of lawsuits against doctors is not financial, but poor communication between patients and doctors. In many cases, patients resort to litigation because the information they have received from their doctors is insufficient and/or not understood. (14)

The advantages of alternative methods over traditional judicial systems are many – they are emotionally sparing and less expensive; they take significantly less time to resolve disputes; they allow for more open communication between the parties in the dispute; their flexibility makes them more effective in the dynamic, complex, and emotional nature of medical disputes. (15)

History of alternative dispute resolution methods

Although the boom of alternative dispute resolution methods was in the 1960s, their history can be traced back long before then. In China, during the Zhou dynasty around 2000 BC, a position of mediator was established in all government administrations. Currently, the role of mediators in China is not only to resolve conflicts but also to carry out primary prevention by not allowing them to arise. (16) In ancient Greece, alternative methods were also used to resolve conflicts. In 400 BC, due to the overloading of the courts in Athens, arbitration was introduced as an alternative to the judicial process. The procedure was formal, and the arbitrator was chosen by lottery for each individual case. His main function was to resolve the problem, and if that was not possible, to direct the dispute to a traditional court. It was mandatory for every 16-year-old boy to go through the position of an arbitrator. (17) During the Middle Ages, arbitration gained increasing popularity in Europe as an alternative method for resolving disputes in international trade, due to the lengthy traditional judicial process. (18) Among the countries with the greatest contribution to the development of the modern concept of alternative dispute resolution methods is the United States. In the 1920s, the US Congress passed a federal law on arbitration, giving legal weight to this process. The newly adopted law obliges the parties to comply with the agreement, authorizes the courts to enforce arbitral decisions. In 1926, the American Arbitration Association was established, which aims to inform arbitrators and parties to the dispute about alternative methods of conflict resolution and the procedures for their implementation. At an academic level, in the 1980s, some universities in the United States began to offer courses and bachelor's

programs in the field of alternative dispute resolution. (19)

In the 1960s and 1970s, there was a rapid increase in medical malpractice lawsuits against doctors in the USA. As a result of this crisis, it was one of the first countries to notice the application and benefits of these methods for resolving conflicts in healthcare. (20) The first wave of reforms in the USA began in the 1970s and 1980s. Its goal was to reduce the number, severity, and costs of medical malpractice lawsuits against doctors. Almost all states made changes to their local legislation, adopting alternative methods for resolving disputes in medical claims. (21) Three main methods are used as an alternative to the traditional judicial system: arbitration, mediation, and screening panels. (5) Some of the first states to make legislative changes were Maryland and New Mexico. In 1962, New Mexico adopted a law for screening panels as a voluntary alternative method for resolving conflicts in healthcare, and in the 1970s, it became mandatory. From 1976 to 1996, 2,100 medical malpractice claims were heard, and three quarters /of the cases were settled before going to trial. (4) In 1976, the General Assembly of the State of Maryland passed a law regarding medical arbitration. The law states that any legal action against a physician with a value greater than \$5000 must be reviewed by an arbitration jury, consisting of a lawyer who serves as the jury chair and has legal expertise, a physician who provides expert opinions on medical issues, and a member of the public who, in contrast to the other two jury members, lacks expert knowledge. (22) In 1969, the arbitration project Southern California begins under the guidance of the Association of Hospitals and the Medical Association. Lawsuits under \$20,000 were resolved by a single arbitrator – a lawyer, and larger lawsuits were settled by a jury

consisting of three members – a lawyer, a doctor to provide expert opinion on medical issues, and another member who did not have expert knowledge in contrast to the other two members of the jury. The process followed the rules of the American Arbitration Association. The results of a comparative study between hospitals using arbitration and those using the conventional judicial system in California show that arbitration is more financially and time-efficient in reaching a settlement. (20) Numerous medical institutions are implementing mediation – the University of Michigan, Johns Hopkins, Rush Medical Center, the University of Pittsburgh, and Drexel Medical Center. According to a study, the cost savings from mediation on average amount to \$50,000 in court fees. (2) Other proven advantages of mediation over the judicial system are the time it takes to reach an agreement, which varies from a few hours to a few months in more formal cases, and the satisfaction of the parties' expectations in the dispute, which occurs in 90% of cases in mediation. Legal fees also decrease significantly. The average preparation time for an attorney for mediation is 2.5 hours, while for a trial it is 36 hours. (15) The mediation program at Drexel Medical Center began in 2004 with a success rate of resolving disputes in 85% of cases. In the same year, a mediation program also began at the University Hospital in Pittsburgh with a success rate of resolving cases in 88% of cases and saving defense costs of \$1 million. 35 states adopt legislation for negotiation between doctors and patients, which is reflected in early recognition of medical errors and apologies. (2)

The reform in Germany began in the 1970s with the increase in lawsuits against doctors. The country moves towards a formal alternative dispute resolution method by creating arbitration boards. At present, there

are 12 arbitration boards in Germany. A key moment for them is the voluntary nature of the process, with parties to the conflict retaining the right to turn to traditional courts. To date, the step towards arbitration boards is considered a success. In 2009, approximately 11,000 claims were filed for consideration of lawsuits. (23)

In the UK, attempts at reforms happened much later. In 2014, the National Health Service of UK launched a pilot program for resolving medical malpractice claims through mediation. A total of 49 cases were included, 47 of which reached an agreement through mediation, 1 was resolved before the process, and 1 was withdrawn. Following the success of the pilot program, in December 2016, the National Health Service began offering mediation services for claims. From the start of the services until March 2019, 606 cases were considered. 74% of the cases were resolved on the first day of the process or within 28 days. (24)

In Bulgaria, in 2004, the Law on Mediation was adopted, which regulates the relations related to this process. According to it, mediation is a voluntary and confidential procedure for out-of-court settlement of disputes and is conducted by a non-convicted person who is not deprived of the right to practice a profession or activity, has passed a mediator training course, and is registered in the Unified Register of Mediators. (25) In 2005, the National Association of Mediators was registered. (26) However, in Bulgaria, these alternative methods are still not well known in the context of healthcare, and there is a real need for their popularization and application.

Conclusion

Alternative methods for conflict resolution have their origins in ancient times, but they have only recently entered the field of

healthcare and are becoming a successful practice. Their increasing application for resolving conflicts between doctors and patients is based on their advantages over traditional legal disputes. Historically, they have proven to be an effective, more cost-effective and faster method for achieving consensus in the dynamic and complex healthcare environment, achieving a high degree of satisfaction among all interested parties. In Bulgaria, these alternative methods are still not well known, and there is a real need for their popularization and application.

References

1. Shamir Y. Alternative dispute resolution approaches and their application. 2002.
2. Sohn DH, Bal BS. Medical malpractice reform: the role of alternative dispute resolution. *Clin Orthop Relat Res*. 2012;470(5):1370-8.
3. Kaddoura S. ADR in the Medical Field: Premises and Promises. . 2020.
4. Fraser JJ, Jr., Committee on Medical L. American Academy of Pediatrics: Technical report: Alternative dispute resolution in medical malpractice. *Pediatrics*. 2001;107(3):602-7.
5. Nussbaum L. Trial and Error: Legislating ADR for Medical Malpractice Reform. *Maryland Law Review*. 2017;76(2).
6. Khodapanahandeh SHS. Efficiency of Using "Alternative Dispute Resolution" Method in Medical Negligence Claims. *Advances in Natural and Applied Sciences*. October 2014;8(13):1-5.
7. Gerardi D. Using mediation techniques to manage conflict and create healthy work environments. *AACN Clin Issues*. 2004;15(2):182-95.
8. Brown H, Simanowitz A. Alternative dispute resolution and mediation. *Qual Health Care*. 1995;4(2): 151-8.
9. Kassim PJ, Ibrahim A. Mediating medical negligence claims in Malaysia: An option for reform. *Malayan Law Journal Articles*. 2008;2008(4).
10. Mello MM, Chandra A, Gawande AA, Studdert DM. National costs of the medical liability system. *Health Aff (Millwood)*. 2010;29(9):1569-77.
11. Studdert DM, Mello MM, Sage WM, DesRoches CM, Peugh J, Zapert K, et al. Defensive medicine among high-risk specialist physicians in a volatile malpractice environment. *JAMA*. 2005;293(21): 2609-17.
12. Hambali SN, Khodapanahandeh S. A review of medical malpractice issues in Malaysia under tort litigation system. *Glob J Health Sci*. 2014;6(4):76-83.
13. Gallagher TH, Waterman AD, Ebers AG, Fraser

- VJ, Levinson W. Patients' and physicians' attitudes regarding the disclosure of medical errors. *JAMA*. 2003;289(8):1001-7.
14. Meruelo NC. Mediation and medical malpractice: the need to understand why patients sue and a proposal for a specific model of mediation. *J Leg Med*. 2008;29(3):285-306.
15. Szmania S. JA, Mulligan M. Alternative dispute resolution in medical malpractice: A survey of emerging trends and practices. *Conflict Resolution Quarterly*. oct. 2008;26(1):71-96.
16. Barrett JT, Barrett J. *A History of Alternative Dispute Resolution: The Story of a Political, Social, and Cultural Movement*: Wiley; 2004.
17. A. P. Traditional approaches in alternative dispute resolution: a brief overview. *conflict studies quarterly*. 2014,v 2014(7):34-48.
18. Rivkin DW. The Impact of International Arbitration on the Rule of Law: The 2012 Clayton Utz/University of Sydney International Arbitration Lecture. *Arbitration International*. 2013;29(3):327-60.
19. McManus M. SB. Brief History of Alternative Dispute Resolution in the United States. *CADMUS*. 2011;1(3):100-5.
20. Mitchell CN, McDiarmid S. Medical Malpractice: A Challenge to Alternative Dispute Resolution. *Canadian journal of law and society*. 2014;3:227-45.
21. Kinney ED. Malpractice reform in the 1990s: past disappointments, future success? *J Health Polit Policy Law*. 1995;20(1):99-135.
22. MacAlister JKaS, Alfred L. Jr. Health Claims Arbitration in Maryland: The Experiment Has Failed. *University of Baltimore Law Review*. 1985;14(3).
23. M. S. Medical Malpractice and Compensation in Germany. *Chicago-Kent Law Review*. 2013;86(3): 179-210.
24. J. V. Mediation in healthcare claims – an evaluation. NHS; 2020.
25. Закон за медиация, (Обн., ДВ, бр. 110 от 17.12.2004 г).
26. Национална асоциация на медиаторите, <https://nam-bg.org/>.

Application of the Pilates Method for Prevention and Control of Postural Disorders and Spinal Curvatures in Adolescents

Gergana Koleva¹, Samuela Davidova¹, Selen Durmushalieva², Petya Kasnakova²

1. Medical College, Medical University of Plovdiv

2. Department of Health Care Management, Faculty of Public Health, Medical University of Plovdiv

Introduction: Modern living conditions, the improper nutrition, reduced physical activity in adolescents are factors that cause impaired posture, various deformations of the spine and chest. Postural disorders and spinal distortions at young age are a serious medico-social problem that adversely affects physical and mental development. **The aim** of the study is to conduct a screening for detection of postural disorders and spinal distortions among adolescents and to compile a program based on the Pilates method to improve motor activity in case of disordered posture and spinal curvatures. **Materials and methods:** The study covered 90 people aged 6 to 25 held in November 2022 in Plovdiv. The functional examination included anamnesis, examination in combination with special tests for spinal dysfunctions. **Results and discussion:** An analysis was made of the results of the respondents' diagnostics, grouped according to the type of spinal dysfunctions and distortions. A program based on the Pilates method was drawn up and approved in accordance with the findings of the functional examination. Original rehabilitation algorithms for the prevention of impaired posture have been promoted, including physical exercises to correct the deformity from unencumbered position and antigravity exercises to stimulate postural reflexes in combination with breathing exercises. **Conclusion:** Successful prevention and treatment of spinal curvature depends on early detection, identification of causes, type, degree and severity. The possibilities for the prevention of spinal problems at a young age, which problems are the main reason for back pain in adulthood, have been analyzed.

Keywords: the Pilates method, spinal distortions

Introduction

A sedentary lifestyle is widespread among all age groups of the population worldwide [1]. Measures to increase the physical activity of the population are one of the most economical and effective approaches for better public health in response to the main risk factors, such as unhealthy diet and inactivity [2]. According to data from the National Study on Risk Factors for Chronic Non-Communicable Diseases (2021–2025) in Bulgaria, a sedentary lifestyle is widespread among all age groups and insufficient physical activity is reported among the population. According to the Global Action Plan for Physical Activity 2018–2030, the implementation of activities to increase physical activity will enable improvements in the nation's health, quality of life and general well-being [3]. The problem of low physical activity among children in Bulgaria between the ages of 10–19 is

particularly serious. Almost half of them (46%) have never practiced sports or other physical activity. The results of the research show that 48.2% of boys and 25.5% of girls are physically active almost every day during their free time, 33.8% of them play sports 2–3 times a week. Every third student plays sports once a week or less, or does not do sports at all, with the share of girls being almost twice as high as that of boys [3]. According to a study by the Institute for Social Research, the most common reasons for immobility are insufficient free time (50%); the impossibility of additional financial expenses (14%); the lack of a close/convenient place for sports to the home/workplace (12%). In a Eurobarometer study, the main reason for the low level of physical activity in our country is the lack of sufficient opportunities for sports and physical activity (46%). Only 4% of Bulgarians are members of sports clubs and 3% of fitness and health

clubs [4]. Approaches to promoting physical activity have changed dramatically in recent years. For several decades, almost all strategies have been designed to educate and motivate young people to be physically active in order to promote health and prevent a number of diseases. The prevention of postural and spinal disorders is an important factor in public health care due to their significant increase. The reason for this drastic increase in spinal deformities is rooted in the modern sedentary lifestyle since childhood, leading to reduced motor activity. According to Filkova (2017), the applied methods for building and improving the motor stereotype for correct posture in conventional kinesiotherapy do not give the desired result. This is a reason to look for and apply new methods that are more effective and motivating for children [5].

The Pilates method is a modern mix of physical exercises, also called new fitness gymnastics [6]. Motor practice helps shape the figure, control weight, builds muscle strength, endurance, and motor qualities. Physical fitness is the first requisite of happiness. Our interpretation of physical fitness is the maintenance of a uniformly developed body with a sound mind fully capable of naturally, easily, and satisfactorily performing our many and varied daily tasks with spontaneous zest and pleasure (Pilates 1945). In Joseph Pilates' book, *Return to Life Through Contrology*, he writes that his method is not merely a physical fitness regimen of mindlessly repeated exercises, but rather a holistic approach to well-being and a lifelong process of refinement [7].

The Pilates system of movement is a holistic exercise program that emphasizes whole-body conditioning. According to Osar and Bussar (2015), this is one of the characteristics that make Pilates such a natural method for exploring fascial fitness

concepts. Dynamic, whole-body movements and stretches are inherent in the Pilates repertoire, a departure from the single-muscle focus of traditional stretching and weight training. A fascial approach to exercise tends to focus on stretching and training long myofascial chains of muscles in movements that are more functional and intuitive [8]. Pilates is a great tool to assist or even enhance a physiotherapy program when someone is recovering from an injury [9]. By strengthening the deepest muscles of the core, optimizing alignment, and creating correct movement patterns [10]. The pilates method can be used by patients with low back pain to improve pain, function and aspects related to quality of life (functional capacity, pain and vitality) [11].

The aim of the study is to conduct a screening for detection of postural disorders and spinal distortions among adolescents and to compile a program based on the Pilates method to improve motor activity in case of disordered posture and spinal curvatures.

Materials and methods

The study covered 90 people aged 6 to 25 held in November 2022 in Plovdiv. of them, 64 (71.1%) were girls and 26 (28.9%) were boys. The average age of the examined persons was 14.6 years. The functional examination included anamnesis, examination in combination with special tests for spinal dysfunctions: cervical, thoracic and lumbar mobility, tests of Ott, Tom Mayer, Schober, Moshkow.

Results and discussion

Regarding somatoscopy for posture assessment, postural disorders, pathological asymmetries and muscle hypotrophy are identified in the analysis of the results. During the examination, a relaxed/incorrect/posture was observed in 57.8% (n = 52) and muscle imbalance in the area of the shoulder

girdle and shoulder blades. The largest percentage of asymmetry is in the area of the shoulder blades – 45.6% (n = 41). We found scoliotic posture in 35.6% (n = 32) of them. The values obtained by us regarding the prevalence of postural disorders are similar to the data from the specialized literature. They are close to the results of a study by S. Filkova (2017), which revealed the frequency of asymmetries in the area of the shoulder girdle and shoulder blades [5].

We found increased thoracic kyphosis in 23.3% (n = 21), kyphoscoliosis – 24.4% (n = 21) and lumbar lordosis – 16.7% (n = 15) of the examined. The deformity is usually associated with periods of rapid growth and may appear during puberty in girls as a malformation in breast development. In this period of their development, different types of orthostatic deformities have already formed, resulting from the student lifestyle – incorrect carrying of school bags; uncomfortable but fashionable shoes; prolonged stay in an incorrect seat, etc.

Divided by gender, the number of girls among the studied adolescents with impaired posture prevails by 38%. The results of the study place first the girls with disturbed /relaxed/ posture (69.2%). Most often, the

posture is the result of weak muscles, changes in different parts of the spine – scoliosis (62.5%), kyphosis (52.3%) and lordosis (53.3). In boys, kyphoscoliosis (59.1%) and minor asymmetry of the shoulder girdle (29.6%) were observed most often. There is no correlation between gender and the type of distortion, motor activity and posture in everyday life are important (Figure 1).

A screening study by R. Pascaleva (2017; 2021) in children of preschool age proves the wide spread of impaired posture – postural disorders in 65.41% and 12.2% – overweight, which confirms the thesis of a number of authors about insufficient motor activity in adolescents [12, 13].

A postural assessment is necessary to check whether the problems are related to the lower limbs, pelvis, shoulder girdle, torso, upper limbs or only functional causes are present [13]. The physical development of adolescents is correlated with their motor and nutritional regime, with their constitution, with hardening, with the early detection, prevention and treatment of acute and chronic diseases that accompany growth. Early diagnosis, systematic monitoring, treatment and proper movement regime contribute to a favorable outcome in children [14].

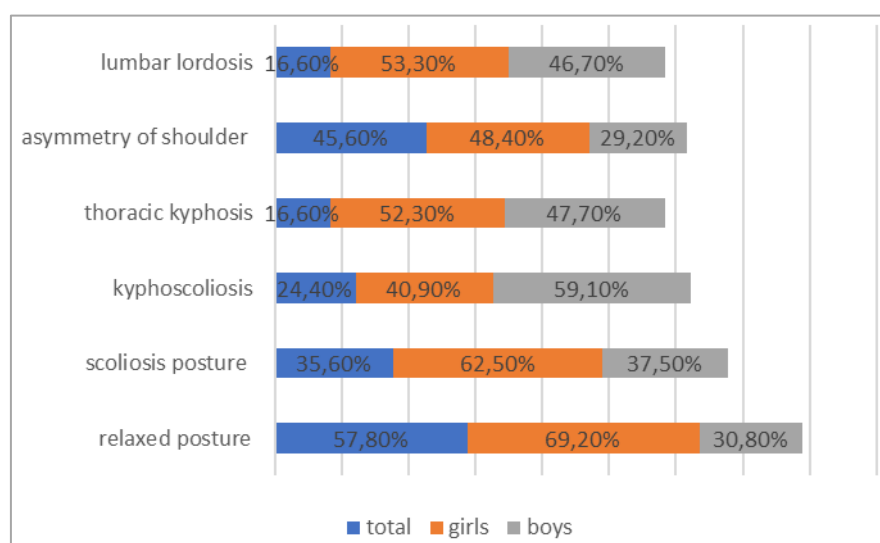


Figure 1. Distribution of the studied orthostatic deformities according to type

At the present day, the treatment of this disease has as its goal the prevention or correction of spinal deformities [15]. Positive results are also observed from programmed corrective training on postural disorders in the lumbar and thoracic region [16]. A program based on the Pilates method was drawn up and approved in accordance with the findings of the functional examination. Original rehabilitation algorithms for the prevention of impaired posture have been promoted, including physical exercises to correct the deformity from unencumbered position and antigravity exercises to stimulate postural reflexes in combination with breathing exercises.

According Filkova and Usheva (2017), “Good posture” is the position of the body that imposes minimal stress on muscles and negligible load on joints in order to maintain balance. This is achieved by keeping the muscle balance necessary for proper development and functioning of the locomotor system [17].

The developed Pilates methodology was presented in an attractive and motivating way for the teenagers, so that they would be interested in continuing the activities even after completing the course.

Evidence is presented in studies that motor activity, physical environment conditions and other factors in children are important for their health in an active age, and Pilates programs can be recommended as an appropriate exercise for impaired posture and musculoskeletal disorders [18].

Conclusion

Correct human posture is one of the signs of good physical development and health. It provides the necessary conditions for the normal functioning of the internal organs, maintains muscle balance and creates favorable mechanical conditions for the

development of the musculoskeletal system. Cultivating the habit of proper posture from childhood has a lasting influence throughout life. The possibilities for the prevention of spinal problems at a young age, which problems are the main reason for back pain in adulthood, have been analyzed.

The prevention of impaired posture and various postural disorders is directly related to the development of initiatives for the affirmation of healthy practices, engagement and the creation of an infrastructure for the practice of therapeutic physical exercises, mass sports and various motor activities on a national level.

References

1. World Health Organization. (2018). Global action plan on physical activity 2018–2030: more active people for a healthier world. World Health Organization. <https://apps.who.int/iris/handle/10665/272722>. License: CC BY-NC-SA 3.0 IGO
2. WHO. Global strategy on diet, physical activity and health. Geneva: World Health Organization, 2004. http://www.who.int/dietphysicalactivity/strategy/eb11344/strategy_english_web.pdf.
3. National program for the prevention of chronic non-communicable diseases 2021-2025. https://www.mh.government.bg/media/filer_public/2021/08/09/nacionalna-programa-prevenciq-hnb-2021-2025_htc24ZU.pdf
4. Public attitudes towards the construction of free outdoor fitness areas. ISIM, 2011. Available at: mbmd.net/Handler_ResourceEdit_DownloadResource?param
5. Filkova S. Prevention of spinal curvatures in pre-school children. Author's abstract of a dissertation work for awarding the educational and scientific degree PhD, Varna, 2017:78.
6. Siler B. The Pilates Body . New York, 2000:15-16.
7. Pilates, J. H. Return to life through contrology. Miami, FL: Pilates Method Alliance. 1945.
8. Osar E, M. Bussard. Functional Anatomy of the Pilates Core: An Illustrated Guide to a Safe and Effective Core Training, North Atlantic Books, Berkeley, California, 2015:7-13.
9. Wood S. Pilates for rehabilitation. Champaign, IL: Human Kinetics, 2019:13.
10. Akbas E, E. U. Erdem. Does Pilates-based approach provide additional benefit over traditional physiotherapy in the management of rotator cuff tendinopathy? A randomized controlled trial. *Annals of Sports Medicine and Research*. 2016, 3(6): 1083.

11. Natour J, L. Araujo Cazotti, L. Ribeiro, et al. Pilates improves pain, function and quality of life in patients with chronic low back pain: A randomized controlled trial. *Clinical Rehabilitation* 2015, 29 (1): 59.
12. Paskaleva R. Prevention and control of postural disorders in childhood - a mission possible. Monograph. EX-PRESS Publishing House Gabrovo 2021:93-99.
13. Paskaleva R. Diagnosis and rehabilitation of spinal deformities - new approach in practical training. *Medicine & Science in Sports & Exercise*, 2017, 12 (2): 2855-2861.
14. Paskaleva R, V. Ivanova, V. Pavlova. Early diagnostics and prevention of spinal deformities in children of pre-school age - an innovative approach in the practical training of the students. *KNOWLEDGE - International Journal*, (23.1), 2018:487-493.
15. Scaramuzzo, Laura. Special Issue: Spinal Deformity: Diagnosis, Complication and Treatment in Adolescent Patients. *Journal of Clinical Medicine* 12, no. 2 2023: 525. <https://doi.org/10.3390/jcm12020525>
16. Bogdanović Z, F. Mavrić, A. Mavrić. Effects of a Programmed Corrective Training on Postural Disorders in Lumbar and Thoracic Region. *International Journal of Sports and Physical Education*, 3, (1), 2017:7-13.
17. Filkova S, N. Usheva. Influence of exercises with sports hammock functional durability supporting posture muscles, *Scripta Scientifica Salutis Publicae*, 2016 2:177.
18. Lee S, C. Lee, D. O'Sullivan, et al. Clinical effectiveness of a Pilates treatment for forward head posture. *Journal of Physical Therapy Science* 28 (7) 2016: 2009-13.

Association between Shift Work and Cardio-metabolic Diseases: a Literature Review

Margarita Todorova, Stanislava Harizanova, Desislava Bakova

Department of Hygiene, Faculty of Public Health, Medical University of Plovdiv

Introduction: Shift work is associated with an adverse risk profile for high mortality and chronic diseases. Despite growing evidence, a significant portion of the workforce in our society remains concentrated in shift and night work. A combination of unfavorable characteristics in nutritional behaviour accompanied by circadian rhythm abnormalities is observed among the shift workers. **Aim:** The aim of this review is to examine the long-term cardio-metabolic health consequences among shift workers. **Materials and methods:** A search was conducted in PubMed, Google Scholar and ScienceDirect Database, as well as official websites of various governmental and non-governmental institutions by using keywords and word combinations. **Results and discussion:** The combination of a disturbed sleep-wake circadian cycle and an unhealthy diet has been found to promote the development of numerous metabolic disorders, such as cardiovascular disease, insulin resistance, diabetes, dyslipidemia, and metabolic syndrome. Studies have shown that cases of overweight and obesity are more prevalent in shift workers than in fixed-day workers. In addition, The International Agency for Research on Cancer classifies shift work as a probable carcinogen. **Conclusion:** Shift work should be considered an occupational hazard. More research is needed to clarify its underlying pathological mechanisms. This will allow researchers to focus on preventive approaches and contribute to the reduction of modern society's morbidity.

Keywords: *Shift work, night-shift work, risk factors, cardio-metabolic diseases, chronic diseases*

Introduction

The most recent scientific evidence highlights the detrimental effects of contemporary work schedules on human well-being. Findings indicate that annual exposure to shift and night work often leads to a combination of unhealthy eating habits and disrupted circadian rhythms, which might contribute to the onset of cardio-metabolic ailments, namely cardiovascular diseases (CVD), hypertension, and type 2 diabetes (T2D) among employed individuals. Gathered scientific data in this review demonstrate a significant prevalence of metabolic syndrome, central and visceral obesity, hypertension and dyslipidemia within the population of shift and night workers.

Aim

This literature aims to clarify the correlation between shift work and cardio-metabolic diseases in conjunction with a pro-inflammatory diet and disrupted endogenous circadian rhythms – two of the most widely debated detrimental factors prevalent among

the cohort of individuals engaged in shift and night work.

Materials and methods

Systematic research was conducted in PubMed, Google Scholar, ScienceDirect Database as well as official websites of various governmental institutions using keywords and word combinations. Inclusion criteria were articles in English, available in full text, published between 2007 and 2022.

Discussion

Overweight and obesity

Obesity and overweight are defined according to the value of the body mass index (BMI) value (kg/m^2) or the weight of the individual divided by their height squared. According to WHO, obesity in adults is defined as a BMI greater than or equal to 30, while overweight is defined as a BMI within the range of 25.0 to 29.9. (1) Obesity constitutes a component of metabolic syndrome and is closely linked to the development of diabetes, hypertension, coronary heart disease,

stroke, obstructive sleep apnea, and certain types of cancer. (2) In 2017, globally, obesity and overweight were responsible for 2.4 million deaths and 70.7 million disability-adjusted life years among women and 2.3 million deaths and 77.0 million disability-adjusted life years among men. (3)

The latest scientific evidence reveals that the prevalence of overweight and obesity is higher among individuals engaged in shift and night work compared to those working fixed day shifts. (4) Results from a comprehensive meta-analysis encompassing 28 studies show that shift and night work increase the risk of obesity and overweight by 23%, along with a 35% higher likelihood of developing abdominal obesity. Moreover, metabolic consequences and their severity vary based on the specific type of non-standard work schedule: permanent night workers exhibit a 29% more elevated risk of obesity when in comparison with workers on rotating night shifts. (5) Liu et al. report that continuous night shifts correlate with a higher frequency of overweight while rotating night shifts heighten the risk of obesity. (6) It is suggested that the combination of unhealthy dietary behavior and chronic circadian desynchronization are the main causative factors for the higher frequency of obesity among night and shift workers. Desynchronization symptoms include lack of sleep, irregular timing of food intake and anomalies in daily and nighttime activity, which are typical for night shift workers and are often associated with obesity. (7) A cross-sectional study of 1593 participants by Givens et al. reports finds a positive correlation between sleep disturbances and obesity in shift workers. (8) The exact pathological mechanism behind this phenomenon is not clear. More high-quality longitudinal studies are needed to clarify this connection.

Metabolic syndrome

Metabolic syndrome (MetS) manifests as a combination of metabolic abnormalities: hypertension, obesity, insulin resistance, and atherogenic dyslipidemia. It has been reported that mortality in individuals with MetS is 46% higher compared to that in healthy individuals. (9)

Bacquer et al. conducted a six-year prospective study that followed 1,529 shift workers from several large Belgian companies and public administrations which resulted in 23.8% of the shift workers developing Metabolic Syndrome (MetS) after the six-year period. (10) In the systematic review of Canuto et al., ten out of eight studies show a positive correlation between shift work and the frequency of MetS. (11) A meta-analysis of 12 studies by Sooriyaarachchi et al., compared the risk of adverse health outcomes between groups of medical workers on rotating shifts and fixed day shifts and reports that workers on rotating and night shifts are twice as likely to develop MetS compared to fixed-day workers. (12) In another meta-analysis, Khosravipour et al. report that workers on rotating shifts have a higher likelihood of developing MetS than those hired on other types of work schedules. (13) The cross-sectional study of Bayon et al. shows that the frequency of MetS in individuals continuously working on night shifts is higher among men compared to women, whereas women working at night suffer from a higher risk of elevated triglyceride levels compared to men. (14) The cross-sectional study by Yu et al. is the first of its kind conducted among young workers – the study of 3,317 individuals aged between 20 and 40 shows that the group of female employees working shifts is associated with a higher risk of metabolic syndrome, while no significant association is

observed within the group of male shift workers. (15)

Insulin resistance and type 2 diabetes

The relationship between disrupted glucose metabolism and circadian disruption caused by long-term shift work is of significant scientific interest. Wefers et al. were the first to investigate the direct impact of disrupted circadian rhythms on insulin sensitivity in skeletal muscles: a circadian disruption was induced in fourteen healthy, non-smoking, non-shift-working men. The following skeletal muscle biopsy and molecular analysis found that on a molecular level, physiological processes were not synchronized with the induced circadian disruption which led to elevated levels of postprandial plasma glucose, despite elevated levels of plasma insulin. (16) In another laboratory study, Morris et al. found a decrease in glucose tolerance among shift workers in a laboratory study, with a 5.6% increase in postprandial glucose levels. (17) Silva-Costa et al. found a positive connection between shift work and increased fasting glycemia and higher levels of glycosylated hemoglobin (18).

Gan et al. conducted a meta-analysis examining 12 observational studies, which found that shift workers have a 9% higher risk of developing type 2 diabetes than those working on a fixed daytime schedule, with men being at a higher risk than women. (19) The meta-analysis of Gao et al. reports that shift workers are at a 10% higher risk of developing type 2 diabetes compared to fixed-day workers, with a trend towards increased risk associated with the annual duration of shift work. (19)

It is suggested that when exposed to shift-work, individuals with a late chronotype (i.e., whose biological night occurs later on a hormonal and metabolic level in the 24-hour cycle of light/darkness) suffer less

from circadian rhythm disruptions compared to workers with an early chronotype. (20) Chronotype-work schedule misalignment is associated with an elevated risk of metabolic disturbances, including a higher susceptibility to type 2 diabetes in early chronotypes working frequent night shifts. (20) On the other hand, studies show that early morning shifts are unfavorable for the circadian rhythms of late chronotypes. (21) Considering these results, the chronotype of each worker would be an important component in developing health prevention strategies, and its inclusion in future studies on shift work as a risk factor for type 2 diabetes will lead to more consistent and robust results.

The study by Pan et al. is one of the most accurate on the connection between shift work and type 2 diabetes: the 20-year study followed 69,269 women and found that the risk of developing type 2 diabetes increases with the duration of shift work: 5% after 1–2 years, 20% after 3–9 years, 40% after 10–19 years, and almost 60% after ≥ 20 years. The study is part of the Nurses' Health Study, the largest prospective study on health and chronic diseases among nurses which is expected to confirm the relationship between diet, shift work, and metabolic diseases, and develop new prevention strategies and policies for shift workers. (22)

Cardiovascular disease, dyslipidemia and hypertension

Although the connection between shift work and CVD is well-documented by epidemiological studies, the exact mechanisms leading to this adverse phenomenon are not clear. According to Torquati et al., night shifts increase the risk of cardiovascular incidents and mortality respectively by 17% and 20%. (23) An increased risk of atrial fibrillation (the most common arrhythmia in congestive heart failure) has been found in

workers exposed to prolonged night shifts. (24) Burgueno et al. observed increased levels of the protein resistin (an inflammatory biomarker associated with atherosclerosis) in rotating shift workers. (25) The meta-analysis of Lu et al. found that shift workers are predisposed to leukocytosis and elevated levels of plasma resistin and MetS. (26)

The state of the lipid profile is one of the main mediators of the risk of CVD. When it comes to the relationship between dyslipidemia and shift work – studies present us with diverse results. Tucker et al. conducted the first prospective study on the association between non-standard work schedules and dyslipidemia. The study followed two large cohorts (11,998 men and 49,944 women) and found that night shift workers had a higher frequency of purchasing medications for dyslipidemia (mainly statins), providing evidence for a link between non-standard work schedules and adverse lipid profiles. (27) Dutheil et al. found that permanent night shift workers have higher levels of total cholesterol, a higher risk of dyslipidemia, and significantly lower levels of HDL cholesterol compared to fixed day shift workers. (28) In contrast to these findings, earlier studies do not find a connection between lipid profile components and shift work. (18, 29) Loef et al. report that workers on shift and night schedules with work experience of 20 years or more have significantly lower levels of total cholesterol and LDL cholesterol. (30) These conflicting results show a need for future and more in-depth scientific research when it comes to the effects of shift work on the lipid profile.

The rhythmic secretion of melatonin in the human body is closely related to the course of circadian rhythms and plays an important role in the maintenance of normal

blood pressure. (31) Shift workers are exposed to light, physical activity and ingestion of food during night hours which disrupts the rhythmic melatonin secretion and leads to compromised circadian rhythms and poor blood pressure control. (32) Morris et al. induce a state of short-term circadian deviation in healthy, fixed-day time workers. This 8-day experiment leads to increased systolic blood pressure (by 1.4 mmHg from the baseline level) and an increase in diastolic blood pressure (by 0.8 mmHg from the baseline level), as well as a rise in the values of the CRP protein (an inflammatory biomarker) in the participants. (17) The meta-analysis by Manohar et al., which included 27 studies, shows a significant association between rotating shift work and an increased risk of developing hypertension. (33) A study on 1075 shift workers and 878-day workers from a petrochemical plant found a correlation between work experience in a specific shift schedule and the frequency of hypertension. Workers with more than 20 years of shift work experience had a significantly higher risk of developing hypertension. (32) To examine the long-term health effects of shift work, Guo et al. studied 26,463 retired employees divided into five categories based on the duration of their work experience in shiftwork conditions: 1) never worked shifts, 2) 1–4 years, 3) 5–9 years, 4) 10–19 years, and 5) over 20 years. The study found that short-term exposure to shift work (less than 10 years) mainly affects sleep quality, whereas long-term exposure (over 10 years) is associated with an increased risk of diabetes and hypertension. (34) It should be noted that shift work predisposes unhealthy behavior like smoking and inadequate dietary patterns (35) which can be seen as additional risk factors for the development of CAD, unhealthy lipid profile and hypertension.

Conclusion

Without a doubt, shift work is considered an occupational adverse factor. Compared to employees on fixed daily schedules, those exposed to night and shift work have a higher risk of cardio-metabolic diseases. The population group of shift and night workers is more likely to experience disruptions in endogenous circadian rhythms and unhealthy eating habits. There is an urgent need to provide evidence-based nutritional recommendations based not only on “what shift workers should eat”, but also on “how, when, and why”. Effective nutritional recommendations designed for shift and night workers would help to compensate for the consequences of circadian disruption, unnatural night-time activity, and night-time food intake.

References

1. Lin X, Li H. Obesity: Epidemiology, Pathophysiology, and Therapeutics. Vol. 12, Frontiers in Endocrinology. Frontiers Media S.A.; 2021.
2. World Health Organization. Global status report on noncommunicable diseases 2014. 280 p.
3. Dai H, Alsalhe TA, Chalghaf N, Riccò M, Bragazzi NL, Wu J. The global burden of disease attributable to high body mass index in 195 countries and territories, 1990–2017: An analysis of the Global Burden of Disease Study. Vol. 17, PLoS Medicine. Public Library of Science; 2020.
4. Brum MCB, Dantas Filho FF, Schnorr CC, Bertolotti OA, Bottega GB, Da Costa Rodrigues T. Night shift work, short sleep and obesity. *Diabetol Metab Syndr*. 2020 Feb 10;12(1).
5. Sun M, Feng W, Wang F, Li P, Li Z, Li M, et al. Meta-analysis on shift work and risks of specific obesity types. Vol. 19, Obesity Reviews. Blackwell Publishing Ltd; 2018. p. 28–40.
6. Liu Q, Shi J, Duan P, Liu B, Li T, Wang C, et al. Is shift work associated with a higher risk of overweight or obesity? A systematic review of observational studies with meta-analysis. *Int J Epidemiol*. 2018 Dec 1;47(6):1956–71.
7. Antunes LC, Levandovski R, Dantas G, Caumo W, Hidalgo MP. Obesity and shift work: Chronobiological aspects. *Nutr Res Rev*. 2010 Jun;23(1):155–68.
8. Givens ML, Malecki KC, Peppard PE, Palta M, Said A, Engelman CD, et al. Shiftwork, sleep habits, and metabolic disparities: Results from the Survey of the Health of Wisconsin. *Sleep Health*. 2015 Jun 1;1(2):115–20.
9. Hui WS, Liu Z, Ho SC. Metabolic syndrome and all-cause mortality: A meta-analysis of prospective cohort studies. *Eur J Epidemiol*. 2010;25(6):375–84.
10. De Bacquer D, Van Risseghem M, Clays E, Kittel F, De Backer G, Braeckman L. Rotating shift work and the metabolic syndrome: A prospective study. *Int J Epidemiol*. 2009;38(3):848–54.
11. Canuto R, Garcez AS, Olinto MTA. Metabolic syndrome and shift work: A systematic review. Vol. 17, Sleep Medicine Reviews. 2013. p. 425–31.
12. Sooriyaarachchi P, Jayawardena R, Pavey T, King NA. Shift work and the risk for metabolic syndrome among healthcare workers: A systematic review and meta-analysis. Vol. 23, Obesity Reviews. John Wiley and Sons Inc; 2022.
13. Khosravipour M, Khanlari P, Khazaie S, Khosravipour H, Khazaie H. A systematic review and meta-analysis of the association between shift work and metabolic syndrome: The roles of sleep, gender, and type of shift work. Vol. 57, Sleep Medicine Reviews. W.B. Saunders Ltd; 2021.
14. Bayon V, Berger M, Solelhac G, Haba-Rubio J, Marques-Vidal P, Strippoli MP, et al. Impact of night and shift work on metabolic syndrome and its components: A cross-sectional study in an active middle-to-older-aged population-based sample. *BMJ Open*. 2022 Feb 15;12(2).
15. Yu KH, Yi YH, Kim YJ, Cho BM, Lee SY, Lee JG, et al. Shift work is associated with metabolic syndrome in young female Korean workers. *Korean J Fam Med*. 2017;38(2):51–6.
16. Wefers J, Van Moorsel D, Hansen J, Connell NJ, Havekes B, Hoeks J, et al. Circadian misalignment induces fatty acid metabolism gene profiles and compromises insulin sensitivity in human skeletal muscle. *Proc Natl Acad Sci U S A*. 2018 Jul 24;115(30):7789–94.
17. Morris CJ, Purvis TE, Mistretta J, Scheer FAJL. Effects of the internal circadian system and circadian misalignment on glucose tolerance in chronic shift workers. *Journal of Clinical Endocrinology and Metabolism*. 2016 Mar 1;101(3):1066–74.
18. Silva-Costa A, Rotenberg L, Coeli CM, Nobre AA, Härter Griep R. Night work is associated with glycemic levels and anthropometric alterations preceding diabetes: Baseline results from ELSA-Brasil. *Chronobiol Int*. 2016 Jan 2;33(1):64–72.
19. Gan Y, Yang C, Tong X, Sun H, Cong Y, Yin X, et al. Shift work and diabetes mellitus: A meta-analysis of observational studies. Vol. 72, Occupational and Environmental Medicine. BMJ Publishing Group; 2015. p. 72–8.
20. Vetter C, Devore EE, Ramin CA, Speizer FE, Willett WC, Schernhammer ES. Mismatch of sleep and work timing and risk of type 2 diabetes. *Diabetes Care*. 2015 Sep 1;38(9):1707–13.
21. Juda M, Vetter C, Roenneberg T. Chronotype modulates sleep duration, sleep quality, and social jet lag in shift-workers. *J Biol Rhythms*. 2013 Apr;28(2):141–51.
22. Pan A, Schernhammer ES, Sun Q, Hu FB. Rotating night shift work and risk of type 2 diabetes: Two

- prospective cohort studies in women. *PLoS Med*. 2011 Dec;8(12).
23. Torquati L, Mielke GI, Brown WJ, Kolbe-Alexander T. Shift work and the risk of cardiovascular disease. A systematic review and meta-analysis including dose-response relationship. Vol. 44, *Scandinavian Journal of Work, Environment and Health*. Nordic Association of Occupational Safety and Health; 2018. p. 229–38.
 24. Linz D, Arzt M, Baumert M. Keep your rhythm during rotational night work shifts! Vol. 42, *European Heart Journal*. Oxford University Press; 2021. p. 4189–91.
 25. Burgueño A, Gemma C, Gianotti TF, Sookoian S, Pirola CJ. Increased levels of resistin in rotating shift workers: A potential mediator of cardiovascular risk associated with circadian misalignment. *Atherosclerosis*. 2010 Jun;210(2):625–9.
 26. Lu YC, Wang CP, Yu TH, Tsai IT, Hung WC, Lu IC, et al. Shift work is associated with metabolic syndrome in male steel workers-the role of resistin and WBC count-related metabolic derangements. *Diabetol Metab Syndr*. 2017 Oct 16;9(1).
 27. Tucker P, Härmä M, Ojajarvi A, Kivimäki M, Leineweber C, Oksanen T, et al. Associations between shift work and use of prescribed medications for the treatment of hypertension, diabetes, and dyslipidemia: A prospective cohort study. *Scand J Work Environ Health*. 2019;45(5):465–74.
 28. Dutheil F, Baker JS, Mermillod M, De Cesare M, Vidal A, Moustafa F, et al. Shift work, and particularly permanent night shifts, promote dyslipidaemia: A systematic review and meta-analysis. *Atherosclerosis*. 2020 Nov 1;313:156–69.
 29. Esquirol Y, Perret B, Ruidavets JB, Marquie JC, Dienne E, Niezborala M, et al. Shift work and cardiovascular risk factors: New knowledge from the past decade. Vol. 104, *Archives of Cardiovascular Diseases*. 2011. p. 636–68.
 30. Loefer B, Van Baarle D, Van Der Beek AJ, Beekhof PK, Van Kerkhof LW, Proper KI. The association between exposure to different aspects of shift work and metabolic risk factors in health care workers, and the role of chronotype. *PLoS One*. 2019 Feb 1;14(2).
 31. Baker J, Kimpinski K. Role of melatonin in blood pressure regulation: An adjunct anti-hypertensive agent. Vol. 45, *Clinical and Experimental Pharmacology and Physiology*. Blackwell Publishing; 2018. p. 755–66.
 32. Yeom JH, Sim CS, Lee J, Yun SH, Park SJ, Yoo CI, et al. Effect of shift work on hypertension: Cross sectional study. *Ann Occup Environ Med*. 2017 Apr 11;29(1).
 33. Manohar S, Thongprayoon C, Cheungpasitporn W, Mao MA, Herrmann SM. Associations of rotational shift work and night shift status with hypertension: A systematic review and meta-analysis. Vol. 35, *Journal of Hypertension*. Lippincott Williams and Wilkins; 2017. p. 1929–37.
 34. Guo Y, Liu Y, Huang X, Rong Y, He M, Wang Y, et al. The Effects of Shift Work on Sleeping Quality, Hypertension and Diabetes in Retired Workers. *PLoS One*. 2013 Aug 16;8(8).
 35. Bae MJ, Song YM, Shin JY, Choi BY, Keum JH, Lee EA. The Association Between Shift Work and Health Behavior: Findings from the Korean National Health and Nutrition Examination Survey. *Korean J Fam Med*. 2017;38(2):86.

A 23-year-old Man with a Rash after Returning from Italy

A. H. Syed

Mentors: R. Komitova, S. Krumova, P. Argirova, O. Boykinova

Faculty of Medicine, Medical University of Plovdiv

Monkeypox (previously monkeypox), an African zoonosis has been known for decades. Sadly, it only became a topic of interest after it rapidly spread outside Africa in 2022. We described a case (homosexual male) of mpox after returning from Italy, following the pattern of recent mpox cases. All of his samples tested positive for the mpox virus. The patient made an uneventful recovery. We discussed the unusual, atypical, and confusing aspects of current mpox outbreaks. Vaccines and antiviral agents have been in our toolbox since the beginning of the mpox epidemics (based on animal data). However, to prevent the reemerging infection from becoming a pandemic, effective and equitable use of available tools is essential.

Keywords: monkeypox, epidemics, MSM

Introduction

As the world recovers from the shock of the COVID-19 pandemic and reflects on the lessons learned from the failure to contain the global SARS-CoV-2 outbreak, a new infectious disease threat has remerged – an orthopoxvirus African infection – mpox (previously known as monkeypox) (1). Unprecedented and unexpected outbreaks of human monkeypox cases since May 7, 2022 rapidly spread across Europe, the Americas, and Australia. As of January 2023, the number of confirmed cases are 84 639 in 111 countries and 86 deaths (2). Sadly, the mpox had only become a topic of interest once transmitted outside Africa. There are several unusual, atypical, and perplexing aspects of these outbreaks. We described a case of following the pattern of recent MOX vases in some European countries.

Case presentation

A 23-year-old previously healthy man was admitted to the infectious disease department, University hospital, Plovdiv, in July 2022 after being referred from another hospital after 2-day treatment for severe pharyngitis and high fever. A rash appeared on the second day of his hospital stay. He presented after 5 days of high fever, malaise, myalgia and sore throat. In this hospital the physical examination revealed a temperature of 39°C,

blood pressure of 105/65 mmHg, pulse of 116 beats per minute, and oxygen saturation of 97% on room air. Pulmonary and cardiac examination revealed no abnormalities. There were scattered small papules on his face, trunk, and extremities and enoral lesion on the left tonsil. The left submandibular lymph nodes were palpable and tender. He reported having had unprotected homosexual intercourse 15 days earlier in Italy, from where he returned 10 days ago. In light of recent cases in several European countries, including Italy, an mpox infection was suspected. A monkeypox virus was positive on the skin and throat swabs, as well as in semen, urine, stools and sputum (NCIPD, Sofia). His blood borne virus screen test was negative for HCV, HBV and HIV. He also tested negative for syphilis. A few days after admission, he was informed by his partner that he had a confirmed mpox. The patient remained febrile, and new lesions continued to develop and evolved into vesicles until day 8th, after which all lesions turned into pustules and began to crust. No specific treatment except ceftriaxone for suspected bacterial skin superinfection was administered. He was discharged on day 11th and advised to isolate at home until all lesions desquamated.

At 4-month follow-up the patient was doing well.

Discussion

We described a case of mpox following the pattern of recent mpox cases in some European countries.

Classically, before 2022, most data on human mpox were reported from central and western Africa. Animal-to-human was the predominant way of transmission and rodents and other mammals were likely reservoirs. Children, pregnant women, and immunocompromised were most at risk.

The mpox (monkeypox) virus was first isolated and identified in 1959 when monkeys shipped from Singapore to a Denmark research facility fell ill (3). However, the first confirmed human case was in 1970 when the virus was isolated from a child in the Democratic Republic of Congo suspected to have smallpox. (4).

Typically, what is already known (or thought to be known) about the diseases is as follows:

After an incubation period of 7–14 days a prodrome and lymphadenopathy appear followed by a rash. The mpox rash is centrifugal (predominantly on the face and distal extremities). The lesions evolve simultaneously through papular, vesicular, pustular, and crust phases over 14–21 days. They are characterized as firm and deep-seated, and 2 to 10 mm in size. Lethality rates vary depending on mpox virus clades included (1–10%) and are higher in Central Africa (5, 6, 7).

The available information is incomplete, as most of the cases were not laboratory confirmed because of a scarcity of local diagnostic infrastructure, challenges with civil unrest, and the existing healthcare system.

The mpox has been on the rise since the 1970s, and as of 2017, more cases had been diagnosed than in the previous 40 years. Previous vaccination against smallpox was estimated to be 85% effective in preventing

mpox. However, eradicating smallpox and the subsequent suspension of vaccination paved the way for mpox to gain clinical relevance. This rise is most likely attributable to natural and man-made factors (8, 9).

The current epidemics are precedential in several ways (is mpox a new name for a new disease?) (10, 11). For the first time, the outbreak has spread outside Africa to 5 continents. Most cases have no apparent epidemiological ties to the endemic regions (recent travel to endemic areas, interaction with wild animals imported from endemic areas).

The affected population is mainly, though not solely, gay and bisexual men who have sex with men (GBMSM). The dominant mode of transmission is through close contact during sex. Patients with mpox often experience a localized rash. The genitalia or anus are usually affected and have a clustering of lesions. The latter are believed to be due to the sexual nature of transmission. Usually, there is no flu-like syndrome. Lesions show pleomorphism, presenting at different stages simultaneously. Single lesions are not uncommon. Vesicles and pustules may be smaller than classically described. **Our patient's recent trip to Italy, his high-risk sexual behaviour, and characteristic skin manifestations alerted the treating physicians to proceed with further investigations.** Complications, including bacterial superinfections, myocarditis, and encephalitis, are rare. Proctitis, not usually associated with mpox, is common. Initially, some cases have been erroneously misdiagnosed as sexually transmitted diseases. As in our case, the current mpox is a self-limiting illness, often requires only symptomatic treatment, and mortality remains low (0,05%).

Mpox should be suspected in any patient presenting with a new **unspecified** rash and lymphadenopathy, esp. with recent travel to

central or West Africa, part of Europa where mpox has been reported, or in those with any known contacts with mpox. The collection of two specimens, each from multiple lesions from different locations, is required. RT-PCR on lesion materials or body fluids can be used. Samples should first be tested for orthopoxvirus species and then positive samples further tested with mpox-specific PCR.

Vaccines and antiviral agents are in our toolbox from the beginning of the mpox outbreaks, whereas the COVID-19 toolbox was empty when COVID-19 emerged (12)

Currently, there are no specific clinically proven treatments for mpox infection. Tecovirimat, the preferred antiviral, was approved for the treatment of smallpox, not monkeypox, as part of bioterrorism preparedness (based on data in animals). The medication is safe and tolerable, with minor side effects but not readily available in many countries. It is indicated for the immunocompromised and those with severe diseases (widespread rash, severe pain, and/or system symptoms). Treatment is yet to be evaluated in a randomized controlled trial and a few are underway (13). Others, less commonly used, are cidofovir, brincidofovir, and vaccinia immune globulin (14, 15).

The uncomplicated mpox course in the presented case required only symptomatic treatment apart from ceftriaxone for suspected bacterial superinfection.

A new smallpox vaccine (MCA-BN) approved for mpox contains a life-attenuated non-replicating virus. In Europe is used under the brand name Imvanex, and in the US – Jynneos. The recommended regimen consists of 2 doses (4 weeks apart) subcutaneously, and the peak in immunity develops 2 weeks after the second dose. It is recommended for a specific high-risk population (16).

Conclusion

Mpox is one of many and not the last reemerging disease. It reminds us that an old neglected disease can spread to new regions affecting a different population presenting with novel clinical presentations.

Notably, the new name (to avoid stigmatization) reflects the new natural history and clinical presentation. with much to explore including effective treatment strategies. Efforts should be made to address stigma within the MSM community and equitably use available treatment and immunization.

References

1. Zumla A, Valdeiros SR, Haider N, et al. Monkeypox outbreaks outside endemic regions: scientific and social priorities. *Lancet Infect Dis*. 2022 Jul;22(7):929-93
2. US Centers for Disease Control and Prevention. 2023 monkeypox outbreak global map. 2023. www.cdc.gov/poxvirus/monkeypox/response/2023/world-map.html (accessed January 15, 2023)
3. von Magnus P, Andersen EK, Perersen KB et al. A pox-like disease in cynomolgus monkeys. *Acta Pathol Microbiol Scand* 1959;46:156-76
4. Ladnyj, I. D., Ziegler, P. & Kima, E. A human infection caused by monkeypox virus in Basankusu Territory, Democratic Republic of the Congo. *Bulletin of the World Health Organization*, 1972 46 (5), 593–59
5. Chen N, Li G, Liszewski MK, et al. Virulence differences between monkeypox virus isolates from West Africa and the Congo basin. *Virology*. 2005 Sep 15;340(1):46-63V
6. Huhn GD, Bauer AM, Yorita K, et al. Clinical characteristics of human monkeypox, and risk factors for severe disease. *Clin Infect Dis*. 2005 Dec 15;41(12):1742-5
7. Jezek Z, Szczeniowski M, Paluku KM, et al. Human monkeypox: clinical features of 282 patients. *J Infect Dis*. 1987 Aug;156(2):293-816
8. Fine PE, Jezek Z, Grab B, Dixon H. The transmission potential of monkeypox virus in human populations. *Int J Epidemiol*. 1988 Sep;17(3):643-50.
9. Happi C, Adetifa I, Mbala P, et al. Urgent need for a non-discriminatory and non-stigmatizing nomenclature for monkeypox virus. *PLoS Biol*. 2022 Aug 23;20(8):e3001769
10. Mitjà O, Ogoina D, Titanji B, et al. Monkeypox. *Lancet*. Online first November 17, 2022 [https://doi.org/10.1016/S0140-6736\(22\)02075-X](https://doi.org/10.1016/S0140-6736(22)02075-X)
11. Thornhill JP, Barkati S, Walmsley S, et al. Monkeypox virus infection in humans across 16 countries – April–June 2022. *N Engl J Med* 2022; 387:679–91.

12. Laine C, Moyer DV, Cotton D. Monkeypox: Challenging Clinical Questions. *Ann Intern Med*. 2022 Dec;175(12):1754-1755
13. Study of Tecovirimat for Human Monkeypox Virus (STOMP) [clinical trial]. Accessed at <https://clinicaltrials.gov/ct2/show/NCT05534984> on 11 October 2022.
14. Warner BM, Klassen L, Sloan A, et al. In vitro and in vivo efficacy of tecovirimat against a recently emerged 2022 monkeypox virus isolate. *Sci Transl Med* 2022; published online Nov 1. <https://doi.org/10.1126/scitranslmed.ade764>
15. Stittelaar KJ, Neyts J, Naesens L, et al. Antiviral treatment is more effective than smallpox vaccination upon lethal monkeypox virus infection. *Nature* 2006; 439:745–47
16. US Centers for Disease Control and Prevention. Monkeypox vaccine guidance. June 2, 2022. www.cdc.gov/poxvirus/monkeypox/clinicians/smallpox-vaccine.html (accessed Nov 6, 2022)

II. Biomedical session

Oxygen Uptake Efficiency Slope (OUES) as a Useful Indicator for Assessment of the Functional Capacity at Submaximal Effort During Cardio-Pulmonary Exercise Testing

Aglika Byandova, Zdravko Taralov, Peter Dimov

Department of Pathophysiology, Faculty of Medicine, Medical University of Plovdiv

Introduction: Cardio-pulmonary exercise testing (CPET) is a valuable tool in evaluating exercise tolerance, functional capacity and ventilatory response. However, maximal effort is needed to obtain the main parameters. **Aim:** The aim of this article is to review some of the possible applications of the oxygen uptake efficiency slope (OUES) as a marker of functional capacity at submaximal level in patients with chronic heart and lung conditions. **Materials and methods:** Using the Scopus database, an advanced search was made to identify articles pertaining to the topic. Initially, 619 articles were found and were narrowed down to under 30 based on the inclusion criteria (age, medical history, use of submaximal effort during testing). **Discussion:** During CPET, a variety of data can be extrapolated including oxygen consumption, CO₂ production, ventilation and ventilatory efficiency. OUES was first introduced as an index of cardio-pulmonary functional reserve. It is derived as the relation between the oxygen uptake (VO₂) and the common logarithm of the minute ventilation (VE). Calculated in such a way, OUES presents a linear relationship between the two parameters. OUES has been proven an objective, effort-independent parameter for assessing the functional reserve at submaximal effort. **Conclusion:** OUES is a useful parameter to assess the functional capacity for groups of patients who find it hard, or impossible to perform a CPET at maximal effort, and therefore cannot reach a VO₂ max, mainly – children, chronically ill patients, as well as athletes in mid-training season.

Keywords: *cardio-pulmonary exercise testing, oxygen uptake efficiency slope, ventilatory efficiency, sub-maximal effort*

Introduction

Cardio-pulmonary exercise testing (CPET) is a comprehensive test that assesses the cardiovascular, respiratory and muscular systems' function during exercise performance. It is a valuable tool in evaluating exercise tolerance, functional capacity and ventilatory response. Moreover, CPET can be a helpful diagnostic tool, aiding clinicians in monitoring the progression of chronic conditions, such as heart and lung disease [1]. During CPET, the individual typically exercises on a stationary bike or a treadmill while connected to specialized equipment. The exercise intensity is gradually increased until the individual reaches their maximum effort or until certain clinical endpoints are reached. During CPET, a variety of physiological parameters can be extrapolated. Some measurements that are being taken

are: minute ventilation (VE) in l/min, oxygen uptake (VO₂) and carbon dioxide production (VCO₂), as well as heart rate (HR) and oxygen saturation (SpO₂). Additional values and indexes that can be calculated from the data, include respiratory exchange rate (RER), oxygen pulse (O₂ pulse) and ventilatory equivalents, as well as several useful thresholds, like the anaerobic threshold (AT) and the respiratory compensation point (RCP) [2]. Data can be easily represented in a graphical formation, with relationships between the different parameters shown in a clear fashion.

Aim

The aim of this article is to review some of the advantages and drawbacks of oxygen uptake efficiency slope (OUES) as a useful marker of functional capacity at submaximal

level in patients with chronic cardiac and respiratory conditions.

Materials and methods

Using the Scopus and PubMed databases, an advanced search was made to identify articles pertaining to the topic. Initially, 619 articles were found and were narrowed down to under 30 based on the inclusion criteria (age, medical history, use of submaximal effort during testing). The chosen articles reflected the time period between 1996 and 2015.

Results and discussion

Oxygen uptake efficiency slope is a newer index of respiratory function, that reflects the relationship between the oxygen uptake (VO_2) and minute ventilation (VE) during exercise [3, 4]. It is calculated by

plotting the oxygen uptake (VO_2) against the minute ventilation (VE) during exercise, and then calculating the slope of this relationship. Specifically, OUES is the slope of the linear regression line that is fit to the VO_2 -VE relationship during incremental exercise (figure 1). A steeper slope indicates a more efficient oxygen uptake, whereas a lower one, means that more ventilation is needed, for a given oxygen quantity to be used by the body. The OUES is expressed in units of $\text{ml O}_2/\text{min}$ per unit of VE (l/min) [5].

Calculating OUES

OUES can be further calculated via a mathematical equation (figure 2). In the given formula, the values “a” and “b” present constants, determined by the patient’s anthropomorphic characteristics. The value “a” represents OUES itself [6].

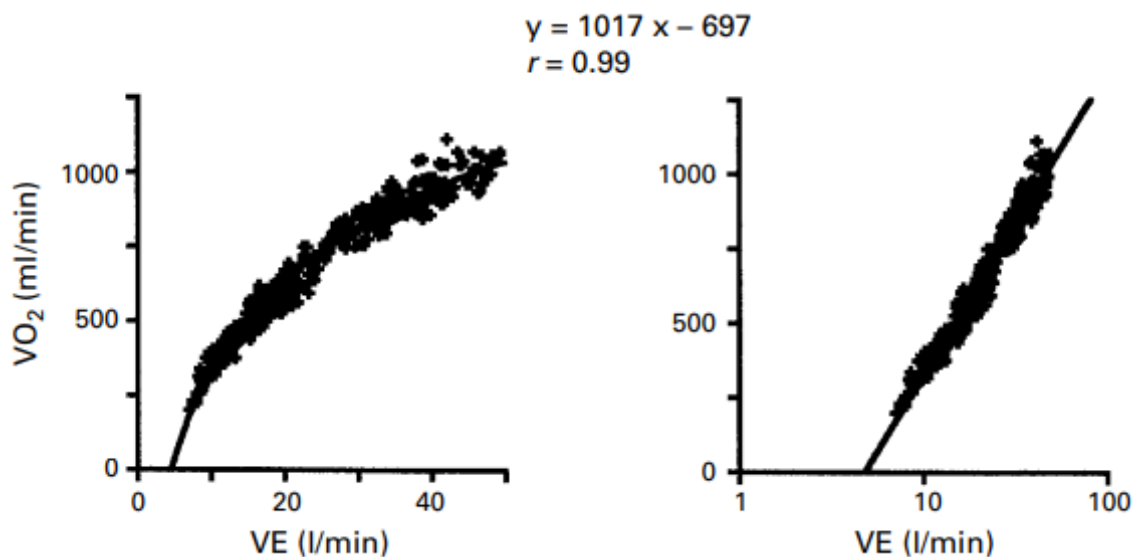


Figure 1. Relationship between oxygen uptake (VO_2) and minute ventilation (VE) during incremental exercise in a representative subject.

Baba R, Nagashima M, Nagano Y. Role of oxygen uptake efficiency slope in evaluating exercise tolerance. Archives of Disease in Childhood, 1999; pp 73-75

$$\text{VO}_2 = a \times \log_{10} \text{VE} + b$$

Figure 2. Mathematical formula for calculating OUES; a = OUES

Advantages of OUES

Research has shown OUES to be a useful parameter, for several different reasons. One of the main advantages of OUES is that it does not need maximal exercise effort from the patient's side, in order to be calculated. OUES remains relatively stable through the entire duration of the exercise test [7], and therefore can be reliably extrapolated even with CPET performed at submaximal level, or prematurely interrupted. Studies have shown that, while shortening the exercise protocol affects both VO_2max and the VE/VCO_2 slope, the same cannot be said about OUES [8]. This is especially useful for testing populations with either a lower functional reserve and existing cardiac or ventilatory limitations (such as patients with chronic heart or lung disease [9] or for testing populations with a lower rate of compliance and motivation, such as pediatric subjects [10, 11].

Moreover OUES has proven to be an objective parameter, since it can be mathematically determined (via the formula stated above) from a set of exercise data, rather than needing a subjective interpretation of the graphic data.

There is a significant correlation between OUES and VO_2max , calculated at maximal and submaximal (75% and 85%) aerobic running speed [12]. Therefore, OUES is an accurate enough substitute for VO_2max in cases where VO_2max was not reached during the exercise protocol.

Results have shown that OUES is reproducible and reliable, supporting the clinical usefulness of this index – an index of replicability for OUES around 20% has been established [13].

Disadvantages of OUES

While OUES is a useful measure of cardiorespiratory fitness and exercise efficiency, there are some limitations and potential disadvantages to consider. A major drawback of this index is the technical complexity of the parameter, since it requires specialized equipment, in order to be measured accurately. This can make it challenging to use in clinical and research settings that do not have access to these resources. Moreover, the lack of standardization between different protocols, makes it difficult to compare the results across different studies and populations. Since OUES values can be affected by body size, age, and sex, this can make it challenging to interpret results in different populations or to compare results across studies [14]. More research is needed to determine reference values for OUES across different age groups, as well as how OUES is influenced by the onset of puberty and how it reflects exercise training in children, and the difference in values between healthy children and children with chronic diseases [15, 16].

While OUES has been shown to be a useful predictor of mortality and cardiovascular events in some populations [17], its predictive value may be limited in others [18]. Additionally, it may not be as useful as other measures of cardiorespiratory fitness or exercise capacity in certain populations or clinical situations, for example individuals with severe exercise limitations, or those with medical conditions that affect the oxygen uptake and ventilation.

Conclusion

OUES is a useful parameter in CPET, however, since it is a rather new index, more research needs to be done in order to determine, whether OUES can be adequately used as a prognostic index in different patient groups, as well as how it changes in different populations, including the overweight and obese. Moreover, data obtained using treadmill exercise and bicycle ergometer needs to be compared, in order to deduce whether the two methods can be used interchangeably. Further research is needed for a better insight of OUES in the pediatric population. Overall, while OUES can be a useful measure of cardiorespiratory fitness and exercise efficiency, it should be used in conjunction with other measures and interpreted in the context of the individual's overall health status and other relevant factors.

References

1. Albouani K, Egred M, Lhmar A, Wright D. J. Cardiopulmonary exercise testing and it's application. *Heart* 2007; 93:1285–1292
2. Herdy A, Ritt L, Stein R. Cardiopulmonary exercise test: Background, applicability, and interpretation. *Brazilian Archives of Cardiology* 2016; 107 (5): 467–481
3. Baba R, Nagashima M, Nagano Y. Role of oxygen uptake efficiency slope in evaluating exercise tolerance. *Archives of Disease in Childhood*, 1999; pp 73–75
4. Baba R. The oxygen uptake efficiency slope and it's value in the assessment of cardiorespiratory functional reserve. *Congestive heart failure*, 2000; 6:256–258, 276
5. Baba R, Nagashima M, Goto M, et al. Oxygen intake efficiency slope: a new index of cardiorespiratory functional reserve derived from the relationship between oxygen consumption and minute ventilation during incremental exercise. *Nagoya Journal of Medical Science* 1996; 59:55–62
6. Flaconi L, Guidetti L, Baldari C, Gallotta MC, Meucci M. Oxygen uptake efficiency slope in healthy normal weight young males: an applicable framework for calculation and interpretation. *PeerJ* 2022, 10:e13709
7. Van Laethem C, Bartunek J, Goethals M, Nellens P, Andries E, Vanderheyden M. Oxygen uptake efficiency slope, a new submaximal parameter in evaluating exercise capacity in chronic heart failure patients. *American Heart Journal* 2005;149:175–180
8. Hollenberg M, Tager IB. Oxygen uptake efficiency slope: an index of exercise performance and cardiopulmonary reserve requiring only submaximal exercise. *Journal of the American College of Cardiology* 2000; 36:194–201
9. Van Laethem C, Van De Veire N, Backer B, et al. Response of the oxygen uptake efficiency slope to exercise training in patients with chronic heart failure. *European Journal of Heart Failure* 2007; 9:625–629
10. Marinov B, Mandadzhieva, Kostianev S. Oxygen uptake efficiency slope in healthy 7- to 18-year-old children. *Pediatric exercise science*, 2007, 19, pp 159–170
11. Marinov B, Kostianev S. Exercise performance and oxygen uptake efficiency slope in obese children performing standardized exercise. *Acta Physiologica et Pharmacologica Bulgarica* 2003; 27:59–64.
12. Pichon A, Jonville S, Denjean A. Evaluation of the interchangeability of VO₂max and oxygen uptake efficiency slope. *Canadian Journal of Applied Physiology* 2002; 27: 589–601
13. Baba R, Kubo N, Morotome Y, Iwagaki S. Reproducibility of the oxygen uptake efficiency slope in normal healthy subjects. *Journal of Sports Medicine and Physical Fitness*. 1999;39:202–206
14. Carter H, Jones AM, Barstow TJ, Burnley M, Williams CA, Doust JH. Oxygen uptake kinetics in treadmill running and cycle ergometry: a comparison. *Journal of Applied Physiology* 2000; 89:899–907
15. Akerman M, Brussel M, Hulzebos E. The oxygen uptake efficiency slope. What do we know? *Journal of Cardiopulmonary Rehabilitation and Prevention* 2010; 30:357–373]
16. Harkel A, Takken T. Oxygen uptake efficiency slope in children: its role in exercise testing. *European Journal of Preventative Cardiology*, 2019, vol. 26(2), pp 171–173]
17. Peterman JE, Novelli DS, Fleenor BS, Whaley MH, Kaminsky LA, Harber MP. Oxygen Uptake Efficiency Slope as a Predictor of Mortality Risk: The Ball State Adult Fitness Longitudinal Lifestyle Study (BALL ST). *Journal of Cardiopulmonary Rehabilitation and Prevention* 2022; Nov 17. doi: 10.1097/HCR.0000000000000744. Epub ahead of print. PMID: 36394515
18. Giardini A, Specchia S, Gargiulo G, Sangiorgi D, Picchio FM. Accuracy of oxygen uptake efficiency slope in adults with congenital heart disease. *Int J Cardiol*. 2009 Mar 20;133(1):74-9. doi: 10.1016/j.ijcard.2007.11.092. Epub 2008 Feb 1. PMID: 18242739

Corresponding author

Aglia Aleksandrova Byandova, MD
Department of Pathophysiology
Medical University of Plovdiv
Bul “Vasil Aprilov” 15A, 4002 Plovdiv
+ 359 889 362 589
Email: abyandova@pathophysiology.info

Review of the Proposed Pathophysiological Mechanisms for Postural Orthostatic Tachycardia Syndrome as Part of Long-Covid Syndrome

Petar Georgiev¹, Krasimir Avramov², Zdravko Taralov²

1. Faculty of Medicine, Medical University of Plovdiv

2. Department of Pathophysiology, Faculty of Medicine, Medical University of Plovdiv

Introduction: Postural Orthostatic Tachycardia Syndrome (POTS) is a dysautonomia that is long-recognized to impair the quality of life, but now it is also acknowledged as a major part of Long-COVID Syndrome (LCS). The symptoms of POTS deteriorate the daily functioning of patients. Moreover, since its incidence is increased, it is essential to understand the pathophysiological mechanisms that induce it after SARS-CoV-2 infection. **Aim:** This review aims at exploring the proposed mechanisms that trigger POTS after acute SARS-CoV-2 infection in relation to the already well-established pathogenesis of the condition. **Material and Methods:** A search in the PubMed database was performed utilizing the following keywords: postural orthostatic tachycardia and long covid syndrome. The results were filtered to include articles from 2019 to 2023 and 25 articles were selected. **Results and Discussion:** Both POTS and LCS involve the dysfunction of multiple systems. As with other dysautonomias, the etiology for POTS could be a viral agent – in this case – SARS-CoV 2. Several mechanisms are discussed – immune-mediated alteration in different receptors, cellular toxicity by the viral spike protein, and neuroinvasion directly through the olfactory nerve or indirectly through the gastrointestinal system. Furthermore, other components of LCS set the ground for POTS manifestation – physical deconditioning, chronic fatigue, hypovolemia, sustained proinflammatory state, etc. **Conclusion:** Although some of the hypothesized mechanisms are not unfamiliar, there are also novel findings to explain the development of POTS as part of LCS. Improvement in their understanding allows for the development of suitable preventive and rehabilitation strategies.

Keywords: *postural orthostatic tachycardia, long covid syndrome*

Introduction

Postural Orthostatic Tachycardia Syndrome (POTS) is a dysautonomia characterized by an increase in heart rate (HR) of at least 30 beats per minute within 10 minutes of standing up from a supine position, as observed in a head-up tilt test (HUTT) for adults over 19 years old, or a minimum of 40 beats per minute for younger patients (1). POTS has been long recognized to impair the quality of life, but now it is also acknowledged as a major part of Long-COVID Syndrome (LCS) (2). LCS is a complex condition that impacts multiple organ systems, with an incidence of 10–30% in non-hospitalized cases, 50–70% in hospitalized cases, and approximately 10–12% in vaccinated cases, although the majority of cases occur in non-hospitalized individuals with mild illnesses (2). Symptoms of LCS encompass a wide range, including chest pain,

palpitations, cough, dyspnea, fatigue, cognitive impairment, memory loss, disordered sleep, and more. Importantly, LCS shares many clinical similarities with myalgic encephalomyelitis/chronic fatigue syndrome (ME/CFS) (2), (3). Notably, patients with ME/CFS often meet the diagnostic criteria for POTS (1), (2), (4). Furthermore, dysautonomias such as POTS lead to orthostatic intolerance (OI), which refers to the inadequate activation of the autonomic nervous system (ANS) to meet the body's demands, resulting in the failure or hyperactivity of either the sympathetic nervous system (SNS) or parasympathetic nervous system (PNS) (5). While other types of OI, such as orthostatic hypotension and vasovagal syncope, involve a drop in blood pressure upon standing, POTS does not exhibit such a characteristic (6). In POTS, the heart rate often increases to 120 beats per minute or higher,

while the mean arterial pressure (MAP) remains stable (6). POTS primarily affects female patients of childbearing age, with a female-to-male ratio of 5:1 (1), (7). The worldwide prevalence is estimated to be between 500,000 and 3 million individuals, with reports from autonomic clinics globally indicating an increased incidence that parallels the spread of the COVID-19 pandemic (8). Notably, approximately 2.5% of Post-COVID-19 patients develop dysautonomia, suggesting that at least 65 million people worldwide are affected by LCS (2), (9). However, establishing accurate prevalence is challenging due to study limitations, such as varying diagnostic criteria, patient characteristics, observational design, and small sample sizes (8). Common symptoms of POTS exhibit characteristics of reduced cerebral blood flow and a hyperadrenergic state, including orthostatic tachycardia, palpitations, dizziness, brain fog, presyncope, migraine, fatigue, sleep disturbances, gastrointestinal (GI) symptoms, and overlap with LCS and ME/CFS, making the differential diagnosis challenging (10). Moreover, considering the increased incidence of POTS during the pandemic and its significant impact on socio-economic aspects of patients' lives, including disability, inability to work or requiring reduced working hours, anxiety development, and reduced quality of life, the importance of understanding the pathological mechanisms leading to the disease becomes evident (10). Several mechanisms have been investigated, given the complex nature of the disease, but the proposed ones include immune-mediated alteration in different receptors, cellular toxicity caused by the viral spike protein, and direct or indirect neuroinvasion through the olfactory nerve or the gastrointestinal system, with some new

findings emerging in relation to the ongoing COVID-19 pandemic.

Aim

This review aims at exploring the proposed mechanisms that trigger POTS after acute SARS-CoV-2 infection in relation to the already well-established pathogenesis of the condition.

Material and methods

A search in the PubMed database was performed utilizing the following keywords: postural orthostatic tachycardia and long covid syndrome. The results were filtered to include articles from 2019 to 2023 and 29 articles were selected.

Results and discussion

POTS is classified as one of the various types of orthostatic intolerance (OI) (6). When assuming an upright position, approximately 800 mL of blood volume shifts from the upper body to the lower body and splanchnic circulation. To counteract the resulting decrease in arterial pressure caused by reduced venous return and stroke volume, several mechanisms come into play (Fig. 1). Among these mechanisms, autonomic reflexes, such as sympathetic stimulation and activation of the renin-angiotensinogen-aldosterone system (RAAS), play a crucial role (Fig. 2) (11). POTS is believed to consist of five subtypes, which can overlap and require different treatment approaches: 1) hyperadrenergic; 2) neuropathic; 3) hypovolemic; 4) joint-hypermobility-related; and 5) immune-related (12). Moreover, in Post-COVID POTS, several pathological mechanisms are proposed to be involved, which may overlap and interact with each other (8).

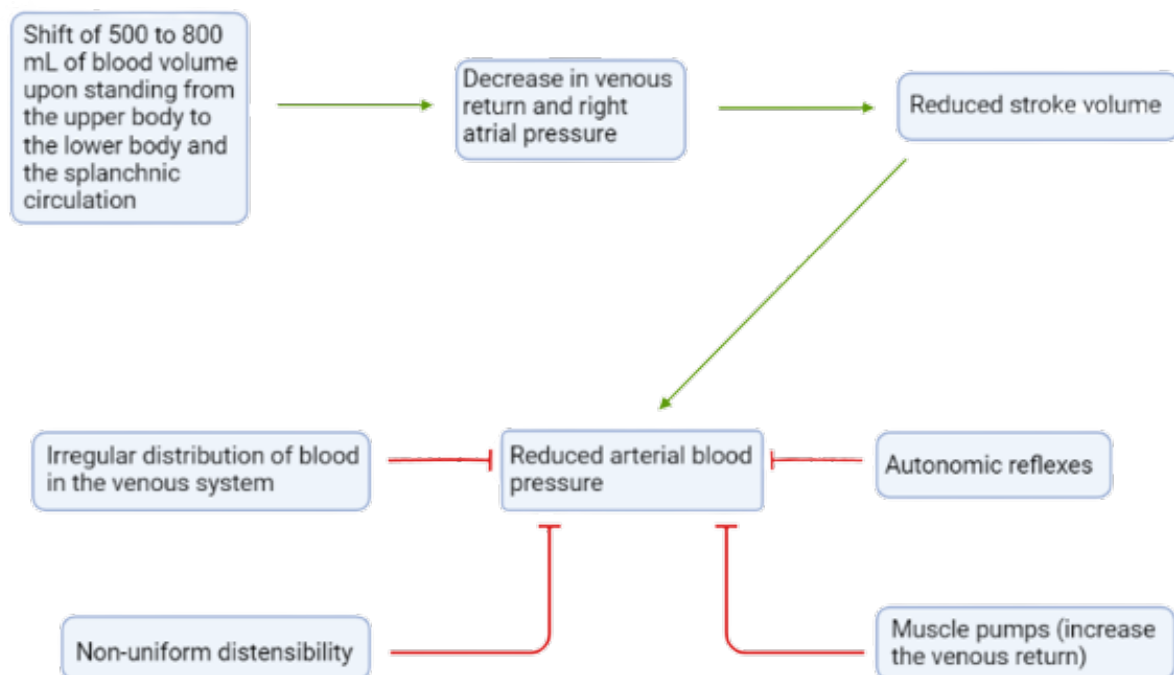


Figure 1. Orthostasis results in a shift of up to 800 mL of blood volume from the upper body to the lower body and the splanchnic circulation. To counteract the subsequent reduced arterial pressure, resulting from a decrease in venous return and reduced stroke volume, several mechanisms play a role including irregular distribution of blood in the venous system, non-uniform distensibility, muscle pumps, and of biggest importance autonomic reflexes.

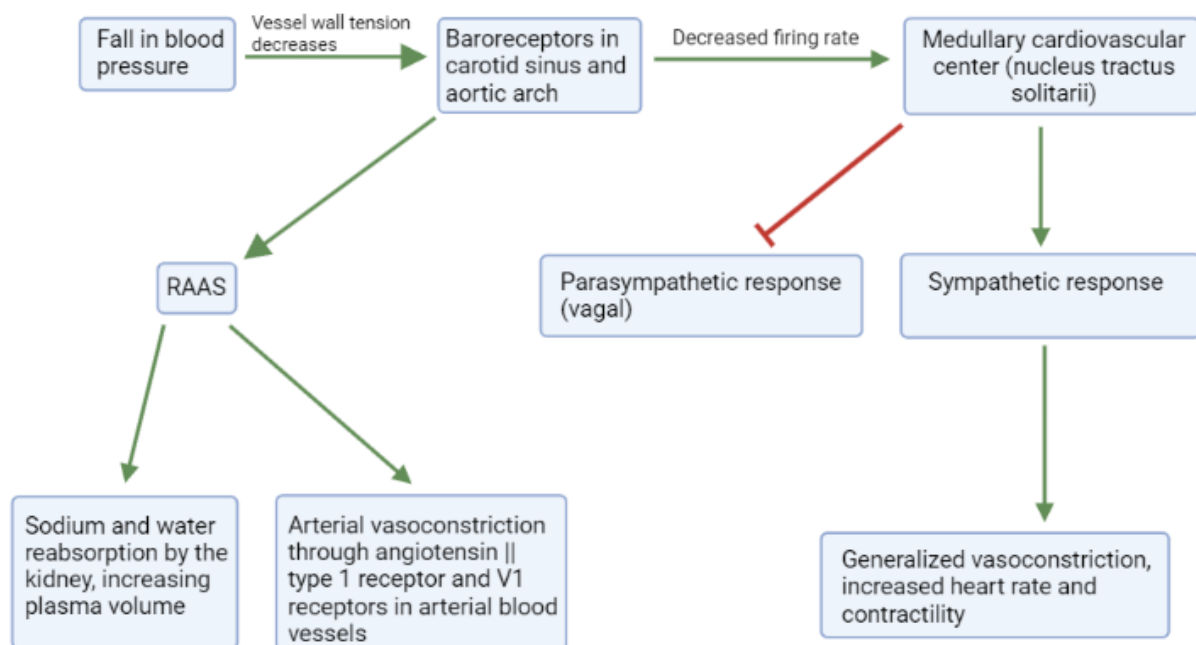


Figure 2. Autonomic reflexes include sympathetic stimulation and activation of the renin-angiotensinogen-aldosterone system (RAAS). Baroreceptors in the carotid sinus and aortic arch are activated upon sensing vessel wall tension decrease, which mirrors the fall in blood pressure. Activated baroreceptors in turn activate RAAS and sympathetic response, the sum of the two leading to fluid retention, vasoconstriction, increase in heart rate, and contractility, which counteract the fall in blood pressure.

One of the proposed pathological mechanisms in POTS involves immune-mediated alterations in various receptors. POTS patients have a higher prevalence of anti-nuclear antibodies, anti-phospholipid antibodies, and comorbid autoimmune disorders like Hashimoto's thyroiditis, rheumatoid arthritis, and celiac disease compared to the general population (13). SARS-CoV-2 infection may trigger the production of autoantibodies targeting different receptors, leading to hyperadrenergic reactions and tachycardia upon standing (8). Standard enzyme-linked immunosorbent assays (ELISA) have shown that POTS patients exhibit seropositivity for antibodies against ganglionic N-type and P/Q-type acetylcholine receptors, alpha 1, beta 1, and beta 2 adrenergic receptors, muscarinic M2 and M4 receptors, angiotensin II type 1 receptors, and opioid-like 1 receptors (13). However, the presence of these antibodies alone does not fully explain the disease's pathogenesis, as they can also be found in healthy individuals, necessitating further investigation into autoantibody activity (14). Interestingly, there appears to be an inverse correlation between autoantibodies and protective antibodies in long COVID, with women being less likely to seroconvert and more likely to serorevert, reflecting the 5:1 ratio of POTS affecting women to men (2). Additionally, elevated levels of inflammatory markers have been observed in POTS patients (13), which aligns with the increased levels of IL-1 β , IL-6, TNF, and IP10 observed in LCS patients (2). Another finding is the association between the downregulation of circulating proprotein convertases subtilisin/kexin type (PCSK)-3, i.e., proconvertase furin and POTS, with proconvertase furin having a proteolytic function for cytokines and chemokines such as anti-inflammatory transforming growth factor (TGF)-1 and secreted

TNF-family receptors (7). Another study of 34 POTS patients showed increased plasma concentration of IL1 β , IL21, TNF α , INF γ , and CD30, which are immune markers of the innate immune response, that could point at T-cell mediated autoantibody secretion (15) (16), which is in line with the observed increase of CD4⁺ T cells in LCS patients (2). Furthermore, a pilot study by the same group found elevated IL-1 family cytokines IL-1 β and IL-18 levels in POTS patients (16). Interestingly, POTS is associated with platelet delta granule storage pool-deficient (δ -SPD), which could develop as a consequence of chronic inflammation seen in these patients and result in symptoms such as easy bruising, epistaxis, heavy menstrual bleeding in women (15). Moreover, case-control study of plasma proteomic profiling in POTS patients revealed significant upregulation of glycoproteins 1B (GP1BA and GP1BB), filamin A (FLNA), Tubulin beta-1 (TUBB1), Integrin-linked kinase (ILK), beta-parvin (PARVB), beta-2-microglobulin (B2M), myosin light chain 1/3, skeletal muscle isoform (MYL1), myosin regulatory light chain 12B (MYL12B) and downregulation of Cartilage oligomeric matrix protein (COMP), Thrombospondin-4 (TSP-4), WD repeat-containing protein 1 (WDR1) and Lumican (LUM) is observed in POTS patients (17). GP1BA/GP1BB, FLNA have a role in platelet aggregation and activation, whereas COMP inhibits these functions (17). ILK, PARVB, and TSP-4 play a role in the regulation of cardiac contractility, with ILK and TSP-4 being further involved in processes of hypertrophy and remodeling. B2M is part of the major histocompatibility complex class I (MHC I) and is associated with inflammation. LUM is involved in the promotion of innate immunity and is hypothesized that its downregulation leads to impaired immune response (17). Myosin light

chain 1/3, skeletal muscle isoform (MYL1) is expressed on fast-twitch (type II) skeletal muscle fibers and has a role in myogenesis. Impaired regulation of this process leads to deconditioning like-changes such as decreased muscle mass, which set the ground for deconditioning (17) – recall a finding in POTS patients is lower BMI as well as deconditioning. Myosin regulatory light chain 12B (MYL12B) initiates the polymerization of vascular smooth muscle, which could relate to increased adrenergic activity in POTS (17). COMP downregulation may result in defective hemostasis and thrombosis (17). Furthermore, an interesting finding is that POTS patients have higher plasma levels of growth hormone (GH). Higher GH may be due to disruption of the secretory pathways of GH caused by elevated inflammatory cytokines. TNF-alpha and IL-1 are known to cause IGF-1 resistance, hence higher GH levels, which may explain lower BMI among POTS patients, having in mind the stimulatory effect of GH on lipolysis. Additionally, ocreotide which is used to treat hypersomatotropism in acromegaly by inhibition of GH, is shown to be an effective treatment of POTS by its inhibitory effect on splanchnic pooling (18).

POTS can be caused by vaccination (19), which is in line with a finding that COVID-19-vaccinated individuals had higher odds for POTS diagnosis than referent conventional primary care diagnoses, nevertheless still lower odds than diagnosis after SARS-CoV-2 infection (20).

All aforementioned findings suggest autoimmunity could be a key mechanism in Post-COVID POTS.

On the other hand, another proposed mechanism is cellular toxicity by the virus. Upon entering cells through the angiotensin-converting enzyme 2 receptor (ACE-2) causing structural damage is done to multiple

organ systems including cardiovascular, pulmonary, kidney systems, etc ultimately resulting in tachycardia (8). This may be due to RAAS dysregulation, hyperinflammation, and hypercoagulability with thrombosis (8). Furthermore, the spike protein is thought to cause neurotoxicity through endothelial damage, autoimmunity mechanisms, or neuroinflammation, which could explain some symptoms of POTS such as chronic fatigue and brain fog (8).

Similarly, SARS-CoV-2 can invade the central nervous system (CNS) and autonomic nervous system (ANS) through direct and indirect pathways. Direct CNS and ANS invasion occurs through the olfactory nerve, neuronal pathways, blood circulation, and angiotensin-converting enzyme 2 in the brainstem. Indirect invasion of ANS occurs through the enteric nervous system and its sympathetic afferents following a gastrointestinal tract (GIT) infection (8). Traditionally, POTS is thought to be a dysautonomia, with 50% of patients exhibiting small fiber neuropathy on quantitative sudomotor axon reflex test (QSART), although evidence of central involvement exists (21). Moreover, higher total choline and myoinositol were present on magnetic resonance (MR) spectroscopy in the dorsal medulla, where cardiovascular and breathing centers are located, in adolescents with POTS, syncope, and orthostatic hypotension which suggests the presence of neuroinflammation (21). Furthermore, there is evidence of cardiovagal and central baroreflex damage in two patients with LCS suggesting the involvement of the ANS and CNS due to these reflexes centers being located in the dorsal medulla (22), as well as the presence of SARS-CoV-2 and spike proteins in 33 deceased COVID patients there and the olfactory mucosal-neuronal junction (8). What's more, brain MRI showed lower white matter volume in a

couple of locations including the precentral gyrus and paracentral lobule, right pre- and post-central gyrus, paracentral lobule, and superior frontal gyrus in POTS patients in addition to a significant negative correlation between left insula volume and trait anxiety and depression scores (21).

Deconditioning is also discussed as a potential mechanism. Effects of the acute infection and LCS are thought to induce deconditioning due to excessive bed rest, night sweats, fever, and nausea (8), (23) resulting in reduced exercise capacity and physical intolerance. An interesting finding in POTS patients is reduced myoglobin levels which may be a result of immobilization and limited physical activity (18). Aforediscussed biomarkers may play a role as well. Furthermore, patients with POTS had smaller hearts coupled with reduced blood volume compared with healthy controls, leading to the authors proposing the name “Grinch syndrome” as a reference to the main character in the famous children’s movie “How the Grinch Stole Christmas!”, who had a heart that was two times smaller.

Finally, fear conditioning is proposed as well. POTS patients have higher somatic vigilance, more pronounced anticipatory tachycardia right before tilt testing, faster heart rate when standing, higher plasma catecholamine levels, and an indication of cerebral hypoperfusion compared to controls (24). Furthermore, POTS patients have lower cerebral tissue oxygen saturation (SctO₂) during tilt testing compared to controls and interestingly OI symptoms are not directly related to lower upright SctO₂ values (25). The effect of pandemic stress on somatic symptoms should be taken into consideration. Although psychic component can be present, POTS should not be misdiagnosed as a primary psychiatric disorder.

Conclusion

POTS and Post-COVID POTS are multifactorial disorders of the autonomic nervous system that reduce a patient’s life quality. Autoimmunity following acute infection with SARS-CoV-2, cellular toxicity by the viral spike protein, neuroinvasion, LCS symptoms and fear conditioning are explored as potential mechanisms with further research needed to decipher the unknowns around POTS.

References

1. Cutsforth-Gregory JK, Sandroni P. Clinical neurophysiology of postural tachycardia syndrome. *Handbook of Clinical Neurology*. 2019 Jan 1;429–45.
2. Davis, H.E., McCorkell, L., Vogel, J.M. et al. Long COVID: major findings, mechanisms, and recommendations. *Nat Rev Microbiol* 21, 133–146 (2023). <https://doi.org/10.1038/s41579-022-00846-2>
3. Ryabkova VA, Gavrilova NY, Fedotkina TV, Churilov LP, Shoenfeld Y. Myalgic Encephalomyelitis/Chronic Fatigue Syndrome and Post-COVID Syndrome: A Common Neuroimmune Ground? *Diagnostics* (Basel). 2022 Dec 26;13(1):66. doi: 10.3390/diagnostics13010066. PMID: 36611357; PMCID: PMC9818907.
4. Vernon SD, Funk S, Bateman L, Stoddard GJ, Hammer S, Sullivan K, Bell J, Abbaszadeh S, Lipkin WI, Komaroff AL. Orthostatic Challenge Causes Distinctive Symptomatic, Hemodynamic and Cognitive Responses in Long COVID and Myalgic Encephalomyelitis/Chronic Fatigue Syndrome. *Front Med (Lausanne)*. 2022 Jun 23;9:917019. doi: 10.3389/fmed.2022.917019. PMID: 35847821; PMCID: PMC9285104.
5. Eshak N, Abdelnabi M, Ball S, Elgwairi E, Creed K, Test V, Nugent K. Dysautonomia: An Overlooked Neurological Manifestation in a Critically ill COVID-19 Patient. *Am J Med Sci*. 2020 Oct;360(4):427–429. doi: 10.1016/j.amjms.2020.07.022. Epub 2020 Jul 17. PMID: 32739039; PMCID: PMC7366085.
6. https://emedicine.medscape.com/article/902155-overview?icd=login_success_email_match_norm – Orthostatic intolerance
7. Spahic JM, Ricci F, Aung N, Axelsson J, Melander O, Sutton R, Hamrefors V, Fedorowski A. Proconvertase Furin Is Downregulated in Postural Orthostatic Tachycardia Syndrome. *Front Neurosci*. 2019 Mar 29;13:301. doi: 10.3389/fnins.2019.00301. PMID: 31001074; PMCID: PMC6455076.
8. Ormiston CK, Świątkiewicz I, Taub PR. Postural orthostatic tachycardia syndrome as a sequela of COVID-19. *Heart Rhythm*. 2022 Jul 16;19(11): 1880–9. doi: 10.1016/j.hrthm.2022.07.014. Epub ahead of print. PMID: 35853576; PMCID: PMC9287587.

9. Carmona-Torre F, Mínguez-Olaondo A, López-Bravo A, Tijero B, Grozeva V, Walcker M, Azkune-Galparsoro H, López de Munain A, Alcaide AB, Quiroga J, Del Pozo JL, Gómez-Esteban JC. Dysautonomia in COVID-19 Patients: A Narrative Review on Clinical Course, Diagnostic and Therapeutic Strategies. *Front Neurol*. 2022 May 27;13:886609. doi: 10.3389/fneur.2022.886609. PMID: 35720084; PMCID: PMC9198643.
10. Czosek, Lauren BSN, RN. Postural orthostatic tachycardia syndrome: Recognition and treatment. *Nursing* 53(2):p 18-22, February 2023. | DOI: 10.1097/01.NURSE.0000905696.14880.53
11. Orthostasis – Alejandro Campos Munoz; Shekhar Vohra; Mohit Gupta
<https://www.ncbi.nlm.nih.gov/books/NBK532938/>
12. Taub PR, Zadourian A, Lo HC, Ormiston CK, Golshan S, Hsu JC. Randomized Trial of Ivabradine in Patients With Hyperadrenergic Postural Orthostatic Tachycardia Syndrome. *J Am Coll Cardiol*. 2021 Feb 23;77(7):861-871. doi: 10.1016/j.jacc.2020.12.029. PMID: 33602468.
13. Blitshteyn S, Whitelaw S. Postural orthostatic tachycardia syndrome (POTS) and other autonomic disorders after COVID-19 infection: a case series of 20 patients. *Immunol Res*. 2021 Apr;69(2):205-211. doi: 10.1007/s12026-021-09185-5. Epub 2021 Mar 30. Erratum in: *Immunol Res*. 2021 Apr 13;: PMID: 33786700; PMCID: PMC8009458.
14. Hall J, Bourne KM, Vernino S, Hamrefors V, Kharraziha I, Nilsson J, Sheldon RS, Fedorowski A, Raj SR. Detection of G Protein-Coupled Receptor Autoantibodies in Postural Orthostatic Tachycardia Syndrome Using Standard Methodology. *Circulation*. 2022 Aug 23;146(8):613-622. doi: 10.1161/CIRCULATIONAHA.122.059971. Epub 2022 Jun 29. PMID: 35766055; PMCID: PMC9390234.
15. Gunning WT 3rd, Stepkowski SM, Kramer PM, Karabin BL, Grubb BP. Inflammatory Biomarkers in Postural Orthostatic Tachycardia Syndrome with Elevated G-Protein-Coupled Receptor Autoantibodies. *J Clin Med*. 2021 Feb 6;10(4):623. doi: 10.3390/jcm10040623. PMID: 33562074; PMCID: PMC7914580.
16. Gunning WT, Kramer PM, Cichocki JA, Karabin BL, Khuder SA, Grubb BP. Platelet Storage Pool Deficiency and Elevated Inflammatory Biomarkers Are Prevalent in Postural Orthostatic Tachycardia Syndrome. *Cells*. 2022 Feb 23;11(5):774. doi: 10.3390/cells11050774. PMID: 35269395; PMCID: PMC8909348.
17. Johansson M, Yan H, Welinder C, Végvári Á, Hamrefors V, Bäck M, Sutton R, Fedorowski A. Plasma proteomic profiling in postural orthostatic tachycardia syndrome (POTS) reveals new disease pathways. *Sci Rep*. 2022 Nov 21;12(1):20051. doi: 10.1038/s41598-022-24729-x. PMID: 36414707; PMCID: PMC9681882.
18. Medic Spahic J, Ricci F, Aung N, Hallengren E, Axelsson J, Hamrefors V, Melander O, Sutton R, Fedorowski A. Proteomic analysis reveals sex-specific biomarker signature in postural orthostatic tachycardia syndrome. *BMC Cardiovasc Disord*. 2020 Apr 22;20(1):190. doi: 10.1186/s12872-020-01465-6. PMID: 32321428; PMCID: PMC7178975.
19. Eldokla AM, Numan MT. Postural orthostatic tachycardia syndrome after mRNA COVID-19 vaccine. *Clin Auton Res*. 2022 Aug;32(4):307-311. doi: 10.1007/s10286-022-00880-3. Epub 2022 Jul 23. PMID: 35870086; PMCID: PMC9308031.
20. Kwan, A.C., Ebinger, J.E., Wei, J. et al. Apparent risks of postural orthostatic tachycardia syndrome diagnoses after COVID-19 vaccination and SARS-CoV-2 Infection. *Nat Cardiovasc Res* 1, 1187–1194 (2022). <https://doi.org/10.1038/s44161-022-00177-8>
21. Blitshteyn S. Is postural orthostatic tachycardia syndrome (POTS) a central nervous system disorder? *J Neurol [Internet]*. 2022;269(2):725–32. Available from: <http://dx.doi.org/10.1007/s00415-021-10502-z>
22. Chung TH, Azar A. Autonomic nerve involvement in post-acute sequelae of SARS-CoV-2 syndrome (PASC). *J Clin Med [Internet]*. 2022 [cited 2023 Apr 26];12(1):73. Available from: <https://www.mdpi.com/2077-0383/12/1/73>
23. Rinaldo RF, Mondoni M, Parazzini EM, Pitari F, Brambilla E, Luraschi S, Balbi M, Sferrazza Papa GF, Sotgiu G, Guazzi M, Di Marco F, Centanni S. Deconditioning as main mechanism of impaired exercise response in COVID-19 survivors. *Eur Respir J*. 2021 Aug 26;58(2):2100870. doi: 10.1183/13993003.00870-2021. PMID: 33926969; PMCID: PMC8082950.
24. Lucy Norcliffe-Kaufmann, Jose-Alberto Palma, Jose Martinez, Celeste Camargo, Horacio Kaufmann, Fear conditioning as a pathogenic mechanism in the postural tachycardia syndrome, *Brain*, Volume 145, Issue 11, November 2022, Pages 3763–3769, <https://doi.org/10.1093/brain/awac249>
25. Isabella Kharraziha, Hannes Holm, Erasmus Bachus, Olle Melander, Richard Sutton, Artur Fedorowski, Viktor Hamrefors, Monitoring of cerebral oximetry in patients with postural orthostatic tachycardia syndrome, *EP Europace*, Volume 21, Issue 10, October 2019, Pages 1575–1583, <https://doi.org/10.1093/europace/euz204>

Insomnia – the False Face of Two of the Most Common Sleep Disorders – 2 Case Studies

Dana Stoyanova¹, Krasimir Avramov², Teodor Georgiev², Aneliya Draganova², Kiril Terziyski²

1. Medical University of Plovdiv

2. Department of Pathophysiology, Faculty of Medicine, Medical University of Plovdiv

Introduction: Despite the epidemiological inconsistencies reported for insomnia, the available data suggests a pronounced socially significant impact of this sleep disorder. Here we present two clinical cases, which exemplify insomnia as a leading symptom which can mask other underlying sleep disturbances.

Aim: The objective of this article is to illustrate the necessity of an integrative approach to the assessment of a patient with the complaint of insomnia. **Materials and methods:** Both clinical cases involve patients, whose main complaint was reported as their perception of a decreased quality and quantity of sleep. Patient interviews and physical examinations raised suspicion of concurrent sleep disorders. Full polysomnographic studies (PSG) were performed, and patients were instructed to fill in sleep diaries. Consequently, patient 1 was referred for cardiological examination and patient 2 for additional blood tests. **Results and discussion:** The PSG results revealed the presence of obstructive sleep apnea in patient 1 and restless legs syndrome – in patient 2. These conditions are long acknowledged to cause secondary insomnia. Nonetheless, the current consensus for the recognition of insomnia do not require obligatory PSG, which can hinder the diagnostic process. Failure to recognize underlying sleep disorders could lead to inappropriate treatment of insomnia. In addition, the latter can aggravate the condition. **Conclusion:** Currently there exists controversy in the classification of insomnia, hence the uncertainty in the approach to these patients. We hope that these clinical cases will accentuate the importance of comprehensive evaluation of these patients and will demonstrate the advantages of PSG.

Keywords: SLEEP, INSOMNIA, OSA, RLS

Introduction

Chronic insomnia disorder (CID) is defined as a sleep disorder characterized by difficulty in initiation or maintenance of sleep and/or decreased sleep duration, associated with daytime dysfunction, that last for a minimum of 3 months with symptoms occurring at least 3 nights a week¹. It is further classified as primary, when there is no apparent psychiatric/medical condition or substance use/abuse causing it, or secondary¹. Insomnia also appears to be the major symptom of many sleep disorders, which can further obscure their diagnosis and impede the appropriate treatment. This often results in further exacerbation of the underlying disease and the insomnia itself, creating a vicious cycle.

In the following clinical cases 2 of the most common such disorders are presented and namely – obstructive sleep apnea (OSA) and restless legs syndrome (RLS). OSA is characterized by episodes of hypopnea or

apnea during sleep, associated with an arousal and a $\geq 3\%$ oxygen desaturation¹. RLS – a sleep related movement disorder, causes paresthesias in the lower limbs at nighttime, that are relieved with moving of the legs¹. Both of these diseases could present with insomnia as a main symptom.

Case presentation

Patient 1, a 36-year-old male, was referred to the Sleep Laboratory at the Department of Pathophysiology, Medical University of Plovdiv. He reported difficulties falling asleep and frequent awakenings over the past 2 years, attributing them to work-related stress, anxiety, and the COVID pandemic. The patient also experienced loud snoring and daytime sleepiness. At the time of examination, he was being treated with Hedonin and Melatonin. Hedonin helped him fall asleep faster, but it had no impact on sleep consolidation. The patient had no accompanying diseases and was in good general condition, with a BMI of 29.3.

Before undergoing polysomnography (PSG), the patient maintained a standard sleep diary for 14 days. The diary revealed relatively good sleep hygiene, with consistent bedtime between 22:00–23:00 and wake-up time at 7:00 during weekdays. On weekends, the patient would delay waking up by an hour. Interestingly, there was no discernible pattern of a good-night's sleep following a poor night's sleep, which was also reported by the patient. Analysis of the sleep diary showed a prolonged mean sleep latency of 75 minutes and decreased sleep efficiency of 66%.

The most important results from the subsequent PSG are presented on Table 1.

Further assessment revealed reduced TST and sleep efficiency, high WASO, elevated PLMS index, and decreased N3 sleep stage. Additionally, an increase in AHI, ODI, and an average desaturation drop of 3.7% suggested a diagnosis of moderate obstructive sleep apnea (OSA)¹. The patient's

complaints of insomnia were considered secondary to OSA.

The treatment approach involved discontinuing Hedonin and initiating continuous positive airway pressure (CPAP) therapy, along with providing detailed guidance on maintaining good sleep hygiene. Initially, the patient responded well to CPAP therapy, showing significant improvements in sleep maintenance, with over 4 hours of device usage and a notable reduction in AHI to 1.1. However, the patient discontinued CPAP treatment after three days, resulting in a recurrence of trouble falling asleep.

Subsequently, an electric cranial stimulation device (DAVID Delight Pro) was prescribed as a non-invasive method to enhance sleep quality and promote relaxation. The patient used the device regularly for three months, which positively impacted his anxiety levels but did not noticeably improve sleep quality. Despite his current condition, the patient continues to refuse CPAP therapy.

Table 1. Main polysomnographic data

	Patient 1	Patient 2
TST	6 h 13 min	4 h 55 min
Sleep efficiency	77.4%	58.4%
Arousal index in TST	16.7/h (Respiratory – 5.6/h)	40.6/h (Respiratory – 9.3/h, PLM – 19.9/h)
AHI	21.2	11.0
ODI	19.8	9.7
WASO	97.5 min	113 min
Sleep Latency	11.3 min	97.9 min
PLMS Index	11.9/h	39.5./h
N3	33%	8.5%
REM	23.2%	17.4%
Average desaturation drop	3.7%	3.3%

Legend: TST – total sleep time, AHI – apnea hypopnea index, ODI – oxygen desaturation index, WASO – wake after sleep onset, PLMS – periodic limb movements

Patient 2, a 76-year-old male, reported insomnia that began 10 years ago following a stressful experience. The insomnia was not influenced by seasonal change, sleeping in a new environment or sleep quality from the previous night. He struggled with difficulty falling asleep, frequent awakenings, and daytime sleepiness. Nocturia was present, but there were no night sweats, snoring, or sleep apnea. The patient preferred sleeping with his head elevated and had no leg edema. After being prescribed Hedonin 25 mg, he started experiencing unpleasant leg sensations and an urge to move and massage his legs in the evening. Additional medications, including Remirta, Pregabalin, and others, provided little relief for the insomnia with Remirta additionally aggravating the RLS. Comorbid conditions include arterial Hypertension treated with Doxazosin 4 mg, Roxiper 2.5 mg, Cordaron and gout for which he took Milurit.

The patient has a BMI of 34.2 which falls in the obesity range. PSG revealed reduced TST, sleep efficiency, and increased WASO, indicating insomnia. A high arousal index due to PLM suggested RLS¹, which was supported by a decrease in N3 sleep². Additionally, indicators of OSA – increased AHI and ODI¹, were present. Iron levels were in the normal range.

Treatment involved discontinuing Hedonin and increasing the dosage of Pregabalin to 150 mg, which provided some relief for the leg sensations. However, the improvement in insomnia symptoms was insufficient. Subsequently, the Pregabalin dosage was further increased to 225 mg. While the paresthesias in the lower limbs were successfully treated, the insomnia persisted. As an additional approach, an electric cranial stimulation device (DAVID Delight Pro) was introduced. After two weeks of therapy,

the patient reported partial but significant alleviation of insomnia. Although early morning awakening remained a discomfort, the patient acknowledged feeling and functioning better overall.

Discussion

Both patients are examples of insomnia as an accompanying or secondary illness in the context of another sleep disorder. Patient 1 corresponds to the diagnosis of secondary insomnia due to OSA or post-COVID insomnia further exacerbated by OSA. Patient 2 exhibits characteristics of primary insomnia that has been exacerbated by RLS, following incorrect therapy with Hedonin.

In the first patient's case COVID could have played a dual role in the pathogenesis of insomnia. On one hand the COVID pandemic has been linked to higher levels of stress and more frequent occurrence of symptoms of anxiety and depression which could possibly lead to insomnia³. That is also evident in the patient's history as he attributed the beginning of insomnia symptoms to anxiety related to work and the COVID pandemic.

On the other hand, infection with SARS-CoV-2 has been associated with the development of insomnia through various mechanisms, such as the systemic inflammatory response known as the "cytokine storm". This response is characterized by elevated levels of Interleukin 1 beta and TNF alpha, which have been shown to decrease GABA levels and the count of its receptors. GABA is the major inhibiting signaling molecule in the CNS and therefore plays a crucial role in sleep induction. Consequently, low GABA levels could explain the COVID induced insomnia.^{4,5,6}

The patient's insomnia could have played a major role in his weight gain as reduced

sleep duration stimulates hunger and food intake thus overcompensating for the increased energy expenditure during the extended wakefulness period⁷. Reward mechanisms are supposedly involved with evidence of increased levels of two endocannabinoids – 2-arachidonoylglycerol (2-AG) and its structural analog 2-oleoylglycerol (2-OG), which play a role in appetite control⁷. Increased BMI is the major risk factor for developing OSA with approximately 60% of moderate to severe OSA being attributed to obesity¹. The pathogenic mechanism involves narrowing of the upper airways (in overweight and obese patients – due to adipose tissue deposition) which causes increased resistance of the UA and consequently additional work for the pharyngeal muscles that open the UA during inspiration. During sleep muscle tone decreases and the UA collapse periodically, partially or fully, causing hypopneas/apneas which lead to reduced PO₂ and increased PCO₂ and then to arousal for the UA to reopen⁵. These arousals result in inefficient and nonrestorative sleep and insomnia.

Irrespective of the exact mechanism of involvement of OSA in the pathogenesis of insomnia in the patient (primary cause or an aggravating factor later in its progression), the treatment of the OSA is crucial for the managing of the insomnia symptoms. The cortical arousals secondary to the breathing disturbances are well known to disrupt significantly sleep architecture and, in some patients, may lead to difficulties in sleep maintenance and secondary insomnia⁵.

With the second patient an emphasis should be put on the therapy with Hedonin (quetiapine) which is an atypical antipsychotic that acts as a dopamine, serotonin and adrenergic antagonist, as well as an H1 receptor antagonist. Histamine binding to H1

receptors has been shown to cause depolarization in human cortical neurons thus increasing cortical arousal and promoting wakefulness, while H1 blockade induces sedation⁸. In contrast to other H1 antagonists, which show some efficacy in increasing quality of sleep, quetiapine has been shown to be ineffective in the treatment of insomnia⁹. Furthermore, because of its antidopaminergic effect it plays a crucial role in the exacerbation of RLS as is the case with Patient 2. One of the main hypotheses for the etiology of RLS is that it is a consequence of reduced postsynaptic responsiveness to dopamine which suffices throughout the day but with the physiological circadian drop in dopamine secretion at nighttime leads to the expression of RLS. This reduced postsynaptic response compensates for the increased presynaptic activity in individuals with decreased membrane-bound DAT (dopamine transporter) and striatal D2 receptors, which coupled with the dopamine antagonism of quetiapine further exacerbate the condition⁵. Additionally, since RLS may be secondary to Fe deficiency, it was important to exclude such a condition⁵. The presence of RLS for a significant period after cessation of Hedonin indicates the necessity to treat it pharmacologically according to the guideline which, as evident in the second clinical case, has a positive impact on the insomnia symptoms as well.

Conclusion

Insomnia presents a significant diagnostic and therapeutic challenge in modern medical practice, owing to its multifaceted nature as both a symptom and a distinct disorder. Accurately discerning between these two dimensions is paramount for the effective management of both insomnia and its underlying etiology.

References

1. American. International Classification of Sleep Disorders. 2014.
2. Geng C, Yang Z, Zhang T, Xu P, Zhang H. Polysomnographic nighttime features of Restless Legs Syndrome: A systematic review and meta-analysis. *Frontiers in Neurology*. 2022 Aug 25;13.
3. Cénat JM, Blais-Rochette C, Kokou-Kpolou CK, Noorishad PG, Mukunzi JN, McIntee SE, et al. Prevalence of symptoms of depression, anxiety, insomnia, posttraumatic stress disorder, and psychological distress among populations affected by the COVID-19 pandemic: A systematic review and meta-analysis. *Psychiatry Research*. 2021 Jan;295:113599.
4. Nikbakht F, Mohammadkhanizadeh A, Mohammadi E. How does the COVID-19 cause seizure and epilepsy in patients? The potential mechanisms. *Multiple Sclerosis and Related Disorders*. 2020 Nov;46:102535.
5. Kryger MH, Roth T, Dement WC. Principles and Practice of Sleep Medicine E-Book. Philadelphia Elsevier Ann Arbor, Michigan Proquest; 2016.
6. Gottesmann C. GABA mechanisms and sleep. *Neuroscience [Internet]*. 2002;111(2):231–9. Available from: www.ncbi.nlm.nih.gov/pubmed/11983310
7. Hanlon EC, Tasali E, Leproult R, Stuhr KL, Doncheck E, de Wit H, et al. Sleep Restriction Enhances the Daily Rhythm of Circulating Levels of Endocannabinoid 2-Arachidonoylglycerol. *Sleep*. 2016 Mar 1;39(3):653–64.
8. Reiner PB, Kamondi A. Mechanisms of antihistamine-induced sedation in the human brain: H1 receptor activation reduces a background leakage potassium current. *Neuroscience [Internet]*. 1994 Apr 1 [cited 2022 Dec 18];59(3):579–88. Available from: www.sciencedirect.com/science/article/abs/pii/0306452294901783
9. Prof Andrea Cipriani, PhD FDC MD Gian Loreto D'Alò, MD † Edoardo G Ostinelli, MD † Marco Ciabattini, MD Valeria Di Franco, MD Norio Watanabe, PhD Ayse Kurtulmus, MD Anneka Tomlinson, PhD Zuzana Mitrova, BA Prof Francesca Foti, PhD Cinzia Del Giovane, PhD Digby J Quesed, MD Prof Phil J Cowen, MD Prof Corrado Barbui, MD Laura Amato, MD Orestis Efthimiou, PhD. Comparative effects of pharmacological interventions for the acute and long-term management of insomnia disorder in adults: a systematic review and network meta-analysis. *The Lancet [Internet]*. 2022 Jul 16;400(10347). Available from: [https://www.thelancet.com/journals/lancet/article/PIIS0140-6736\(22\)00878-9/fulltext](https://www.thelancet.com/journals/lancet/article/PIIS0140-6736(22)00878-9/fulltext)

A Rare Case of Hepatosplenic Fungal Infection in Previously Healthy Child

Diyana Najar¹, Atanas Uchkunov², Liubov Chochkova-Bukova³

1. Faculty of Medicine, Medical University of Plovdiv

2. Department of Paediatrics, University Hospital "Pulmed", Plovdiv

3. Department of Paediatrics, University Hospital "St. George", Plovdiv

Hepatosplenic fungal infection (HSF) is a distinct form of invasive fungal infection with main involvement of the liver, spleen, and occasionally the kidney. It's a condition most commonly observed in children with malignant hematologic diseases or immunosuppression. We report a case of a 7-year-old girl who first presented with fever of 39.5°C to her GP. After watchful waiting for 2 days with no improvement the GP started her on Amoxiclav. The fever persisted and thus Clarithromycin was started, again with no improvement in the next 4 days which led to her hospitalization in the Paediatric Clinic of UMHAT "Saint George". Different laboratory and imaging studies were done. An abdominal ultrasound showed multiple round hypoechoic lesions in the spleen which were confirmed with a CT scan. The patient was switched to triple therapy with Metronidazole, Targocid and Fluconazole. This led to an afebrile period of 5 days after which she was again febrile with night sweats, simultaneously new lesions were found in the liver. Serum Total Candida antibodies came positive and she was started on Voriconazol, with good therapeutic effect. She remained permanently afebrile, the splenic and hepatic lesions gradually reduced and disappeared over a period of four months. Cat scratch disease, Visceral larva migrans, Toxoplasmosis, infective endocarditis, vasculitis and primary immune deficiency were excluded. This report shows a very unusual case of HSF. Being that rare especially in a generally healthy child makes it important for clinicians to recognize the condition and treat it properly.

Keywords: *Hepatosplenic fungal infection, splenic and hepatic lesions, fever of unknown origin*

Introduction

Hepatosplenic fungal infection (HSF) is recognized as a distinct form of chronic disseminated fungal infection with main involvement of the liver, spleen, and occasionally the kidneys. The condition is seen mainly in patients with hematologic malignancies especially acute leukemias. The exact pathogenesis is unknown, but studies have shown that invasive candidiasis is related to intestinal colonization, host defense at the mucosa and intestinal mucosal damage. That leads to blood invasion and fungi reaching the portal splenic bloodstream, forming small abscesses in the liver and/or spleen.

Case study

History

Our case entails a previously healthy 7-year-old girl without any comorbidities, from a normal pregnancy with normal

development to this point. She is fully vaccinated without any side reactions reported.

Patient has a history of typical infections for the age group, she is not an often-sickly child, recurring eczema for the past 2 years, treated with topical corticosteroids and antihistamines. She has a clinically healthy younger sister.

It was reported that the patient had contact with a domestic cat, dogs, a rabbit, and chicken. Around a week before the symptoms occurred, she was scratched by a cat.

The patient presented a fever of 39.5 to her GP. Other symptoms were not reported. After watchful waiting for 2 days she was started on Amoxiclav, but the fever persisted, thus she was examined at the Pediatric Emergency Department where Clarithromycin was started because of elevated pro inflammatory markers. After 4 days without improvement, the patient was hospitalized in the Pediatric Clinic of UMHAT "St. George".

Examination and laboratory results

Physical examination was unremarkable.

Laboratory results showed leukocytosis and thrombocytosis. ESR, CRP, LDH and uric acid were also elevated. Serum IgE was elevated up to 500 U/l. Candida antibodies were tested and came back positive with titer > 1:320. Bartonella hensalae IgG and IgM were also tested IgM <1:100, IgG 1:640.

	02.10	05.10	07.10	13.10	17.10	21.10
CRP	51.3	42.7	28.3	19.7	47.1	17.6

ESR on the first day of hospitalization was 37 mm/h and 43 mm/h when the patient was discharged.

Cell mediated immunity test was done, showing normal T-lymphocyte count with lowered subpopulation of suppressor and cytotoxic T cells. The percentage of Central memory T cells was elevated, whilst CD8+T cells were also lowered. B-lymphocytes were in reference ranges with elevated naive and lowered non-switched memory B-cells. NK cells were in reference range. Regulatory T-cells and Th17 cells percentages were elevated. CD15 and CD18 adhesion markers were present on the respective cell populations.

Phagocytic population – lower-bound oxidative burst.

Imaging studies

Abdominal ultrasound showed the presence of two hypoechogenic lesions – one 7 mm and one 1 cm. On CT it was confirmed that cystic lesions in the spleen were present and there was no splenomegaly. After 9 days on a new abdominal echography the splenic lesions were increased in number with central decay. In progression, another 8 days later again on abdominal US new lesions were discovered this time in the liver. A couple of new formations were seen again on the next day – they were hypoechogenic, heterogenic, size was around 6 mm in IV, V, VI hepatic segments.



Figure 1. Ultrasound images showing hypoechoic, heterogenous lesions with central decay /Bull's eye/ in the liver and spleen of the patient.

Treatment

Treatment was initially with Ceftriaxon, later on Metronidazol, Targocid, Fluconazol and Bi-Septol were added. After new fever peak the therapy was changed once again with Meronem and Vfend /Voriconazole/. After four month course with the latter splenic and hepatic lesions gradually reduced and disappeared.



Figure 2. CT scan showing hypoechoic lesions in the spleen.

Discussion

Hepatosplenic fungal infection was first described by Bodey et al. in 1969. It occurred almost exclusively in individuals with impaired host defense mechanisms, especially in patients with hemato-oncologic malignancy undergoing antineoplastic chemotherapy, and may occur in patients with chronic granulomatous diseases, Crohn's disease, diabetes mellitus, and patients receiving corticosteroid therapy. The incidence of HSF ranged from 3% to 29.1% in patients with hematologic malignancy undergoing chemotherapy or hematopoietic stem cell transplantation. Some cases had been reported in children since 1980s, but the incidence of HSF in pediatric patient remained unclear.

The revised criteria for invasive fungal infection established by the European Organization for Research and Treatment of Cancer/Mycoses were modified to fit all pediatric groups as the following:

- Proven – defined when there was radiologic evidence of fungal infection with multiple focal lesions in the liver and/or spleen and a specific diagnosis of fungal infection was established by the presence of fungi in deep-tissue biopsy, or aspiration specimens by culture, histological examination, or polymerase chain reaction (PCR) result, or blood culture;
- Probable – HSF was defined with typical imaging evidence and positive serum fungal antigen test (serum galactomannan test for *Aspergillus* spp);
- Possible – HSF was defined only with typical imaging study evidence.

Considering the history, clinical presentation, laboratory and imaging studies we made a differential diagnosis with: Cat scratch disease, Visceral larva migrans, Toxoplasmosis, Infectious endocarditis, Vasculitis, Primary immunodeficiency.

The constellation of fever, distinctive imaging findings /Bull's eye/, high antibody titers against *Candida*, the full involution of lesions in the liver and spleen after a course of Voriconazole was started, it was decided that the diagnosis is Hepatosplenic fungal infection.

Literary references showed that invasive fungal disease are practically not seen in children with an intact immune system. Thus tests on humoral and cell-mediated immunity were ordered. The results showed lower-bound oxidative burst and low levels of Cytotoxic T cells with a normal T-cell count, suggesting an immune dysfunction. Further studies are needed.

Conclusion

Hepatosplenic fungal infections are rare in childhood and mostly overlooked as a possible diagnosis, however their recognition and adequate treatment is crucial for the patient's outcome.

Bibliography

1. Yen, T. Y., Huang, L. M., Lee, P. I., Lu, C. Y., Shao, P. L., & Chang, L. Y. (2011, August). Clinical characteristics of hepatosplenic fungal infection in pediatric patients. *Journal of Microbiology, Immunology and Infection*, 44(4), 296–302. <https://doi.org/10.1016/j.jmii.2010.08.005>
2. De Pauw B, Walsh TJ, Donnelly JP, Stevens DA, Edwards JE, Calandra T, Pappas PG, Maertens J, Lortholary O, Kauffman CA, Denning DW, Patterson TF, Maschmeyer G, Bille J, Dismukes WE, Herbrecht R, Hope WW, Kibbler CC, Kullberg BJ, Marr KA, Muñoz P, Odds FC, Perfect JR, Restrepo A, Ruhnke M, Segal BH, Sobel JD, Sorrell TC, Viscoli C, Wingard JR, Zaoutis T, Bennett JE; European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group; National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. Revised definitions of invasive fungal disease from the European Organization for Research and Treatment of Cancer/Invasive Fungal Infections Cooperative Group and the National Institute of Allergy and Infectious Diseases Mycoses Study Group (EORTC/MSG) Consensus Group. *Clin Infect Dis*. 2008 Jun 15;46(12):1813-21. doi: 10.1086/588660. PMID: 18462102; PMCID: PMC2671227.

3. Aspasia Katragkou, Brian T Fisher, Andreas H Groll, Emmanuel Roilides, Thomas J Walsh, Diagnostic Imaging and Invasive Fungal Diseases in Children, *Journal of the Pediatric Infectious Diseases Society*, Volume 6, Issue suppl_1, September 2017, Pages S22–S31, <https://doi.org/10.1093/jpids/pix055>

Corresponding author

Diyana Najar

Phone number: 0895193100

Address: Elin Pelin 14, Plovdiv, Bulgaria

Email: diyanax9199@gmail.com

Application of User-Friendly 3D Printing for Customized Products in Various Medical Fields

Bozhidar Balabanov¹, Ivana Gerdzhikova¹, Tsenka Grancharova^{2,3}

1. Faculty of Medicine, Medical University of Plovdiv

2. Department of Medical Physics and Biophysics, Faculty of Pharmacy, Medical University of Plovdiv

3. Research Institute at Medical University of Plovdiv

Introduction: 3D printing has revolutionized the field of medicine in various ways. Additive manufacturing allows patient-specific models of anatomical structures to be created such as tools for pre-operative surgical practices. This has the potential to reduce the risks associated with invasive procedures. 3D printing also can be used to create customized implants and prosthetics that are tailored to the patient's individual needs. InVesalius is a free open-source software package for medical image processing and 3D visualization. The program enables users to import and process medical imaging data, creating highly accurate and detailed 3D models of internal structures. Its advanced features and user-friendly interface make it a resource of significant importance in the medical field. **Aim:** Production of patient-specific, sterilizable low-cost 3D printed products with a user-friendly process, suitable for scientific, clinical, educational, and surgical needs. **Materials and methods:** FDM 3D Printer Creality Ender 3 S1 Pro, polylactic acid (PLA), InVesalius software, Meshmixer software, MRI and CT scans from open-access online medical image sites. **Results and discussion:** After checking references, we chose PLA as a suitable polymer for our aim due to its low cost, ease of manufacturing, and sterilizability. The MRI and CT DICOM files used in this study were obtained from open-access medical image sites. With the already-mentioned software, we processed the image data and fabricated 3D models for a wide range of medical applications. **Conclusion:** InVesalius allows medical imaging data to be imported, edited and exported as 3D models in a variety of formats for use in surgical planning, education purposes and research.

Keywords: 3D printing, surgical planning models, patient-specific implants

1. Introduction

From patient-specific anatomical models and implants to 3D bioprinting of human organs, 3D printing has enabled several new possibilities for personalized medicine and greatly improved patient outcomes. One of the main advantages of 3D printing in medicine is the ability to create customized prostheses and implants¹ that perfectly match the unique anatomical characteristics of the individual patients. The high cost of medical 3D printers has restricted their extensive use in healthcare. As a result, many research teams have been working to adapt low-cost “entry-level” 3D printers for medical purposes, seeking to make the technology more accessible. In a study by Osti et al.², researchers explored the potential of this technology to produce low-cost, sterilizable bone models, suitable for clinical use in hospitals with limited resources. Through an in-house, user-friendly process, they were able to produce patient-specific 3D-printed products at a cost

of approximately 130 euros per model. These results demonstrate the potential of 3D printing to improve healthcare outcomes and reduce costs, particularly in regions with limited access to traditional medical technologies.

Another important application of 3D printing in medicine is in surgical planning. By creating patient-specific 3D-printed models of organs, bones, or tumours, surgeons can plan and practice complex surgeries before operating on the patient. This approach helps reduce surgical risks, shorten operation times, and improve surgical outcomes. By leveraging the benefits of 3D printing, surgeons can enhance their skills, optimize their procedures, and ultimately improve the quality of care they provide to their patients. Lau et al.³ reported that there is no difference between the accuracy of low-cost patient-specific 3D-printed models of congenital heart diseases (CHD) and costly 3D-printed models.

3D printing may play a significant role in medical education. Medical students and residents can use these models to learn and practice complex anatomical structures, enhancing their understanding and skills. Ai-Min Wu et al.⁴ showed that a 3D-printed model may improve medical students' understanding of bone spatial anatomy and fractures in some anatomically complex sites and may improve student satisfaction in medical education.

User-friendly 3D printing refers to the ease and accessibility of utilizing this innovative technology for a wide range of users, regardless of their level of expertise or technical background. In our work, user-friendly 3D printing refers to low-cost hardware with the ability for easy modification and free software with a simple and intuitive user interface.

The aim of our work was the making of patient-specific and low-cost 3D printed products by user-friendly process, for scientific, clinical, educational, and surgical needs.

2. Materials and methods

Software: InVesalius 3.1.1; Meshmixer 3.5

3D printer: Fused deposition modelling (FDM) 3D Printer Creality Ender 3 S1 Pro

Filaments: Polylactic acid (PLA); Polyethylene terephthalate glycol (PETG)

Models: The anatomical structures of interest in CT scans or MRI scan were software-based isolated. The scans are obtained from open-access online medical image sites. The selection was converted into an STL file and optimized. The final 3D model was then exported into a .gcode file format and printed.

3D Printing: The nozzle diameter was 0.4 mm; Extrusion temperature was 210°C for PLA and 245°C for PETG; Heated bed required; Printing speed of 70 mm/s.

3. Results and discussion

The CT data from an open-access online medical image library was obtained for the design of 3D-printed models. InVesalius was used for the conversion of Dicom files, which is a standard format used for storing and transmitting medical images, into STL files. Meshmixer was then utilized to optimize, modify, and repair the initial STL files. With the software, we were able to improve our model mesh by filling holes, smoothing the surface, and cutting the 3D model (Fig. 1).



Figure 1. Process of 3D printing of medical models

3.1. Bone models

For the bone model (Fig. 2) we used a CT scan from anonymized public X-ray datasets. Depending on the complexity of the model, the meshing process took about 0.5 to 2 hours. The price of the used PLA filament was 45 lv/kg, and depending on the size of the model, the cost of the models varied from 2 to 9 lv per model. In cases where one side of the body is healthy and the other side is affected, such as in unilateral injuries, the healthy side can serve as a template for 3D printing the missing part. By mirroring the healthy side, a symmetrical model can be designed and printed. To verify the accuracy of the 3D-printed medical models, a common method is positioning a measuring ball at points near the model and measuring the distance between these balls after the printing process.

3.2. Education models

Compared to cadaver anatomy models, 3D printed models do not use hazardous preservation chemicals and do not decompose easily. Unfortunately, these models are expensive, but some of them can be printed by entry-level 3D printers. As an example of an education model, we selected the Circle of Willis. CT scan was obtained to create the bone part and a STL file from an open medical library was used for the arteries (Fig. 3).

3.3. Surgery models

As an example of a surgery model, we chose a model of a heart (Fig. 4) from NIH 3D. USA National Institute of Health 3D is an open portal to download, share, and create

bioscientific and medical 3D models for 3D printing and interactive 3D visualization. The library provides models with various diseases, helping scientists understand complex medical issues. To reduce stringing caused by the complexity of heart models, we increased the retraction speed of the printing of used PETG filament.

3.4. Other applications

3D printing can be used for the creation of other personalized devices such as masks, anti-snoring devices, etc. In addition, it enables the creation of highly customized laboratory tools that can be tailored to specific experimental needs, including sample holders, racks, microfluidic devices, etc.

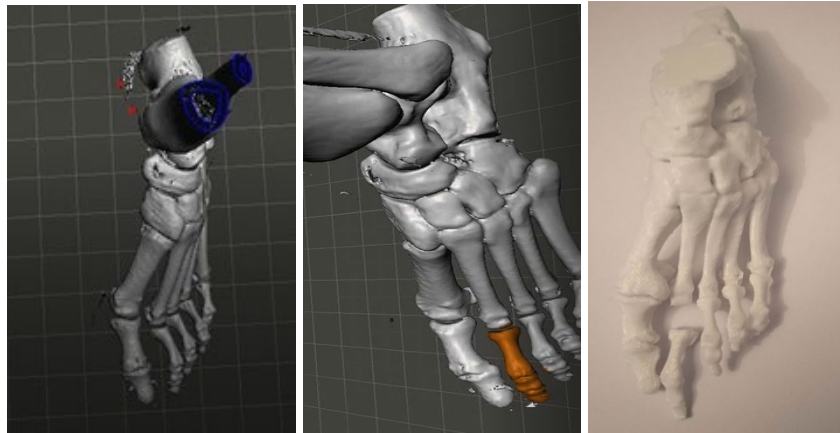


Figure 2. Process of printing of bone model.

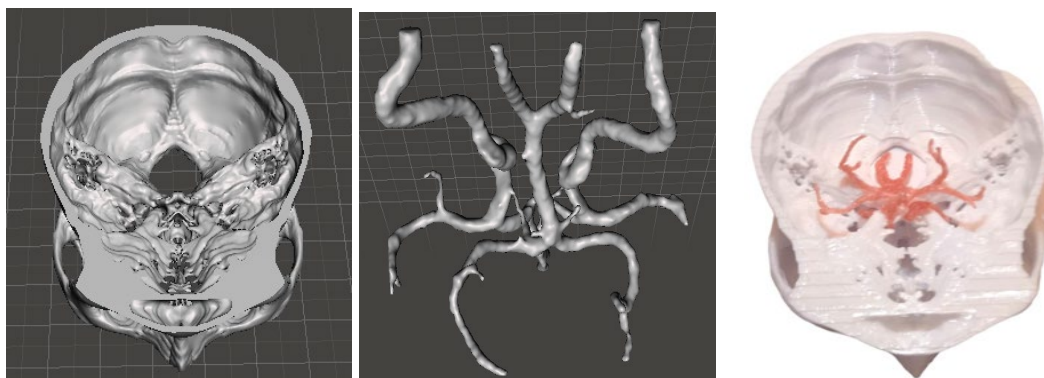


Figure 3. The process of printing the Circle of Willis.

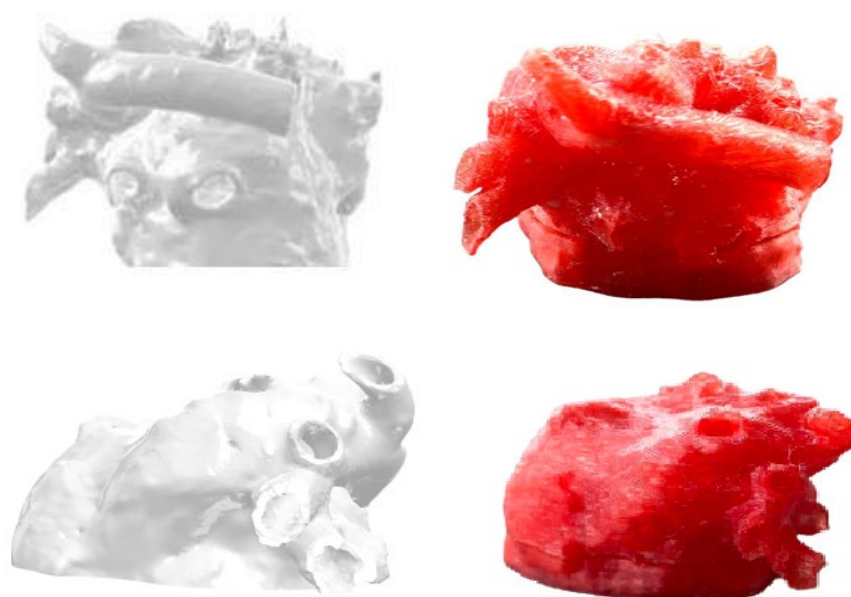


Figure 4. Printing of heart model.

4. Conclusion

With advancements in technology and the availability of open-source software, 3D printing has become more accessible and affordable for smaller centres and hospitals with limited resources. The use of low-cost 3D printing models in medicine has opened a wide range of new possibilities for medical education, surgical planning, and personalized medical devices. With the aid of CT, MRI and specific software, it is possible to reconstruct and produce a personalized model through 3D printing. As technology continues to advance, a more accessible and affordable use of 3D printing is expected for medical and educational purposes in the field of medicine.

References

1. Nikitichev DI, Patel P, Avery J, Robertson LJ, Bucking TM, Aristovich KY, et al. Patient-Specific 3D Printed Models for Education, Research and Surgical Simulation [Internet]. 3D Printing. InTech; 2018. Available from: <http://dx.doi.org/10.5772/intechopen.79667>
2. Osti, F.; Santi, G.M.; Neri, M.; Liverani, A.; Frizziero, L.; Stilli, S.; Maredi, E.; Zarantonello, P.; Gallone, G.; Stallone, S.; Trisolino, G. CT Conversion Workflow for Intraoperative Usage of Bony Models: From DICOM Data to 3D Printed Models. *Appl. Sci.* 2019, 9, 708. <https://doi.org/10.3390/app9040708>
3. Lau I, Wong YH, Yeong CH, Abdul Aziz YF, Md Sari NA, Hashim SA, Sun Z. Quantitative and qualitative comparison of low- and high-cost 3D-printed heart models. *Quant Imaging Med Surg* 2019;9(1):107-114. doi: 10.21037/qims.2019.01.02
4. Wu AM, Wang K, Wang JS, Chen CH, Yang XD, Ni WF, Hu YZ. The addition of 3D printed models to enhance the teaching and learning of bone spatial anatomy and fractures for undergraduate students: a randomized controlled study. *Ann Transl Med* 2018;6(20):403. doi: 10.21037/atm.2018.09.59

Corresponding author:

Tsenka Grancharova
Tsenka.Grancharova@mu-plovdiv.bg

Infantile Amnesia – Empirical Study and Possible Reasons (Review)

Bozhidara Stoencheva¹

Supervisors: Kristina Stoyanova², Drozdstoy Stoyanov³

1. Faculty of Medicine, Medical University of Plovdiv

2. Scientific Research Institute, Medical University of Plovdiv

3. Department of Psychiatry and Medical Psychology and Scientific Research Institute, Medical University of Plovdiv

The first seven years from our life are extremely important from a personal and psychosocial point of view, because this is the main character-defining period. Here comes the question why we remember almost nothing of that time. The phenomenon in which adults cannot recover their memories from earliest childhood is known as infantile amnesia (or childhood amnesia when we talk about children between 3 and 7 years). The purpose of my review is to identify the main suspected reasons for this condition. The methods that the authors of the original articles use to study infants are consisted of baby's limited motor and verbal abilities. The experimental procedures include visual recognition memory (VRM) methods, operant conditioning paradigms and delayed imitation paradigms. For children are used interviews and fMRI, that allows to study the brain connectivity. All experiments confirm the fact that babies can memorize information even before birth, therefore the memories are not simply disappeared, but the processes related to long-term storage or reproduction of the memories are changed or disturbed. Maturation of the brain and the reorganization of its structures stand out as a possible cause. Role in the infantile amnesia have the hippocampus and the cerebral cortex, especially the dentate gyrus. Changes at the neuronal level, the emergence of the self-concept and the acquisition of new knowledge and skills by children also have a connection with the psychosocial development. The infantile amnesia appears to be caused by a combination of factors that are still not fully understood.

Keywords: *infantile amnesia, memory development, childhood memories, hippocampus, cerebral cortex*

Introduction

The character determines what is our behavior in society when we interact with other people. It is formed throughout our life under the influence of the environment and the upbringing, but the childhood remains the main defining period. Then here comes the question of why, since the first seven years are so important from a personal and psychosocial point of view, we remember almost nothing of them. The phenomenon in which adults cannot recover their memories from the earliest childhood – i.e. before 3–4 years of age, is first described by psychologist Caroline Miles in 1895 and is first defined by Freud as infantile amnesia. He also created the term childhood amnesia, which describes the loss of memories in later childhood. In this review, I will use the term infantile amnesia, but it should be borne in mind that the two terms are interchangeable

and related, as they have the same etiology and result – forgetting is present in both, but in a different degree. Infantile amnesia is not specific to humans, but also occurs in animals. Studies on mice of different ages shed light on the neurobiological mechanisms of the process. There is strong evidence that both human and animal infants are capable of remembering, therefore memories are not simply lost, but the processes involved in long-term storage or reproduction of what is remembered have been altered or disrupted. Autobiographical memories are affected. According to Freud, they are inaccessible because they are repressed – the mechanism of repression prevents them from reaching the conscious part of our mind, so we cannot reproduce them. This theory is old and the modern research rejects it. Maturation of the brain and the reorganization of its structures stand out as a possible cause. New studies

show that there are a number of other possible molecular and structural mechanisms on the basis of infantile amnesia – a change in the type of receptors that transmit signals inside cells, in intracellular receptors, in the opioid and neuroendocrine systems. It becomes clear that this phenomenon is not a single factor, but is probably due to a combination of changes.

Aim

The aim of this review is to shed light on the infantile and child amnesia. What are the main possible reasons and hypothesis, which methods are used from the researchers to answer these questions.

Methods

The review is written on the base of articles, found in the databases of Google Scholar, PubMed, Science Direct, Wiley Online Library and the help of Library and Information Center of MU Plovdiv.

What methods are used from the authors of the original articles?

The study of infantile amnesia is not an easy task given the fact that the subjects are infants and the methods of studying memory in adults are not applicable to them. An obstacle is the fact that the tasks set must be of a non-verbal nature – the same problem is present in animal studies. The limited motor capabilities of children under 1 year and the long-term nature of the studies further complicate the process. However, already in the 1970s, scientists managed to prepare appropriate paradigms for the assessment of infantile memory, which were improved and supplemented in the following decades. The used techniques are divided into three groups: visual recognition memory paradigms, operant conditioning paradigms and delayed imitation paradigms.

In 1958 Fantz and Berlyne developed ways of investigating children's perceptions that later became the basis of the two methods for assessing *visual recognition memory (VRM)*. In the first task, the time to look at a new and a familiar stimulus in the form of a visual stimulus is compared. In the second so-called habituation task, a single stimulus is presented many times until the infant's interest in it wanes, then a new stimulus is presented, and the time to loss of interest is measured again. Then the two times are compared.

Through *operant conditioning methods*, the ability to learn in the smallest representatives of our species is investigated. This is the paradigm for high-amplitude sucking - upon reaching a certain speed and interval between suckings, children receive a stimulus in the form of a sound (Eimas, Siqueland, Jusczyk, & Vigorito, 1971), visual (Siqueland & DeLucia, 1969) or taste (Sameroff, 1965) signal. A study by DeCasper and Fifer (1980) tested 3-day-old infants who had to suckle at a certain frequency in order to reproduce their mother's voice – for example, to decrease or increase the interval between suckles. The results are impressive – even though the children had a maximum of 12 hours of postnatal contact with their mothers, they showed a marked preference for their mothers' voices. In another outstanding study by DeCasper and Spence (1986), pregnant women were asked to read the same text to their unborn babies twice a day for 6 weeks prior to their due date. Three days after birth, the scientists gave the newborns the opportunity, by changing the sucking speed, to reproduce the paragraph read to them by their mothers, or another text. The results are clear, regardless of who reads the passage – the mother or another woman – the babies show a clear tendency to change their sucking speed just to hear the familiar text in

front of others. Children who had not heard these passages before birth had the same task – no such dependence was observed in them. No less interesting is the method of Mobile conjugate reinforcement, which is also the most widespread. For this purpose, a strap is attached to the baby's leg, connected to a stand with a mobile phone. When kicking, the child moves the phone – reinforcement period. Each session begins with a no-reinforcement period in which the bar is not connected to the phone and movements remain unrewarded. The paradigm was repeated over a period of time, comparing kicking frequencies in the non-reinforcement stages in each session. For some babies, a periodic reminder before the session is also applied.

Imitation paradigms are based on the tendency of babies to repeat the actions of people from their environment.

Results and discussion

All these experiments confirm the already known fact that babies have the ability to remember, it remains to clarify the question of what causes infantile amnesia. Even before birth, children have the necessary neural networks for perceiving, consolidating, storing and retrieving information. However, the memory system is not mature enough so they cannot retain their memories for a long period of time. According to scientists (Josselyn & Frankland, 2012), the rapid development of the nervous system in children prevents the formation of long-term memories, one possible reason is the replacement of synaptic connections in the memory circuits in the hippocampus.

The hippocampus appears to be critical not only in adults, but also in childhood memory. To clarify its role, researchers use task-based or task-free functional magnetic resonance imaging (fMRI). It is still a challenge to perform task-based fMRI with kids,

because they should stay still for a certain time during the scan. However such a study with children up to 8 years of age investigated which regions are activated when making association-based memories. Activated are regions with increased activity in adults and those, e.g. the orbital frontal gyrus, that are rare in more mature individuals. This leads us to assume that children have a wider or more distributed network for memory encoding. Without assessing the formation of memories in real time, task-free fMRI succeeds by assessing the strength of connectivity between regions to give us information about the integrity and maturity of the memory system. An fMRI study examined the relationships between memory detail and hippocampal size in children and showed that better memory was observed with larger hippocampal head volume in 6-year-olds but not in 4-year-olds (Riggins, Blankenship, Mulligan, Rice, & Redcay, 2015). This proves once again that child development is a very dynamic process, in which age-dependent variations must be taken into account.

Another hippocampal structure directly related to childhood amnesia is the anterior dentate gyrus. A study shows the relation between the volume of the gyrus (CA2-4 subfields), the age and the accuracy of memories in children – at an earlier age, smaller sizes are associated with lower accuracy of memories, and in older children, smaller dimensions are associated with more accurate memories (Canada et al., 2018).

A research of the episodic memory of 4- and 6-year-old children using the Children's Memory Scale standardized test, an episodic memory test and MRI shows that the microstructural state of the white brain matter pathways between the left hippocampus and IPL (inferior parietal lobe), and the right hippocampus and IPL can predict what the

children's performance will be in the test tasks. However, the question remains: what is the mechanism of forgetting as a result of hippocampal neurogenesis – whether it is a question of failed retrieval or storage, has yet to be established.

These changes at the neuronal level should also be considered in relation to psychosocial development and the acquisition of new knowledge and skills by children. This includes increasing the complexity of thinking (categorical reasoning, perspective taking, metamemory and strategy use; Piaget & Inhelder, 1969), the development of language, and the emergence of self-concept (the awareness that things are happening to me). Before the second year, children perceive experiences in themselves. Only then does the memory take on an autobiographical aspect. The emergence of the cognitive self, which succeeds in uniting the memory of experiences with self-awareness, is a key. This process is also associated with increasing the durability of memories. Various studies confirm that the earliest memories in adults coincide with the emergence of the cognitive self. The purpose of memory changes – if younger children and babies do not attach importance to details in events, but extract general information, then for older children the details become important. Preschool classes and going to school play a role in brain development and autobiographical memory.

A study proves that painful experiences in the infantile period can influence the infant's behavior during subsequent painful experiences. The study consists of comparing the pain response to routine vaccination of two groups of male babies – circumcised with and without local anesthesia. The study showed that boys who were circumcised without local anesthesia cried significantly more and experienced greater physical and

emotional distress from the vaccination. However, it is unlikely that this experience will pass into the autobiographical memory. A recent idea is that forgetting is an adaptive process.

Conclusion

Given all that has been said so far, it is clear that behind infantile and childhood amnesia are many related processes. These are phenomena that affect all people, processes directly related to personality development, mental health and even our social functioning as adults. The study of children's memory is of great importance for a complete and comprehensive understanding of memory. One day, this knowledge could help us develop programs that affect memory formation and compensate for childhood amnesia. And why not take it a step further? What if one day, through the information about infantile amnesia and the neuronal traces of memories, we manage to influence this critical period for the formation of the character and thus manage to fight the childhood psychological traumas, which have an impact throughout our lives?

Bibliography

1. S. Freud Three essays on the theory of sexuality Teoksessa J. Strachey (Ed.), The Standard Edition of the Complete Psychological Works of Sigmund Freud, vol. 7, The Hogarth Press, London (1953)
2. Campbell, B. A., & Spear, N. E. (1972). Ontogeny of memory. *Psychological Review*, 79(3), 215–236. <https://doi.org/10.1037/h0032690>
3. Heather Bronwyn Madsen, Jee Hyun Kim, Ontogeny of memory: An update on 40 years of work on infantile amnesia, *Behavioural Brain Research*, 2016, Pages 4-14
4. Harlene Hayne, Infant memory development: Implications for childhood amnesia, *Developmental Review*, Volume 24, Issue 1, 2004, Pages 33-73
5. Fantz, R.L. Pattern vision in young infants. *Psychol Rec* 8, 43–47 (1958). <https://doi.org/10.1007/BF03393306>
6. D.E. Berlyne The influence of the albedo and complexity of stimuli on visual fixation in the human infant *British Journal of Psychology*, 56 (1958), pp. 315-318

7. E.N. Sokolov Perception and the conditioned reflex Macmillan, New York (1963)
8. E.R. Siqueland, C.A. DeLucia Visual reinforcement of nonnutritive sucking in human infants Science, 165 (1969), pp. 1144-1146
9. P.D. Eimas, E.R. Siqueland, P. Jusczyk, J. Vigorito Speech perception in infants Science, 171 (1971), pp. 303-306
10. A.J. Sameroff An apparatus for recording sucking and controlling feeding in the first days of life Psychonomic Science, 2 (1965), pp. 355
11. A.J. DeCasper, W.P. Fifer Of human bonding: Newborns prefer their mothers' voices Science, 208 (1980), pp. 1174-1176
12. A.J. DeCasper, M.J. Spence Prenatal maternal speech influences newborns' perception of speech sounds Infant Behavior and Development, 9 (1986), pp. 133-150
13. Riggins T, Canada KL, Botdorf M. Empirical Evidence Supporting Neural Contributions to Episodic Memory Development in Early Childhood: Implications for Childhood Amnesia. Child Dev Perspect. 2020 Mar;14(1):41-48. doi: 10.1111/cdep.12353. Epub 2020 Jan 19. PMID: 34290824; PMCID: PMC8291724
14. Josselyn SA, & Frankland PW (2012). Infantile amnesia: A neurogenic hypothesis. Learning & Memory, 19, 423–433
15. Riggins T, Blankenship SL, Mulligan E, Rice K, & Redcay E (2015). Developmental differences in relations between episodic memory and hippocampal subregion volume during early childhood. Child Development, 86, 1710–1718
16. Canada KL, Ngo CT, Newcombe NS, Geng F, & Riggins T (2018). It's all in the details: Relations between young children's developing pattern separation abilities and hippocampal subfield volumes. Cerebral Cortex, 29, 3427–3433.
17. Chi T. Ngo, Kylie H. Alm, Athanasia Metoki, William Hampton, Tracy Riggins, Nora S. Newcombe, Ingrid R. Olson, White matter structural connectivity and episodic memory in early childhood, Developmental Cognitive Neuroscience, 2017, Pages 41-53
18. Howe, M.L. (2023), Early Childhood Memories Are not Repressed: Either They Were Never Formed or Were Quickly Forgotten. Top. Cogn. Sci.. <https://doi.org/10.1111/tops.12636>
19. Taddio, A., Katz, J., Ilersich, A. L., & Koren, G. (1997). Effect of neonatal circumcision on pain response during subsequent routine vaccination. Lancet, 349, 599–603.
20. Nørby, S. (2015). Why forget? On the adaptive value of memory loss. Perspectives on Psychological Science, 10, 551–578.
21. Ryan, T. J., & Frankland, P. W. (2022). Forgetting as a form of adaptive engram cell plasticity. Nature Reviews Neuroscience, 23, 173–186.

Corresponding author

Bozhidara Stoencheva
 Medical University of Plovdiv
 e-mail: dari1234567891@gmail.com

Atypical Formation of the Median Nerve in Fossa Axillaris. Case Report

Zlatizara Todorova, Vyara Zaikova

Department of Anatomy, Histology and Embryology, Faculty of Medicine, Medical University of Plovdiv

One of the main nerves in the innervation of the upper limb is the median nerve. The formation of the nerve usually is anteromedial in front of the third part of the axillary artery. Every deviation of the position of the nerve leads to changes in the normal anatomy of the fossa. Our team describes a case in which there is a very distal fusion of the medial and lateral radices which arise from the fascicules of the brachial plexus. Similar formation and location of the median nerve could lead to difficulties in the diagnostic process in this area. When performing different surgical procedures, clinical manipulations and physiotherapeutical procedures it is vital to know exactly the normal and atypical morphologies of the median nerve.

Keywords: axillary, brachial, median nerve

Introduction

Median nerve is a branch of the brachial plexus which originates from C5 to Th1. Its trunks are located in the neck area in the interscalene space. The three trunks of the brachial plexus slightly above or behind the clavicle are divided into anterior and posterior subdivisions. They form three cords – lateral, medial and posterior located around the axillary artery. The median nerve is formed by the fusion of the lateral and medial roots, which originate from the lateral and medial cords of the brachial plexus. Usually this takes place medially to the axillary artery at its third part. This usual formation of the nerve is observed in 70% of cases.

Our team describes a case in which there is a very distal fusion of the medial and lateral roots which arise from the cords of the brachial plexus. Similar formation and location of the median nerve could lead to difficulties in the diagnostic process in this area. When performing different surgical procedures, clinical manipulations and physiotherapeutic procedures it is vital to know both the most common and the atypical morphology of the median nerve.

Case description

The present study took place during the routine dissection classes of the upper limb

which were for the undergraduate medical students at the Department of Anatomy in our university. All formalin-preserved cadavers were dissected on both upper extremities. The skin, superficial fascia, and deep fascia have been removed, the muscles cleaned and we were able to observe the formation of the median nerve and its variations. Our dissection has been carried out on embalmed adult male cadaver of visible age over 65 years. We encountered different formation of the median nerve in right and left arm of the cadaver.

Macroscopic examination of the dissected brachial plexus in the left arm:

Normal position and relation between the trunks of the brachial plexus (fig. 1) and typical formation of the median nerve in the left axilla (fig. 2). Measurements as follow:

Lateral root length – 53 mm

Medial root length – 52 mm

Musculocutaneous nerve length – 37 mm

Macroscopic examination of the dissected brachial plexus in the right arm:

Normal position and relation between the trunks of the brachial plexus (fig. 3) and atypical formation of the median nerve in the right axilla (fig. 4). Measurements as follow:

Lateral root length – 102 mm

Medial root length – 68 mm

Musculocutaneous nerve length – 37 mm



Figure 1. Left arm normal position of the trunks of brachial plexus:

1. superior trunk
2. medial trunk
3. inferior trunk



Figure 3. Left arm normal position of the trunks of brachial plexus:

1. superior trunk
2. middle trunk
3. inferior trunk



Figure 2. Left arm typical formation of median nerve:

1. medial root
2. lateral root
3. median nerve
4. musculocutaneous nerve



Figure 4. Left arm typical formation of median nerve:

1. medial root
2. lateral root
3. median nerve
4. musculocutaneous nerve

Discussion

Atypical formation of the different nerves of the upper extremities has been described by many authors. The median nerve has a great variety of anomalies in its formation and position:

- the typical formation of the median nerve by two roots was found in 70% of the dissected cadavers; three or four roots formation was reported in 30% of the dissections;

	Two roots	Three roots		Four roots	Total
Males	39 (69.64%)	From LC	From MCN	3 (5.36%)	56 (100%)
		9 (16.07%)	5 (8.93%)		
		14 (25%)			
Females	20 (71.44%)	From LC	From MCN	2 (7.14%)	28 (100%)
		4 (14.28%)	2 (7.14%)		
		6 (21.42%)			

LC=lateral cord; MCN=musculocutaneous nerve

- the formation of the median nerve in the axillary fossa was reported in 88.09% of cases and the distal formation in the arm in 11.91% of the reported cases – 8.33% belonging to males and 3.58% to females;

- in the axilla, the nerve was formed lateral to the third part of the axillary artery in 80.95% of cases, with 41.67% of cases belonging to males and 39.28% to females. It was formed anterior to the third part of the axillary artery in 4.76% of cases, with 3.57% of cases belonging to males and 1.19% to females. It formed medially to the third part of the axillary artery in the axilla in only 2.38% of cases, of which 1.19% involved both male and female cadavers;

Site of formation of the median nerve		In males	In females	Total	
1. In the axilla	Lateral to axillary A	35 (41.67%)	33 (39.28%)	68 (80.95%)	74 (88.09%)
	Medial to axillary A	1 (1.19%)	1 (1.19%)	2 (2.38%)	
	Anterior to axillary A	3 (3.57%)	1 (1.19%)	4 (4.76%)	
2. In the arm		7 (8.33%)	3 (3.58%)	10 (11.91%)	

- additional communication between the median nerve and musculocutaneous nerve was observed in 2.38% of cases.

Conclusion

The knowledge about variations in the formation of the median nerve is relevant to anatomists, radiologists and surgeons. Since such variations are commonly encountered they are of great interest and importance in routine surgeries, post-traumatic evaluations, peripheral nerve repairs, and during radical neck dissections where these variations are more prone to iatrogenic injury. The awareness of them can prevent injuries during surgical and anaesthetic procedures around the axilla and the arm and can help in the interpretation of nerve compression causing unexplained clinical symptoms.

References

1. Standring S. Gray's anatomy: the anatomical basis of clinical practice.
2. Drake RL, Vogl W, Mitchell AWM. Gray's anatomy for students.
3. Ghosh B, Dilkash MN, Prasad S, Sinha SK. Anatomical variation of median nerve: cadaveric study in brachial plexus. *Anat Cell Biol.* 2022;55:130–134.
4. Budhiraja V, Rastogi R, Asthana AK. Variations in the formation of the median nerve and its clinical correlation. *Folia Morphologica.* 2012;71:28–30.
5. Beheiry EE. Anatomical variations of the median nerve distribution and communication in the arm. *Folia Morphologica.* 2004;63:313–318.

Corresponding author

Zlatizara Todorova1

Zlatizara.Todorova@mu-plovdiv.bg

+359899399002

The Significance of FOT (Forced Oscillation Technique) in the Diagnosis of Small Airways Dysfunction

Zlatina Zigova¹, Tsvetana Damyanova¹, Martin Georgiev¹, Plamena Stoimenova²

1. Faculty of Dental Medicine, Medical University of Plovdiv

2. Department of Pathophysiology, Medical University of Plovdiv

Introduction: Spirometry is the ‘gold standard’ by which airflow obstruction is assessed but it requires effort and can be difficult for patients to perform. Forced oscillation technique (FOT) is a relatively new method that may hold the key to improving the diagnosis of airway obstruction, helping in the adjustment of therapy and monitoring disease progression. **Aim:** The purpose of this report is to compare the applicability of FOT and Spirometry in respiratory function evaluation. **Materials and methods:** Studies and clinical cases regarding pulmonary function testing are summarized and analyzed. They are found in the following databases – PUBMED, Google Scholar and Science Direct. **Results and discussion:** The Forced oscillation technique is a lung function modality based on the application of an external oscillatory signal in order to determine the response of the respiratory system. The use of FOT should be considered in patients in whom spirometry or other pulmonary function tests cannot be performed. One of the main advantages of this method is that minimal cooperation of the patient is needed and no respiratory maneuvers are required. This technique may be more sensitive than spirometry in identifying disturbances of peripheral airways or the effectiveness of therapy at the long term. **Conclusion:** The Forced oscillation technique has been successfully applied in various paediatric respiratory disorders, such as asthma, cystic fibrosis, and chronic lung disease. Considering these qualities FOT may be used as an alternative of spirometry in heterogeneous ventilatory disturbances of the small airways.

Keywords: *Forced oscillation technique, airway obstruction, heterogeneous ventilatory conditions*

Introduction

The forced oscillation technique (FOT), which applies small pressure oscillations at the mouth during typical breathing, was created to evaluate the impedance of the respiratory system. FOT is considered as a potential alternative method to spirometry. Spirometry is the ‘gold standard’ by which airflow obstruction is assessed but it requires effort and can be difficult for patients to perform. Forced oscillation technique (FOT) may hold the promise of improving the diagnosis of airway obstruction, helping in the adjustment of therapy, and monitoring disease progression in young children.

Aim

The purpose of this report is to compare the applicability of FOT and Spirometry in respiratory function evaluation. The report compares different conditions and the significance that FOT makes in the diagnostic process.

Methods

This literature review analyzes a number of studies and clinical cases related to lung function evaluation especially in the different groups of people that have difficulties in performing the spirometry. They are used the following databases – PUBMED, Google Scholar and Science Direct.

Results

1. FOT in infants and preschool-aged children

The measurement of pulmonary function provides an objective assessment of the state of the respiratory system and useful information for the diagnosis and management of respiratory tract illnesses in adults and children. Many measurements of respiratory function are based on forced expiratory maneuvers. Spirometry has been shown to be feasible in preschool-aged children as young as 2–5 years and can be performed successfully in 70% to 80% of children by the age

of 5 to 6. The above-mentioned method cannot be performed in infants and preschool-aged children. The forced oscillation technique (FOT), measures the impedance of the respiratory system by superimposing small-amplitude pressure oscillations on the respiratory system as well as the resultant oscillatory flow. These techniques have been used in infants, and impedance spectra have been measured above 4 Hz. The forcing function is generally applied through a facemask and includes the impedance of the nose. By applying the forcing function during a pause in breathing produced by invoking the Hering-Breuer reflex, reliable impedance data can be obtained from 0.5 to 20 Hz. Depending on the frequency of the applied pressure wave, the resultant impedance contains different mechanical information. The response to very slow pressure oscillations (<2 Hz), during what has been referred to as the low-frequency forced oscillation technique, allows the noninvasive partitioning of lung function into airway and tissue parameters. In contrast, at very high frequencies, as are used in the high-frequency interrupter technique, the information derived is primarily related to airway wall mechanics, which is particularly important in wheezing disorders.

2. Spirometry in preschool-aged children

To assess repeatability and reproducibility of spirometry measurements, and bronchodilator responsiveness (BDR), Spirometry is performed in healthy 3–6-year-old preschool children and those with asthma. Spirometry was performed before and 20 minutes after administering either inhaled placebo (for repeatability) or 400 μ g salbutamol (for BDR) on two separate occasions (reproducibility) 3–23 days apart in asthmatic preschoolers and healthy controls. Technically successful baseline results were obtained in 86% of children assessed. Paired

data were obtained in 43 asthmatic and 22 controls (median (range) age: 5.1 years). Baseline FEV (0.75) was significantly lower in asthmatic children (mean (SD): 90 (15)% predicted; $p < 0.001$). Within-occasion coefficient of repeatability following placebo was similar in both groups, being 10.4% in asthma and 13.2% in controls for FEV (0.75). Following bronchodilator, FEV(0.75) increased significantly more in asthmatic preschoolers (mean (SD): 15.0 (12) %) than in controls (4.5 (5) %; $p < 0.001$), with no significant difference between groups post-bronchodilator. Between-occasion variability was similar to within-day repeatability in controls, but almost twice as high in asthmatic children.

3. Forced oscillation technique in spinal muscular atrophy

It is challenging to diagnose respiratory impairment in young children due to spinal muscular atrophy (SMA). Children as young as 2 years old can use the nonvoluntary forced oscillation technique (FOT), which is commercially available.

Twelve children (seven male) were recruited; mean age was 6.26 ($\pm$ 2.59) years. Respiratory reactance at 8 Hz (X_{rs8}) (mean z score, +1.41; SD, 1.90; $P < .03$) and respiratory resistance at 8 Hz (R_{rs8}) (mean z score, +0.66; SD, 1.34; $P = .12$) were abnormal. Four children performed spirometry. Linear relationships to X_{rs8} exist: FVC (R^2 , 0.54), unassisted PCF (R^2 , 0.33), assisted PCF (R^2 , 0.43), and AHI (R^2 , 0.32). Over 12 months, X_{rs8z} score worsened (rate of change of +1.08, $P < .001$) and R_{rs8z} score worsened (rate of change +0.51, $P < .001$). No relationship ($P > .05$) was found between clinical characteristics and FOT values. [7]

FOT is feasible in young SMA patients with abnormal reactance and resistance

values on aggregated data, which worsen over a 12-month period. Xrs8 is connected to the respiratory tests used to track development in SMA.

4. Comparison between FOT and Spirometry

Traditional pulmonary function tests such as the ‘gold standard’ – Spirometry, reflect information about global lung function, but obtaining more precise information from the lung periphery has been a challenge. FOT is used to measure respiratory system resistance (Rrs) and reactance (Xrs) during tidal breathing and provides information that cannot be obtained by spirometry. To evaluate the utility of the forced oscillation technique (FOT) bronchodilator responsiveness (BDR) was compared with forced expiratory volume in 1 s (FEV₁).

Data from patients with asthma who withheld bronchodilator medication for at least 8 h before a tertiary airway clinic visit were reviewed. All patients performed FOT and spirometry before and after salbutamol administration, and completed the Asthma Control Test. FOT parameters examined included respiratory system resistance (R5) and reactance (X5) at 5 Hz, and area under the

reactance curve (AX). BDR was defined by standard recommendations for spirometry and based on the 95th percentile of BDR in healthy adults for FOT.

Figure 1 represents the comparison between bronchodilator response (BDR) identified by spirometry and forced oscillation technique (FOT). Shown are the following: cutoff for identifying BDR by spirometry (blue line) and FOT (orange line); region of BDR concordance between spirometry and FOT (orange-shaded areas); change in parameter after bronchodilator administration (postbronchodilator minus prebronchodilator) (Δ). Spirometric BDR is defined as ≥ 200 mL and $\geq 12\%$ improvement in FEV₁ and/or FVC.

Figure 2 represents the association between the presence of BDR and asthma control. Shown are the following: cutoff for poor asthma control (Asthma Control Test score < 20) (blue line); cutoff for BDR by spirometry or FOT (orange line); change in parameter after bronchodilator administration (postbronchodilator minus prebronchodilator) (Δ). ACT = Asthma Control Test; AX = area under the reactance curve, between 5 Hz and resonant frequency; R5 = resistance at 5 Hz; X5 = reactance at 5 Hz.

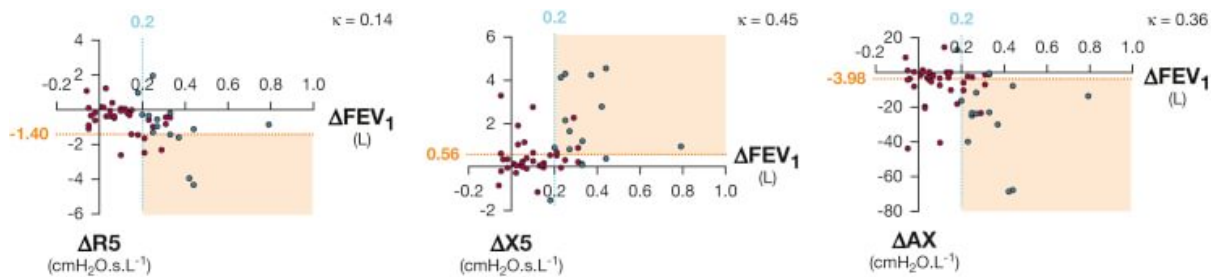


Figure 1.

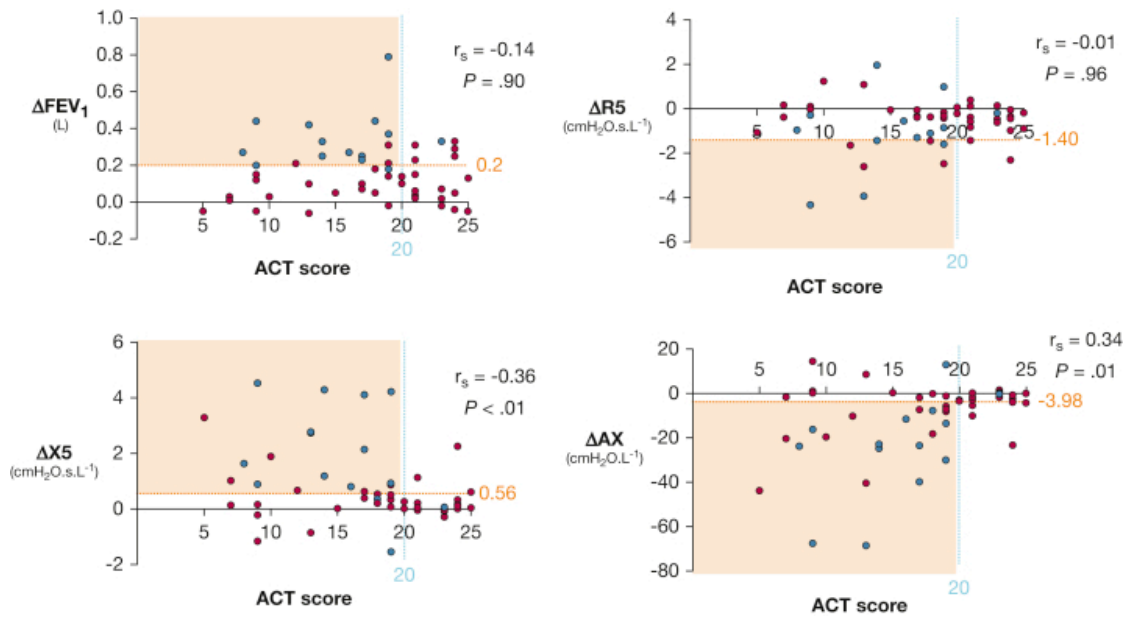


Figure 2.

Results

Fifty-two subjects (18 men; mean age, 53 ± 18 years) were included. BDR was identified more frequently by FOT than spirometry (54% vs 27% of subjects).

The conclusion of this article that the use of FOT can be considered as an alternative to forced expiration for detecting bronchodilatation in asthmatics and patients with COPD.

Discussion

While application of FOT requires minimal cooperation from the subject, data collection and analysis can be technically challenging, and further work is required to establish international guidelines regarding quality control in young children and standardize methods of reporting results. Nevertheless, there are marked differences between the reference ranges for FOT using a variety of techniques, including much higher values obtained when using a modified face-mask rather than a mouthpiece. Although data collected using FOT and Spirometry are similar, the results obtained using these techniques are not interchangeable.

Currently the wide degree of variability between healthy children of similar age or body size limits the extent to which this technique can be used to assess either the presence or the severity of airway disease within individuals. It may, however, be a valuable tool for detecting changes within individuals on the same occasion, particularly when assessing bronchial responsiveness.

Conclusion

It is now evident that FOT measurements of lung function are superior to maximal-effort forced spirometric studies in terms of sensitivity and responsiveness to small airway function. The FOT has been successfully applied in various pediatric respiratory disorders, such as asthma, cystic fibrosis, and chronic lung disease of prematurity. Considering these qualities, Forced oscillation technique can be considered as an alternative for diagnosing patients with COPD.

References

1. Françoise Zerah MD, Anne-Marie Lorino PhD, Hubert Lorino PhD, Alain Harf MD, Isabelle Macquin-Mavier MD. Forced Oscillation Technique vs Spirometry to Assess Bronchodilatation in Patients With Asthma and COPD

2. Makito Yaegashi, Venkata A.K. Yalamanchili, Vaidehi Kaza, Jeremy Weedon, Albert E. Heurich, Michael J. Akerman. The utility of the forced oscillation technique in assessing bronchodilator responsiveness in patients with asthma
3. Toshihiro Shirai, Keita Hirai, Yasuhiro Gon, Shuichiro Maruoka, Kenji Mizumura, Mari Hikichi, Kunihiro Itoh, Shu Hashimoto. Forced oscillation technique may identify asthma-COPD overlap
4. Alice M. Cotte MBBS, Leigh M. Seccombe PhD, Cindy Thamrin PhD, Gregory G. King MBChB, PhD, Matthew J. Peters MBBS, MD, Claude S. Farah MBBS, PhD. Bronchodilator Response Assessed by the Forced Oscillation Technique Identifies Poor Asthma Control With Greater Sensitivity Than Spirometry
5. Peter D. Sly, Wayne J. Morgan, in *Pediatric Respiratory Medicine* (Second Edition), 2008.
- Respiratory Function Testing in Infants and Preschool-Aged Children
6. Michael D. Goldman. Clinical Application of Forced Oscillation
7. Leanne M. Gauld MD, Lucy A. Keeling BExS, Claire E. Shackleton BSc(Hons), Peter D. Sly PhD. Forced Oscillation Technique in Spinal Muscular Atrophy
8. Luis Miguel Borrego, Janet Stocks, Isabel Almeida, Sanja Stanojevic, João Antunes, Paula Leiria-Pinto, José E Rosado-Pinto, Ah-Fong Hoo. Bronchodilator responsiveness using spirometry in healthy and asthmatic preschool children
9. Umme Kolsum, Zoë Borrill, Kay Roy, Cerys Starkey, Jørgen Vestbo, Catherine Houghton, Dave Singh. Impulse oscillometry in COPD: Identification of measurements related to airway obstruction, airway conductance and lung volumes

Alopecia Areata Association with Thyroid Autoimmunity and Dysfunction: Current Review of Literature

Lidiya Todorova¹, Sava Petrov²

1. Department of Dermatology and Venereology, Faculty of Medicine, Medical University of Plovdiv

2. Department of Endocrinology, Faculty of Medicine, Medical University of Plovdiv

Introduction: Alopecia areata (AA) is an autoimmune T-cell mediated non-scarring hair loss which manifests with oval single or multiple bald patches, total loss of scalp hair (alopecia totalis) or bodily hair (alopecia universalis). The prevalence of AA varies from 0.1% to 0.2% of the general population. The aetiology of AA is unclear, but genetic, environmental factors, autoimmunity and endocrine dysfunction may be involved. AA is frequently associated with Hashimoto's thyroiditis and other autoimmune thyroid disorders, diabetes mellitus type 1 and vitiligo. **Aim:** A current review of the literature to establish the association between AA and thyroid autoimmunity and dysfunction. **Materials and methods:** Literature search through PubMed was performed using the following key words: alopecia areata, thyroid autoimmunity, thyroid disorder. A total of 260 articles published between 1993 and 2023 were reviewed and 26 were included based on the relevance to the researched topic. **Results:** There is association of thyroid dysfunction and thyroid autoimmunity in AA patients. The available literature at PubMed shows that the thyroid autoantibodies are frequently higher in AA patients. **Discussion:** AA is associated with high prevalence of thyroid antibodies more than with clinical or laboratory thyroid dysfunction. Also, severe and refractory AA is a potential future risk of autoimmune thyroid disease. **Conclusion:** Routine screening of the thyroid function and test for levels of thyroid antibodies is recommended to be done in individuals with AA.

Keywords: alopecia areata, autoimmune thyroid disorders, thyroid disease, thyroperoxidase antibody, thyroglobulin antibody

Introduction

Alopecia areata (AA) is an autoimmune T-cell mediated disorder characterized by non-scarring hair loss of the scalp or body. Clinically heterogeneous, AA can manifest as a single or multiple well-defined oval bald patches of different size. The hair loss may affect the entire scalp (alopecia totalis (AT)), body (alopecia universalis (AU)); or other bodily parts such as eyebrows and eyelashes [1]. The prevalence of AA varies from 0.1% to 0.2% of the general population [2]. The course of the disease is unpredictable. Approximately 80% of the patients experience spontaneous hair regrowth and sudden relapses. Some patients may have persistent hair loss [1, 3]. The aetiology of AA is unclear, but recent insights on the ethiopathogenesis of AA suggest a genetic and immune predisposition to the disorder as well as environmental factors including viral infections, trauma and psychosocial stress. The loss of the growing hair shafts is linked to the CD4+ and CD8+ T-lymphocytes, which

violate the immune privilege of the anagen hair follicle [1, 3, 4]. AA is often associated with comorbid conditions, including thyroid autoimmunity and dysfunction, atopy, vitiligo, lupus erythematosus, psoriasis, metabolic syndrome, Helicobacter pylori infection, iron and vitamin D deficiency, psychiatric diseases, audiology and ophthalmic abnormalities [5]. The rate of occurrence of thyroid dysfunction, increased presence of thyroid autoantibodies (TAb), and autoimmune thyroid diseases is significantly higher in AA patients than in the general population [6].

Aim

The aim of this current review is to establish the association between AA and thyroid autoimmunity and dysfunction.

Methods

Literature search through PubMed was performed using the following key words: alopecia areata, thyroid autoimmunity, thyroid disorder.

Results

A total of 260 articles published between 1993 and 2023 were reviewed and 26 were included based on the relevance to the researched topic. We divided the used literature into following groups: case reports, retrospective studies, cohort study, and prospective studies.

Case reports

Two case reports matching the criteria were included. The first one discusses AA patient with Graves' disease developed after manifestation of Hashimoto thyroiditis (HT). The patient had elevated thyroglobulin antibodies (Anti-Tg Ab), thyroid peroxidase antibodies (Anti-TPO Ab) and thyroid-stimulating hormone (TSH) [7]. The second case is of a ten-year-old AA patient, with keratoconjunctivitis, keratoconus and HT. The authors suggest an association of keratoconus with multisystem autoimmune disease [8].

Retrospective studies

In a retrospective study, Seyrafi et al. evaluated the prevalence of thyroid function abnormalities in 123 AA patients. Such was found in 8.9% of the study group. Elevated TAb were associated with AA in 51.4% of patients. The authors found no significant association between the AA severity and presence of the antibodies (Ab) [9]. Another study compared the frequency of TAb and the level of triiodothyronine (T3), thyroxine (T4), TSH in 70 AA patients and 30 healthy volunteers. Thyroid functional abnormalities were found in eight (11.4%) patients and positive TAb in 18 (25.7%). Significantly higher frequency of TAb was established in AA patients group than in healthy controls group (25.7% vs. 3.3%; $p < 0.05$). The study does not report connection between disease severity and presence of Tab [10]. Lo Sicco and McGuire, found 36 of 41 patients with

thyroid dysfunction suffering from alopecia: telogen effluvium 29.3%, androgenetic alopecia 27.8%, alopecia areata 24.4%, traction alopecia 9.8% [11]. A nationwide study in South Korea revealed that patients with AA were at an increased risk of Graves' disease and HT, and the associations were stronger in AT and AU [12]. Another study done in the same country investigated 1408 AA patients and found 224 (15.90%) who had thyroid dysfunction and 260 (18.46%) thyroid autoimmunity. Severe AA and mild AA groups showed significant difference in thyroid dysfunction (75.56% vs 13.94%) and thyroid autoimmunity (40.82% vs 18.22%). The study established relationship between thyroid dysfunction and earlier onset and severity of AA [13].

Cohort study

Dai et al. found bidirectional association between AA and thyroid diseases. They first analysed 5929 AA patients and 59,290 controls, to assess the risk of thyroid diseases in AA. Then they included 35,071 thyrotoxicosis patients: 19,227 with Graves' disease, 5460 with thyroiditis, 3352 with HT, to assess the risk of AA. AA patients had an increased risk of all thyroid diseases. Significantly increased risk of developing AA was observed among patients with thyrotoxicosis, Graves' and/or HT [14].

Prospective studies

In 1994 Puavilai et al. conducted a study with 152 AA patients and a control group with the same number of subjects and measured T3, T4, TSH and microsomal Ab levels. The results showed 7.2% prevalence of thyroid disease in the AA group, with 4.2% elevated microsomal Ab and 2.6% small simple goitre. The occurrence of positive microsomal Ab in 4.6% of the patients was not significantly different from the control group

[15]. In a study by Kurtev and Iliev, 46 children with AA (23 girls and 23 boys) were examined. The researchers investigated the size and function of the thyroid gland, and measured T3, T4, TSH, thyroid-releasing-hormone (TRH), Anti-Tg Ab, Anti-TPO Ab. The results showed goitre in 29 children (63%), elevated basal TSH in six of the 29 (13.3%) and hypothyroid-type stimulated secretion (TRH test) in two out of 12 patients. Anti-Tg Ab were increased in 17 (39.5%) and Anti-TPO Ab in 14 (33.3%) children. The team found autoimmune thyroiditis in 22 of 46 AA patients (47.8%) and immune studies revealed increased activated T-lymphocytes [16]. In another study, 63 AA patients were tested for TAb and 28 subjects (44%) were positive. Anti-TPO and Anti-Tg Ab were more frequent, followed by antimicrosomal and antinuclear [17]. Other researchers examined 130 AA individuals and found elevated Anti TPO-Ab titres in 23 patients (17.7%) and six of the patients had higher TSH levels. The prevalence of Anti-TPO-Ab was almost two times higher than in the general population [18]. In 2014, Bakry et al. included in their study 50 AA subjects without any clinical presentation of thyroid disorder. They found 16% that were with subclinical hypothyroidism and statistically significant difference in levels of TSH, T3, T4, Anti-Tg Ab and Anti-TPO Ab between cases and controls [19]. Lyakhovitsky et al examined 78 AA patients with new onset of the hair loss. Thyroid dysfunction and TAb were found in 19 (24%) patients. Hypothyroidism was diagnosed in 12 (15%), one (1%) with an overt hypothyroidism, one (1%) with an overt hyperthyroidism and four (5%) with euthyroid HT [20]. A recent case control study investigated the frequency of TAb and thyroid dysfunction in 54 Spanish AA patients. Pathological levels of Anti-TPO-Ab and/or Anti-TG-Ab titres had 24%

of AA cases. Thyroid dysfunction was found in 22% of AA patients [21]. Kurtipek et al. examined the thyroid gland function of 92 AA and 108 vitiligo patients. They found that there is no statistically significant correlation between AA and thyroid autoimmunity or dysfunction [22]. Similar results showed Kaur et al. The studied population included 110 AA patients and 110 healthy individuals. The authors compared serum levels of T3, T4, TSH, and Anti-TPO Ab in both groups. There was no significant occurrence of thyroid dysfunction and evidence of anti-TPO Ab in AA [23].

The correlation in human leukocyte antigens (HLA) and clinical characteristics of thyroid and islet autoimmunity in 126 AA patients, was estimated via another study. The most frequent autoimmune diseases associated with AA was autoimmune thyroid disease (10.0%), then vitiligo (2.7%) and rheumatoid arthritis (0.9%). The prevalence of thyroid TAb in AA patients was higher compared to controls. The prevalence of islet-related auto-Ab was similar between AA patients and the control group [24]. Saif assessed the importance of thyroid autoimmune screening in AA patients. The study included three groups: 50 AT and AU patients, 50 subjects with partial AA and 50 healthy individuals. TAb were positive in AT/AU by 40%, in mild AA by 14%, and in healthy subjects by 4% [25]. A comparative study between 75 AA patients and 75 healthy individuals, found a prevalence of thyroid disorder in the AA group (17.3%) as compared to the control group (1.3%) ($P = 0.001$). The authors suggest that the grade of the AA severity was higher in patients with abnormal thyroid function. [26]

Discussion

This current review of literature summarizes the evidence of an increased risk of

thyroid conditions in AA patients. Also, studies have found a relationship between AA severity and associated thyroid dysfunction or autoimmunity. Although the precise correlation between AA and thyroid disorders has not been well explained, common genetic background and mutual inflammatory process might do that. A genome-wide association analysis of AA, identified a number of risk loci that were also present in other autoimmune illnesses: cytotoxic T-lymphocyte-associated protein 1, interleukin (IL)-2/IL-21, IL-2RA, genes essential for regulatory T-cell maintenance [27]. Immunological dysregulation in hair follicles caused by CD8⁺ NKG2D⁺ cytotoxic T-lymphocytes is a contributing factor in AA [28]. Human immunological homeostasis is mostly maintained by regulatory T (Treg) cells, whose imbalance results in autoimmunity [29]. Treg cells in people with autoimmune thyroid illnesses are unable to downregulate tissue damage and the autoimmune response [30].

Conclusion

The analysed studies and case reports confirm the connection between thyroid autoimmunity and/or dysfunction to AA. The studies suggest a correlation between severity and duration of AA if the thyroid gland is affected. Most authors recommend routine screening of the thyroid function and test for levels of TAb for AA patients. More studies are needed to clarify the potential mechanisms and interactions underlying between the conditions.

References

1. Villasante A, Miteva M. Epidemiology and burden of alopecia areata: a systemic review. *Clinical, Cosmetic and Investigational Dermatology*. 2015;397. [PMID: 26244028] [DOI:10.2147/CCID.S53985]
2. Kavak A, Baykal C, Ozarmagan G et al. HLA in alopecia areata. *International Journal of Dermatology*, 2000, 39(8), 589–592.
3. Zhou C, Li X, Wang C, Zhang J. Alopecia Areata: an Update on Etiopathogenesis, Diagnosis, and Management. *Clinical Reviews in Allergy & Immunology*. 2021;61(3):403-23. [PMID: 34403083] [DOI:10.1007/s12016-021-08883-0].
4. Suchonwanit P, Kositkuljorn C, Pomsoong C. Alopecia Areata: An Autoimmune Disease of Multiple Players. *ImmunoTargets and Therapy*. 2021;Volume 10:299-312. [PMID:34350136] [DOI:10.2147/ITT.S266409]
5. Lee S, Lee H, Lee CH, Lee WS. Comorbidities in alopecia areata: A systematic review and meta-analysis. *J Am Acad Dermatol*. 2019 Feb;80(2):466-477.e16. doi: 10.1016/j.jaad.2018.07.013. Epub 2018 Jul 18. PMID: 30031145.
6. Lee S, Lee YB, Kim BJ, Lee WS. Screening of thyroid function and autoantibodies in patients with alopecia areata: A systematic review and meta-analysis. *J Am Acad Dermatol*. 2019 May;80(5):1410-1413.e4. doi: 10.1016/j.jaad.2018.10.066. Epub 2018 Nov 14. PMID: 30447313.
7. Aşık M, Binnetoğlu E, Şen H, Tekeli Z, Uysal F, Ukinç K. Graves' disease associated with alopecia areata developing after Hashimoto's thyroiditis. *J Nippon Med Sch*. 2013;80(6):467-9. doi: 10.1272/jnms.80.467. PMID: 24419720.
8. Koçak Altintas AG, Gül U, Duman S. Bilateral keratoconus associated with Hashimoto's disease, alopecia areata and atopic keratoconjunctivitis. *Eur J Ophthalmol*. 1999 Apr-Jun;9(2):130-3. doi: 10.1177/112067219900900210. PMID: 10435426.
9. Seyrafi H, Akhiani M, Abbasi H, Mirpour S, Gholamrezanezhad A. Evaluation of the profile of alopecia areata and the prevalence of thyroid function test abnormalities and serum autoantibodies in Iranian patients. *BMC Dermatol*. 2005 Oct 31;5:11. doi: 10.1186/1471-5945-5-11. PMID: 16259629; PMCID: PMC1280924.
10. Kasumagić-Halilović E. Thyroid autoimmunity in patients with alopecia areata. *Acta Dermatovenerol Croat*. 2008;16(3):123-5. PMID: 18812059.
11. Lo Sicco K, McGuire S, English JC 3rd. A retrospective study of thyroid structural abnormalities in alopecia patients. *Dermatoendocrinol*. 2011 Oct;3(4):251-4. doi: 10.4161/derm.3.4.16838. Epub 2011 Oct 1. PMID: 22259653; PMCID: PMC3256342.
12. Han TY, Lee JH, Noh TK, Choi MW, Yun JS, Lee KH, Bae JM. Alopecia areata and overt thyroid diseases: A nationwide population-based study. *J Dermatol*. 2018 Dec;45(12):1411-1417. doi: 10.1111/1346-8138.14648. Epub 2018 Sep 17. PMID: 30222206.
13. Park SM, Oh YJ, Lew BL, Sim WY. The association among thyroid dysfunction, thyroid autoimmunity, and clinical features of alopecia areata: A retrospective study. *J Am Acad Dermatol*. 2019 Aug;81(2):602-605. doi: 10.1016/j.jaad.2018.04.051. Epub 2018 May 10. PMID: 29753058.
14. Dai YX, Tai YH, Chang YT, Chen TJ, Chen MH. Bidirectional association between alopecia areata and thyroid diseases: a nationwide population-based cohort study. *Arch Dermatol Res*. 2021 Jul;313(5):339-

346. doi: 10.1007/s00403-020-02109-7. Epub 2020 Jul 23. PMID: 32705333.
15. Puavilai S, Puavilai G, Charuwichitratana S, Sakuntabhai A, Sriprachya-Anunt S. Prevalence of thyroid diseases in patients with alopecia areata. *Int J Dermatol.* 1994 Sep;33(9):632-3. doi: 10.1111/j.1365-4362.1994.tb02921.x. PMID: 8002158.
16. Kurtev A, Iliev E. Thyroid autoimmunity in children and adolescents with alopecia areata. *Int J Dermatol.* 2005 Jun;44(6):457-61. doi: 10.1111/j.1365-4632.2005.01971.x. PMID: 15941431.
17. Grandolfo M, Biscazzi AM, Pipoli M. Alopecia areata and autoimmunity. *G Ital Dermatol Venereol.* 2008 Oct;143(5):277-81. PMID: 18833068.
18. Baars MP, Greebe RJ, Pop VJ. High prevalence of thyroid peroxidase antibodies in patients with alopecia areata. *J Eur Acad Dermatol Venereol.* 2013 Jan;27(1):e137-9. doi: 10.1111/j.1468-3083.2011.04420.x. Epub 2012 Jan 6. PMID: 22226373.
19. Bakry OA, Basha MA, El Shafiee MK, Shehata WA. Thyroid disorders associated with alopecia areata in egyptian patients. *Indian J Dermatol.* 2014 Jan;59(1):49-55. doi: 10.4103/0019-5154.123494. PMID: 24470660; PMCID: PMC3884928.
20. Lyakhovitsky A, Aronovich A, Gilboa S, Baum S, Barzilai A. Alopecia areata: a long-term follow-up study of 104 patients. *Journal of the European Academy of Dermatology and Venerology: JEADV [Internet].* 2019 Aug 1; 33(8):1602-9. [PMID: 30887594] [DOI:10.1111/jdv.15582].
21. Díaz-Angulo S, López-Hoyos M, Muñoz-Cacho P, López-Escobar M, González-López MA. High prevalence of thyroid autoimmunity in patients with alopecia areata and vitiligo: a controlled study. *Australas J Dermatol.* 2015 May;56(2):142-3. doi: 10.1111/ajd.12321. PMID: 25900633.
22. Saylam Kurtipek G, Cihan FG, Erayman Demirbaş Ş, Ataseven A. The Frequency of Autoimmune Thyroid Disease in Alopecia Areata and Vitiligo Patients. *Biomed Res Int.* 2015;2015:435947. doi: 10.1155/2015/435947. Epub 2015 Aug 18. PMID: 26355833; PMCID: PMC4555361.
23. Kaur G, Kuldeep CM, Bhargava P, Mathur DK, Sharda S, Chaturvedi P. Insignificant Correlation between Thyroid Hormone and Antithyroid Peroxidase Antibodies in Alopecia Areata Patients in Northern Rajasthan. *Int J Trichology.* 2017 Oct-Dec;9(4):149-153. doi: 10.4103/ijt.ijt_32_17. PMID: 29118518; PMCID: PMC5655622.
24. Noso S, Park C, Babaya N, Hiromine Y, Harada T, Ito H, Taketomo Y, Kanto K, Oiso N, Kawada A, Suzuki T, Kawabata Y, Ikegami H. Organ specificity in autoimmune diseases: thyroid and islet autoimmunity in alopecia areata. *J Clin Endocrinol Metab.* 2015 May;100(5):1976-83. doi: 10.1210/jc.2014-3985. Epub 2015 Mar 3. PMID: 25734250.
25. Bin Saif GA. Severe subtype of alopecia areata is highly associated with thyroid autoimmunity. *Saudi Med J.* 2016 Jun;37(6):656-61. doi: 10.15537/Smj.2016.6.13777. PMID: 27279512; PMCID: PMC4931647.
26. Marahatta S, Agrawal S, Mehata KD. Alopecia Areata and Thyroid Dysfunction Association- A Study from Eastern Nepal. *Kathmandu Univ Med J (KUMJ).* 2018 Apr-Jun;16(62):161-165. PMID: 30636758.
27. Petukhova L, Duvic M, Hordinsky M, Norris D, Price V, Shimomura Y, Kim H, Singh P, Lee A, Chen WV, Meyer KC, Paus R, Jahoda CA, Amos CI, Gregersen PK, Christiano AM. Genome-wide association study in alopecia areata implicates both innate and adaptive immunity. *Nature.* 2010 Jul 1;466(7302):113-7. doi: 10.1038/nature09114. PMID: 20596022; PMCID: PMC2921172.
28. Rajabi F, Drake LA, Senna MM, Rezaei N. Alopecia areata: a review of disease pathogenesis. *Br J Dermatol.* 2018 Nov;179(5):1033-1048. doi: 10.1111/bjd.16808. Epub 2018 Sep 9. PMID: 29791718.
29. DeJaco C, Duftner C, Grubeck-Loebenstein B, Schirmer M. Imbalance of regulatory T cells in human autoimmune diseases. *Immunology.* 2006 Mar;117(3):289-300. doi: 10.1111/j.1365-2567.2005.02317.x. PMID: 16476048; PMCID: PMC1782226.
30. Marazuela M, García-López MA, Figueroa-Vega N, de la Fuente H, Alvarado-Sánchez B, Monsiváis-Urenda A, Sánchez-Madrid F, González-Amaro R. Regulatory T cells in human autoimmune thyroid disease. *J Clin Endocrinol Metab.* 2006 Sep;91(9):3639-46. doi: 10.1210/jc.2005-2337. Epub 2006 Jun 27. PMID: 16804051.

Corresponding author

Д-р Лидия Николова Тодорова
гр. Пловдив, бул. Цар Борис III Обединител 35

МУ-Пловдив

Катедра по дерматология и венерология

гр. Пловдив, бул. Васил Априлов 15А

тел: 0898 570528

e-mail: lidiatodorova@gmail.com

Insomnia with Short Sleep Duration and the Impact on the Cardiovascular System – Current Knowledge and Future Perspectives

Gogilan Udeyana¹, Todor Georgiev², Krasimir Avramov², Aneliya Draganova², Kiril Terziyski²

1. Faculty of Medicine, Medical University of Plovdiv

2. Department of Pathophysiology, Faculty of Medicine, Medical University of Plovdiv

Introduction: Chronic insomnia disorder is known to exert detrimental effects on health, with the short sleep duration phenotype (< 6 hours) being associated with increased risk for cardiovascular complications, compared to the normal sleep duration insomnia. Elucidating the pathogenic links between short sleep duration insomnia and its adverse impact on cardiovascular health would contribute to designing preventive strategies and improving therapeutic outcomes. **Aim:** The aim of this review is to explore the potential pathophysiological mechanisms underlying the cause-effect relationship between chronic insomnia disease and cardiovascular health. **Materials and methods:** A search in the PubMed database was performed utilizing the following key words: short sleep duration insomnia, cardiovascular disease, arterial hypertension, hyperarousal, metabolic syndrome, autonomic nervous system. Studies published between 2000–2023 were included. **Results and discussion:** A growing body of evidence suggests that short sleep duration insomnia is the more biologically severe phenotype of the disorder. It is associated with a higher prevalence of arterial hypertension, coronary artery disease and heart failure. The proposed mechanisms relating insomnia to cardiovascular disorders are the hyperarousal state, increased activity of the sympathetic nervous system, dysregulation of the hypothalamic-pituitary-adrenal axis, systemic inflammation, and metabolic derangements. These are classically acknowledged as important factors in the pathogenesis of various cardiovascular diseases. **Conclusion:** The reviewed data suggests that shortened sleep has a cumulative negative impact on cardiovascular health. Despite the well-supported association, further research is needed to illuminate whether the improvement of sleep duration and quality in chronic insomnia disorder patients would alleviate cardiovascular risk.

Keywords: short sleep duration insomnia, cardiovascular disease, arterial hypertension, hyperarousal, metabolic syndrome

Introduction

Chronic insomnia disorder is known to exert detrimental effects on health, with the short sleep duration phenotype (< 6 hours) being associated with increased risk for cardiovascular complications, compared to the normal sleep duration insomnia. Elucidating the pathogenic links between short sleep duration insomnia (ISSD) and its adverse impact on cardiovascular health would contribute to designing preventive strategies and improving therapeutic outcomes.

Aim

The aim of this review is to explore the potential pathophysiological mechanisms underlying the cause-effect relationship between chronic insomnia disorder and cardiovascular health.

Materials and methods

A search in the PubMed database was performed utilizing the following key words: short sleep duration insomnia, cardiovascular disease, arterial hypertension, hyperarousal, metabolic syndrome, autonomic nervous system. Studies published between 2017–2023 were included.

Results

ISSD has been shown to be associated with an increased risk of various cardiovascular risk factors.

Arterial hypertension

The association between ISSD and arterial hypertension (AH) is well documented. Several comprehensive reviews presented a significant impact of ISSD on blood pressure regulation.

Bathgate et al. (2018) (1) conducted a review of evidence up to 2018 and reported a positive association between IOSSD and high blood pressure. More specifically they reported that individuals with insomnia exhibited higher nighttime systolic blood pressure, increased ambulatory blood pressure variability, and lower systolic blood pressure dipping. These findings suggest that IOSSD may contribute to adverse changes in blood pressure regulation and increase the risk of cardiovascular events. Two studies reviewed found that IOSSD patients are at over three-fold greater risk of reporting AH and twice as likely to be treated with hypertension medication compared to good sleepers.

Li et al (2020) (2) found that the risk for AH is 21% higher among insomnia patients, compared to good sleepers. *It is noteworthy that in this meta-analysis no statistically significant correlation was observed in the Asian populations, indicating that insomnia may impact various ethnic groups in distinct ways.*

Johnson et al (2021) (3) conducted a meta-analysis that reviewed available data to compare objective short sleep duration insomnia (IOSSD) with near normal sleep duration insomnia (INNSD). They investigated the association between both phenotypes and AH, type 2 diabetes, and body mass index. Results showed that individuals with IOSSD were more likely to develop AH compared to those with INNSD. Interestingly, one reviewed study among African Americans compared IOSSD, INNSD and short sleepers and observed increased risk of resistant AH only in short sleepers and in patients with IOSSD, indicating that sleep duration itself may be the decisive factor.

Inflammation

Mendoza et al (2017) (4) explored the correlation between insomnia phenotypes

and systemic inflammation in adolescents. IOSSD was found to be associated with elevated inflammatory biomarkers, as opposed to INNSD. C-reactive protein (CRP) levels were measured highest in those with IOSSD and, at elevated levels of 1.8 mg/L on average, were twice as high as in the other groups. Taking in consideration that the mean age of participants was 17 years, and the fact that all other study groups had CRP levels within the physiologic range of on average (< 1 mg/L), the observed differences may be indicative of potential cardiometabolic risk. Furthermore, other inflammatory biomarkers such as Interleukin-6 (IL-6) and Tumor necrosis factor- α (TNF- α) groups.

Syauqye et al (2019) (5) investigated the relationship of self-reported sleep duration and insomnia with inflammation in middle-aged and older adults with metabolic syndrome. Results showed a U-shaped association of sleep duration with metabolic syndrome and inflammation. Compared with regular sleep duration (6–8 h), short (< 6 h) or long sleep duration (> 8 h) and insomnia symptoms significantly increased the odds ratios of abdominal obesity, high blood pressure, low high-density lipoprotein-cholesterol, high triglycerides, high fasting blood glucose, and high CRP. Interestingly, results suggested that insomnia symptoms did not influence the effects of sleep duration on the components of metabolic syndrome and inflammation. Similar to findings on AH, sleep duration by itself appears to also be the key factor triggering inflammatory processes.

Wang et al (2020) (6) aimed to investigate the link between objective short sleep duration and inflammation in patients with chronic insomnia. The study demonstrated an inversely correlated relationship between sleep duration and inflammatory markers, such as NLR family pyrin domain

containing 3-Inflammasomes (NLRP3-I), caspase-1, Interleukin-1 β , and Interleukin-18. The dysregulation of the inflammasome, which is a component of the innate immune system that contributes to inflammation, was found to be specifically linked to short sleep duration in individuals with chronic insomnia. Results suggest that chronic insomnia and short sleep duration may trigger the development of chronic diseases such as cardiovascular disease (CD) and diabetes by promoting chronic inflammation.

Wang et al (2020) (7) investigated the relationship between chronic insomnia and inflammation in patients with atherosclerotic cerebral small vessel disease. Chronic insomnia was associated with higher levels of circulating interleukin-8 (IL-8). The concentration of IL-8 showed a positive correlation with the Arousal Index and a negative correlation with the duration of sleep. These findings imply that chronic insomnia, particularly sleep fragmentation and short sleep duration, could be a factor in the onset and advancement of cerebral small vessel disease related to atherosclerosis, mediated by the inflammatory pathway.

Cardiovascular complications

Bertisch et al (2018) (8) presented that IOSSD is associated with an increased risk of incident CD and all-cause mortality. Specifically, the study found that individuals with IOSSD had a significantly increased risk of developing CD (+29%) compared to those without insomnia and with normal sleep duration. Interestingly, it was found that insomnia by itself or short sleep by itself was not associated with incident CD, indicating that it is the combination of the two, that is the decisive factor. However, short sleep duration was significantly associated with all-cause mortality. The study also found that subjective ISSD showed a similar risk of CD when compared with IOSSD.

Sigurdardottir et al (2023) (9) conducted a study with objective insomnia phenotyping. IOSSD was found to be one of only 2 insomnia phenotypes that were associated with subclinical myocardial injury as expressed by increased circulating Troponin T (cTnT), the other one being insomnia with obstructive sleep apnea. These 2 phenotypes therefore may be of special relevance to sleep-related susceptibility to CD. Interestingly insomnia alone, insomnia associated with any measure of sleep fragmentation or objective short sleep duration alone were not associated with this outcome, suggesting that only the combination of insomnia with either objective short sleep duration or obstructive sleep apnea describe a clinically relevant phenotype.

Hyperarousal theory

Sympathetic activation is a key factor in the pathophysiology of cardiovascular diseases, and sleep disorders and insufficiencies may contribute to the development of AH, coronary artery disease, heart failure, and arrhythmias through this mechanism.

Jarrin et al (2018) (10) found that patients with IOSSD showed an increased nocturnal heart rate (HR), significantly dampened parasympathetic activation and increased sympathovagal imbalance relative to their counterparts with near normal sleep duration, suggesting cardiovascular autonomic dysfunction. Interestingly, when groups within the same population were formed based on subjective reportings, instead of objective measurements, no differences could be observed between phenotypes.

Cosgrave et al (2020) (11) investigated nocturnal HR and heart rate variability (HRV) in healthy young adult good and poor self-reported sleepers, objectively measuring sleep duration. Significant between-group differences in the way arousals are

distributed across sleep stages were observed. However, these differences were not disclosed when comparing the arousal indexes of the groups. The way the arousal index is calculated does not account for these differences in arousal distribution. It appears that the arousal index is not suited to express and compare these otherwise distinctive differences between groups. Poor sleepers were also found to have a significantly higher mean HR across Stages 2, 3, 4 and rapid eye movement sleep compared to the good sleepers. Interestingly, it was found that among their young and healthy participants only those with ISSD showed a noticeable decrease in HRV, another indication that this phenotype carries the most risk for cardiovascular autonomic dysfunction. Also, objective groupings revealed stronger differences than the original subjective groupings.

Greenlund et al (2022) (12) gave further credence to the hyperarousal model. They found that chronic insomnia has been associated with increased sympathetic activation via plasma norepinephrine and sympathoexcitation from microneurography studies, providing evidence for the hyperarousal theory of insomnia. Interestingly, epidemiological evidence suggests women to be more susceptible to sleep disorder-mediated CD. Studies that had included equal representation of men and women, or specifically focused their aims on sex differences showed that the complex relations between sleep disorders / insufficiencies and sympathetic activity are indeed impacted by sex.

Treatment options

Crönlein et al (2018) (13) examined the effectiveness of cognitive-behavioral therapy for insomnia (CBTI) in patients with short and normal sleep duration. CBTI was found to be equally effective in improving

sleep quality in patients with short sleep duration and those with normal sleep duration.

Vgontzas et al (2020) (14) compared the effects of trazodone and CBTI on patients with IOSSD. Trazodone appeared to lengthen total sleep time both at posttreatment (+ 51 minutes) and at follow-up (+ 50 minutes), respectively, whereas CBT-I did not improve or extend objective sleep duration. Trazodone was also found to reduce cortisol levels whereas CBT-I did not show this effect. However, the effect of trazodone on cortisol levels appeared to weaken over time.

Discussion

This systematic review highlights the association between IOSSD and various cardiovascular risk factors, including AH, inflammation, and cardiovascular complications, suggesting that IOSSD is a significant predictor of poor cardiovascular health outcomes.

The observed association between ISSD and AH is consistent with previous research and supports the notion that ISSD is a significant risk factor for AH. This well-established association with adverse changes in blood pressure regulation is a strong indication that ISSD may increase the risk of cardiovascular events. (1, 2, 3)

Recent research suggests that ISSD contributes to systemic inflammation. Elevated levels of inflammatory biomarkers were found to be associated with ISSD in several studies.

Chronic inflammation triggers the release of proinflammatory cytokines, which damage arteries, promote plaque formation, impair glucose metabolism, and cause dysfunction of adipose tissue, leading to CD and metabolic complications. This implies that ISSD may trigger the development of

cardiovascular and metabolic disease through inflammatory processes. (4, 5, 6, 7)

A growing amount of evidence directly linking IOSSD to cardiovascular complications and all-cause mortality is of significant concern. In this regard it is especially noteworthy that recent studies were only able to find this link in individuals who suffered from insomnia and short sleep duration at the same time. (8, 9) This strongly suggests that, when present in combination, insomnia and short sleep duration trigger a synergistic effect that significantly increases the risk for cardiovascular complications and all-cause mortality.

Moreover, based on the studies reviewed, it can be inferred that IOSSD is the most severe phenotype of insomnia when it comes to its impact on cardiovascular health. (1, 2, 3, 4, 8, 9, 10, 11) In fact, in almost all studies that made a distinction between the different phenotypes of insomnia, IOSSD was found to have the most pronounced effect on cardiovascular health outcomes. (1, 2, 3, 4, 6, 8, 9, 10, 11) Interestingly, in some studies, this subtype was the only one to show a significant difference compared to controls, highlighting its importance in predicting CD risk.

While to this day the mechanisms behind these adverse effects are not completely understood, recent research provides a lot of evidence supporting the hyperarousal theory in this context. The activation of the sympathetic nervous system and the release of adrenaline and noradrenaline increases HR, blood pressure, and leads to a state of physiological arousal that interferes with sleep. This is especially relevant during the night when the body is trying to rest. In addition, chronic activation of the sympathoadrenal system can lead to a state of hyperarousal, which can make it difficult for individuals with insomnia to fall asleep or stay asleep. This may be due in part to a dysregulation of

the body's stress response system, which can lead to increased sensitivity to stressors and difficulty in calming down. Reviewed studies found that patients who suffer from IOSSD show increased sympathetic activity, overall sympathovagal imbalance and exhibit cardiovascular autonomic dysfunction (10, 11, 12). Overall, findings suggest that the hyperarousal theory provides a promising framework for understanding the mechanisms underlying the link between sleep disorders and CD. However, further research is needed to explore this theory and to determine their relative contributions to the pathophysiology of IOSSD and CD.

The results of this review suggest that IOSSD is a potential target for interventions to prevent CD. Sleep hygiene interventions, cognitive-behavioral therapy for insomnia (CBT-I) (13), and pharmacological treatments for insomnia (14) could be useful for treating IOSSD. Future studies should further investigate the effectiveness of these interventions on reducing the risk of CD associated with IOSSD.

During this review we made some noteworthy observations that have important implications for the assessment and management of patients with IOSSD. Some studies found no significant or considerably smaller differences between groups when groups were based on subjective reporting but found significant and more explicit differences when groups were based on objective measurements (5, 10, 11). This indicates that objective measurements of sleep duration are critical in understanding the true impact of sleep on health outcomes. While subjective sleep duration reporting has been commonly used in clinical assessments, our review suggests that relying solely on such reports may not be sufficient to accurately assess the severity of sleep disturbances. Therefore, the use of objective measures, such as

actigraphy or polysomnography, should be considered in clinical practice for a more comprehensive evaluation of sleep disorders and their impact on cardiovascular health.

Additionally, some studies have found significant differences in susceptibility between genders (12) and certain ethnic groups (3). These findings suggest that future research in this area should explore potential gender and ethnic disparities in the assessment and treatment of IOSSD. This could help identify specific subgroups of patients who may require tailored interventions to optimize sleep quality and overall health outcomes.

Conclusion

Our review provides evidence that IOSSD is a significant predictor of poor cardiovascular health outcomes, including AH, inflammation, and cardiovascular complications. Healthcare providers should be aware of these associations when evaluating patients with IOSSD and consider interventions that may improve sleep quality and duration to reduce the risk of CD and its complications. Further research is needed to determine the underlying mechanisms that link IOSSD and CD and to develop effective interventions to reduce the risk of these outcomes.

References

1. Bathgate CJ, Fernandez-Mendoza J. Insomnia, short sleep duration, and high blood pressure: recent evidence and future directions for the prevention and management of hypertension. *Current hypertension reports*. 2018 Jun;20:1-0. <https://doi.org/10.1007/s11906-018-0850-6>
2. Li L, Gan Y, Zhou X, Jiang H, Zhao Y, Tian Q, He Y, Liu Q, Mei Q, Wu C, Lu Z. Insomnia and the risk of hypertension: a meta-analysis of prospective cohort studies. *Sleep Medicine Reviews*. 2021 Apr 1;56:101403. <https://doi.org/10.1016/j.smrv.2020.101403>
3. Johnson KA, Gordon CJ, Chapman JL, Hoyos CM, Marshall NS, Miller CB, Grunstein RR. The association of insomnia disorder characterised by objective short sleep duration with hypertension,

diabetes and body mass index: a systematic review and meta-analysis. *Sleep medicine reviews*. 2021 Oct 1;59:101456.

<https://doi.org/10.1016/j.smrv.2021.101456>

4. Fernandez-Mendoza J, Baker JH, Vgontzas AN, Gaines J, Liao D, Bixler EO. Insomnia symptoms with objective short sleep duration are associated with systemic inflammation in adolescents. *Brain, behavior, and immunity*. 2017 Mar 1;61:110-6. <https://doi.org/10.1016/j.bbi.2016.12.026>

5. Syauqy A, Hsu CY, Rau HH, Kurniawan AL, Chao JC. Association of sleep duration and insomnia symptoms with components of metabolic syndrome and inflammation in middle-aged and older adults with metabolic syndrome in Taiwan. *Nutrients*. 2019 Aug 9;11(8):1848.

<https://doi.org/10.3390/nu11081848>

6. Wang J, Wu X, Liang W, Chen M, Zhao C, Wang X. Objective short sleep duration is related to the peripheral inflammasome dysregulation in patients with chronic insomnia. *Nature and Science of Sleep*. 2020 Oct 19:759-66.

<https://doi.org/10.2147/NSS.S270045>

7. Wang J, Chen X, Men X, Chen M, Tao J, Lu Z. Chronic insomnia is associated with higher circulating interleukin-8 in patients with atherosclerotic cerebral small vessel disease. *Nature and Science of Sleep*. 2020 Feb 11:93-9.

<https://doi.org/10.2147/NSS.S239030>

8. Bertisch SM, Pollock BD, Mittleman MA, Buysse DJ, Bazzano LA, Gottlieb DJ, Redline S. Insomnia with objective short sleep duration and risk of incident cardiovascular disease and all-cause mortality: Sleep Heart Health Study. *Sleep*. 2018 Jun;41(6):zsy047.

<https://doi.org/10.1093/sleep/zsy047>

9. Sigurdardottir FD, Bertisch SM, Reid ML, deFilippi CR, Lima JA, Redline S, Omland T. Association between insomnia phenotypes and subclinical myocardial injury: the Multi-Ethnic Study of Atherosclerosis. *Sleep*. 2023 Apr;46(4):zsac318. <https://doi.org/10.1093/sleep/zsac318>

10. Jarrin DC, Ivers H, Lamy M, Chen IY, Harvey AG, Morin CM. Cardiovascular autonomic dysfunction in insomnia patients with objective short sleep duration. *Journal of sleep research*. 2018 Jun;27(3):e12663. <https://doi.org/10.1111/jsr.12663>

11. Cosgrave J, Phillips J, Haines R, Foster RG, Steinsaltz D, Wulff K. Revisiting nocturnal heart rate and heart rate variability in insomnia: A polysomnography-based comparison of young self-reported good and poor sleepers. *Journal of Sleep Research*. 2021 Aug;30(4):e13278. <https://doi.org/10.1111/jsr.13278>
12. Greenlund IM, Carter JR. Sympathetic neural responses to sleep disorders and insufficiencies. *American Journal of Physiology-Heart and Circulatory Physiology*. 2022 Feb 1;322(3):H337-49. <https://doi.org/10.1152/ajpheart.00590.2021>

13. Crönlein T, Wetter TC, Rupperecht R, Spiegelhalder K. Cognitive behavioral treatment for insomnia is

equally effective in insomnia patients with objective short and normal sleep duration. *Sleep medicine*. 2020 Feb 1;66:271-5.
<https://doi.org/10.1016/j.sleep.2018.10.038>
14. Vgontzas AN, Puzino K, Fernandez-Mendoza J, Krishnamurthy VB, Basta M, Bixler EO. Effects of

trazodone versus cognitive behavioral therapy in the insomnia with short sleep duration phenotype: a preliminary study. *Journal of Clinical Sleep Medicine*. 2020 Dec 15;16(12):2009-19.
<https://doi.org/10.5664/jcsm.8740>

Physiological Demands and Pathophysiological Problems in Ultramarathon Running – a Literature Review

Nikolay Mandadzhiev¹, Katerina Koleva¹, Stoilka Mandadzhieva²

1. Student in Medical University of Plovdiv

2. Department of Pathophysiology, Faculty of Medicine, Medical University of Plovdiv

Introduction: An ultramarathon is a running event exceeding the ‘classical’ distance of 42.125 kilometers. Over the last decade the sport has seen a steady rise in popularity in Bulgaria and currently during the active season a minimum of two big events are hosted every month. **Aim:** The aim of this review is to familiarize medical specialists with the physiological demands of the sport during training and competition, and to focus on the underlying pathophysiology and treatment of the most common medical problems that occur during races. **Materials and methods:** The body of evidence on the topic has been increasing in correlation with the rising popularity of the sport. Three databases were searched for this review – PUBMED, Google Scholar and Science Direct. **Results and discussion:** The major physiological changes that occur during ultra-endurance events are fairly stereotypical and affect the respiratory, cardiovascular, muscular and metabolic systems. The specific physiological demands are related to the low intensity and long duration of the races, which often exceed 4 and last up to 25–30 hours, depending on the distance, level of the athlete, terrain, weather, etc. The most common medical conditions that affect participants, include, but are not limited to: musculoskeletal injuries, GI tract disturbances, heat stroke and heat exhaustion, hypothermia, exercise-associated hyponatremia, blisters, chafing, etc. **Conclusion:** The understanding of the underlying physiology and pathophysiology of ultramarathon running is essential for the effective medical coverage of races and for ensuring the health and longevity of every athlete in the sport.

Keywords: *ultramarathon, running, physiology, pathophysiology*

1. Introduction

An ultramarathon is a running event that exceeds the ‘classical’ marathon distance of 42.195 kilometres. Pheidippides is the central figure of the legend, that gave birth to this modern sport. According to it, when the Greeks defeated the Persians at the battle of Marathon, he ran the distance from the battlefield to Athens, to announce the victory, then collapsed and passed away. What is often omitted from this romantic story is the part where he ran from Athens to Sparta and back, the days before the infamous battle – a total distance of 480 kilometres. 2000 years later the Olympic marathon was born, and we can safely assume Pheidippides is not only the grandfather of the marathon, but also of the ultramarathon. His journey and death were immortalised in one of the most famous ultra races – the Spartathlon (1).

Generally most single-stage races are between the distances of 50 to 200 km, with a duration of 6 to 48 hours (2). The popularity of trail running and ultramarathons has

grown significantly in the past decade in Bulgaria, evidenced by the number of different races, most notably during the summer (3). That has necessitated the expansion of studies and literature that relate to the physiological adaptations to the sport and the medical complications that can occur during training and racing.

To adequately understand the physiological and pathophysiological demands and changes that result from ultramarathon racing we have to mention the great variety of environmental factors that accompany them, mainly, the wide amplitude of temperatures, duration, elevation gain and loss, altitude, humidity, weather changes, etc.

2. Aims

The general aim of this review is to compile the available and most up-to-date information regarding the physiology of ultramarathons, specifically the respiratory, cardiovascular, renal, metabolic and muscular demands and changes, and to further

extrapolate the most common medical complications and the underlying pathophysiology of the imbalance between these systems.

3. Methods and results

For the purpose of this review the medical databases PubMed, Google Scholar and Science Direct were searched with several combinations of the following terms: ‘ultramara-thon’, ‘physiology’, ‘pathophysiology’, ‘en-urance’, ‘cardiovascular’, ‘respiratory’, ‘metabolism’, ‘nutrition’, ‘hyponatremia’, ‘GI distress’, ‘rhabdomyolysis’, ‘injury’, ‘acute kidney injury’. The search yielded 40 articles, including case reports, original re-search, reviews, systematic reviews, and meta-analyses. Additionally, two websites and two books were used.

4. Discussion

4.1. Physiological changes and demands

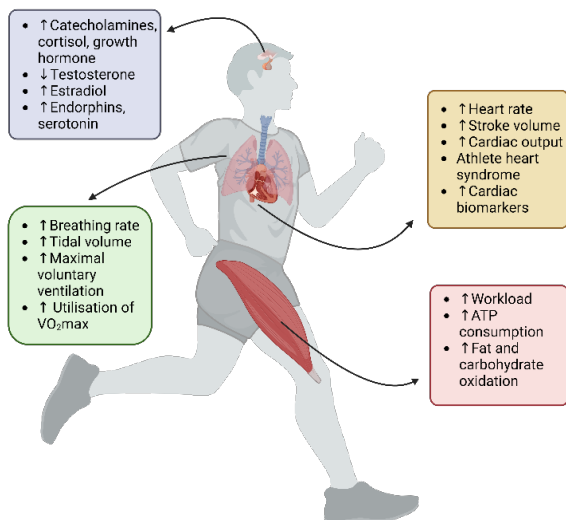


Figure 1. Physiological changes

Respiratory system

The changes that are observed in the respiratory system during any aerobic physical activity are fairly stereotypical and have been thoroughly investigated. There is some variability depending on the intensity of

exercise, which help us determine the differences in various types of running races from short to long, to ultra distances, but nevertheless we see a coherent model of change in respiration, typical for running.

The main concepts that we use to describe respiration are the various lung volumes and capacities. Normally during rest breathing rate is at a mean value of 12 per minute and tidal volume (TV) is in the range of 0.5 litres, which amounts to a minute ventilation of 6 litres. Maximum voluntary ventilation measurements per minute have shown a capacity to reach 100 litres for the general population and up to 200 litres for trained endurance athletes. This goes to show that the respiratory system is well capable of maintaining moderate aerobic physical activity even in the untrained general population (4).

At the starting point of any aerobic exercise lies the increased muscle workload that is needed to move the body, which is intricately connected with the respiratory system. The actively working muscles firstly need the activation of a large number of locomotor neurons which ultimately leads to mass activation in the cerebral cortex. Secondly, the same muscles need a significant amount of energy in the form of ATP, which at low intensity rates, typical for ultramarathons, require the aerobic burning of fat and carbohydrates, ultimately leading to an increased need of oxygen delivery and carbon dioxide removal. This is where above normal activation of the respiratory system is needed to adequately answer to those needs, and this process is comprised of three phases. In phase I the combination of central and peripheral neurogenic stimuli to the centre of breathing in the medulla leads to abruptly increased ventilation – a combined surge in breathing rate and tidal volume. In phase II minute ventilation rises exponentially, reaching a steady state of activation,

which is mainly maintained by the continuing peripheral neurogenic stimuli and the additive effect of chemical stimulation, as a product of increased metabolism. In phase III the steady ventilatory state is fine tuned to the increasing metabolic and chemical by-products of exercise – mainly carbon dioxide and H⁺ concentration, and lactate to a lesser extent (4). Maintaining a high volume of phase III steady state ventilation is a typical trait of ultramarathons, generally as a result of long duration of time on feet and interspersed bouts of higher intensity running, specifically in races with high elevation gain and loss. This has prompted the hypothesis that a decrease in lung function could be expected after such an event and it has been investigated with positive result, where a significant number of studies have shown that to be mainly a combination of inspiratory and expiratory muscle fatigue (5, 6). In some cases lower airway inflammation as a result of ultra endurance running has also been observed and the main mechanism of that is implied to be exercise-induced bronchoconstriction (7). Lastly, in some studies pulmonary edema has been observed after ultramarathon races which were held at high altitude (8). The conclusion that we can draw from these findings is that lung function impairments should be expected and well managed during ultramarathon races.

Cardiovascular system

In endurance exercise the heart sees a typical activation process in accordance with that of the respiratory system – the oxygen, supplied by the lungs, must reach the active workforce – the muscles. The onset of a running activity is met with the following response from the cardiovascular system: the vessels of active musculature are dilated through primarily local chemical mechanisms, namely the release of NO from the endothelium; vasculature in other areas of

the body, that are inactive, is constricted, which leads to decreased venous pooling and increased venous flow to the right atrium; heart rate is markedly increased and for steady-rate running it often reaches 70–80% of maximal heart rate which is generally perceived as moderate intensity – typical for ultramarathons; stroke volume is also markedly increased and ranging anywhere between 100 and 200 ml, mainly due to the aforementioned increased preload and more specifically owing to the Frank-Starling mechanism; all of these changes lead to increased cardiac output as an adequate response to increased workload and it can reach values ranging between 20 to 40 litres per minute, depending on the aerobic fitness of athletes (4, 9). Repeating such aerobic activity in training leads to well documented adaptations comprising the athlete heart syndrome which includes rhythm changes, namely resting bradycardia, augmented vagal tone, left ventricular hypertrophy and increased ventricular and atrial dimensions (10). Maintaining such a level of cardiac activity for between 4 and 30 hours is specific for ultramarathon races and it has been hypothesized that this could lead to impaired cardiac function, based on documented increased levels of cardiac biomarkers in peripheral blood post-race (11). However conclusive evidence on the risk for myocardial injury and dysfunction is still elusive and studies have shown mixed results depending on the duration and intensity of races, where longer and lower in intensity races show lower levels of impaired cardiac function or none at all (6, 12).

Skeletal musculature

Physiologically muscles are described as consisting of two main types of fibre – slow twitch (type I) and fast twitch (type II), which differentiate significantly in their contractability and energetic resource. As in

every endurance sport, in ultramarathon running the predominant muscle fibre type is the slow-twitch fibre, comprising up to 95% of the most actively trained muscle mass (4, 13). Nevertheless, there is no quantitative or qualitative difference in muscle structure, metabolism or function that differentiates ultra endurance exercise from endurance exercise in general.

Endocrine system

The response of the endocrine system at the onset of exercise is stereotypical for endurance exercises of most types. The bout of physical activity is considered as a stressor and the subsequent release of hormones is meant to facilitate the adequate reaction to it. The pituitary gland increases its secretion of growth hormone and ACTH which have an important role in carbohydrate, protein and fat metabolism, and simultaneously releases AVP (ADH) as a response to increased sweating and dehydration. Prolactin is also increased in endurance exercise, which can have a detrimental effect to the menstrual cycle for women who overtrain. Of the thyroid hormones, mainly the level of free T4 is increased. Adrenal hormones, both medullar and cortical, see the most drastic level changes in physical exercise, as they are directly related physiologically to cardiac output, arterial blood pressure, energy expenditure and metabolism. Epinephrine and norepinephrine are both increased, where their levels are intensity-dependent and differ at different levels of VO_2max . Aldosterone levels are elevated, owing mainly to the renin-angiotensin system and cortisol secretion is stimulated through the higher concentration of ACTH. Gonadal hormones, though not directly metabolically active, are also affected. In women estradiol and progesterone levels are increased, and in men testosterone levels have shown a varying change depending on exercise type and intensity, so the

consensus is still equivocal (4). To summarise, more specifically for ultra-endurance exercise are observed increased blood concentrations of growth hormone, ACTH, AVP, epinephrine, norepinephrine, cortisol, aldosterone, estradiol and progesterone, whereas clinically relevant decrease has been noted only for testosterone (2, 14).

Energy expenditure and metabolic requirements

Intuitively ultramarathon running can be perceived as an activity that has extreme levels of energy expended for a given distance. For non-scientific purposes the formula 1 kcal expended per kilogram of body mass per kilometre of level running can give us a general estimation – for an average runner of 75 kg body weight, running a 100 km ultramarathon, the expenditure would be around 7500 kcal. If we assume that mountain ultramarathons of the same distance include on average around 5000 metres of elevation gain, we can add that every 100 m of elevation gained are roughly equal to an additional 1 kilometre of level running, or an average mountain ultramarathon is around 1.5 times as energetically requiring as a level race of the same distance (4, 15). The most widely used scientific methods for measuring energy expenditure are doubly-labelled water method, direct and indirect calorimetry, however their incorporation in on-field studies on ultramarathon running has not been sufficient or possible to produce enough conclusive data (16).

Generally, for any type of endurance exercise the two main energetic resources are fat and carbohydrates, with endogenous stores of fat being estimated as more than 100 000 kcal for an average athlete, while carbohydrates in the form of muscle and liver glycogen are calculated at around 3000 kcal (17). Numerous studies have shown that the oxidation ratio of fat to carbohydrates is

dependent on the exercise intensity, most commonly described as a percentage of VO_2max , and logically in ultramarathon running as a lower-intensity type endurance activity ($< 70\% \text{VO}_2\text{max}$) free fatty acids (FFA) have been observed as the predominantly oxidized fuel in a 70:30 percentage ratio in the latter's favour (13). Nevertheless, the role of glucose is crucial even at low intensities, when endogenous stores are depleted and carbohydrates are not exogenously supplemented, especially in mountain ultramarathons, where bouts of high intensity running are interspersed throughout slower running, owing to the widely varying terrain (18, 19). Current recommendations for carbohydrate intake during racing propose consuming between 60 to 90 grams per hour of multiple transportable carbohydrates, i.e., a mixture of glucose and fructose in ratios between 2:1 and 1:0,8 in favour of glucose. Higher quantities of carbohydrate consumption have been linked with more frequent symptoms of GI distress and are not deemed beneficial (20).

4.2. Pathophysiological problems

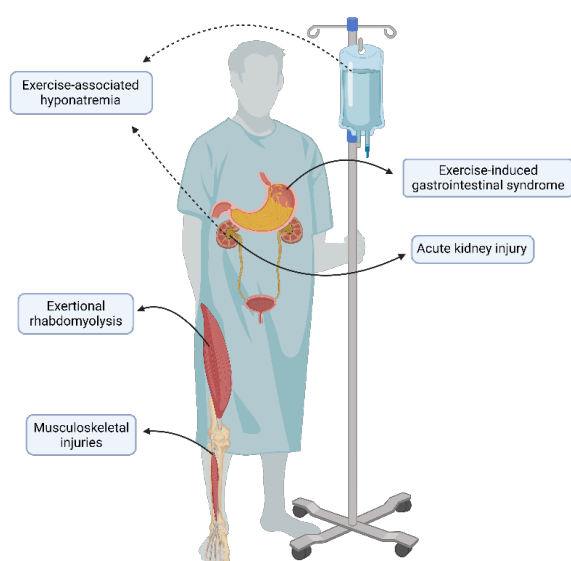


Figure 2. Pathophysiological problems

Exercise-associated hyponatremia

Exercise-associated hyponatremia (EAH) is a common problem for endurance and ultra-endurance events and was once exclusively related to ultramarathons (21). It is described as a plasma sodium level below 135 mmol/l as a result of prolonged aerobic exercise and includes symptoms of cramping, dizziness and weakness to seizures, altered mental status, cerebral and pulmonary edema, coma, and death (2, 22). The pathogenesis requires a sequence of events that happen exclusively during physical activity: increased muscle workload produces substantial amounts of heat that is dissipated mainly through sweating; the increased sweat rate results in a fairly equilibrated loss of water and electrolytes; the dehydration is perceived as thirst and the athlete consumes large quantities of hypotonic fluid without adequately substituting the lost electrolytes which leads to hyperhydration and relative hyponatremia; dehydration also is a signal for the secretion of AVP which leads to water retention by blocking the excretion of urine in the collecting ducts. Another mechanism involved in the excess production of hypotonic liquid is the metabolism of glycogen, which specifically plays an adjuvant role in ultramarathons where most of the liver and muscle glycogen storage is depleted (23, 24). Other hypothesized mechanisms are related to the secretion of IL-6, which happens often with rhabdomyolysis – a problem that is specific to ultra endurance exercise, and that is believed to further activate the secretion of AVP by non-osmotic pathways (25). The most effective measure against EAH is prevention, mostly by education about the condition and its consequences. Physicians as part of the medical coverage teams should be attentive to the environmental factor of ultramarathon races, namely extreme heat and humidity. Athletes

should learn appropriate rehydration and cooling strategies, avoid excessive fluid repletion, which is best done to individually calculated sweat rates, and supplement sodium orally. The specific treatment of symptomatic EAH consists of intravenous administration of hypertonic saline by the following regimen: 100 ml of 3% saline administered every 10 minutes until resolution of encephalopathy symptoms (25).

Gastrointestinal distress

Gastrointestinal distress symptoms are a commonality for runners and endurance athletes and are a frequent symptom in long training runs, marathon and ultramarathon races. They are also one of the most common reasons for poor race performance and race non-completion (26). Most runners present symptoms of nausea, bloating, abdominal cramps, vomiting, diarrhoea and faecal incontinence (22). The appearance of GI distress has also been termed as exercise-induced gastrointestinal syndrome (EIGS). Its pathophysiology is multifactorial but generally consists of two main mechanisms: the onset of steady state exercise is followed by redistribution of blood flow to the active musculature which in turn leads to vasoconstriction and hypoperfusion in the splanchnic region, termed circulatory-gastrointestinal pathway (CGP); the start of strenuous physical activity is also accompanied by a general surge in sympathetic activity and release of stress hormones which affects negatively general intestinal functionality, termed neuroendocrine-gastrointestinal pathway (NGP). The main consequence of the CGP is splanchnic ischemia which leads to epithelial dysfunction – intestinal permeability is enhanced, followed by bacterial translocation and an increase in circulatory endotoxin levels. The NGP is the main reason for a decrease in intestinal motility, delayed gastric

emptying and impaired absorptive transport mechanisms, leading to malabsorption (27). EIGS is one of if not the most exclusively specific condition related to ultramarathon racing. One of the reasons for its high prevalence is the extreme total amount of energy expenditure reaching up to 15–20 000 kcal for a typical 160 km ultramarathon and which requires a very aggressive fuelling strategy during a race as an ergogenic aid to performance (28, 29). The sheer quantities of food and fluid that must be ingested can lead to bloating, flatulence, nausea and vomiting, while non-compliance to the fuelling strategy can also be detrimental – leading to dehydration, that aggravates splanchnic hypoperfusion, and hypoglycaemia (26). Medical coverage at races should be prepared to face the consequences of EIGS, however a specific treatment regimen has not been yet described. The general principles of management are directed at the underlying pathophysiology and include: cessation of exercise or lowering exercise intensity; maintaining an euhydrated state and avoiding the consumption of hyper- and hypotonic fluids; consuming carbohydrates up to 45 g/h as it has been beneficial in ameliorating symptoms in some studies; avoidance of NSAIDs and COX-inhibitors (30). Some general strategies that athletes should follow are: starting exercise and racing in an euhydrated state and maintaining proper hydration levels by ingesting appropriate amounts of isotonic fluids and adequate amounts of carbohydrates, generally between 45–90 g/h; following a gut-training protocol, i.e., practicing specific fuelling strategies during training exclusively, and following the most empirically beneficial plan in racing (27).

Acute kidney injury

Acute kidney injury (AKI) describes a sudden loss of kidney function that is

determined on the basis of increased serum creatinine levels (a marker of kidney excretory function) and reduced urinary output (oliguria) (a quantitative marker of urine production) and is limited to a duration of 7 days (31). The condition has been observed regularly in different studies after ultramarathon races, but there is no consensus on its prevalence, which is ranging from 0% to 85% (32). AKI is often asymptomatic in the stages of injury and remains undiagnosed in most cases (7). The most common biomarkers that are used for diagnosis are serum creatinine levels and eGFR calculations, but some studies have also used novel biomarkers like cystatin C, neutrophil gelatinase-associated lipocalin and kidney injury molecule-1 (33, 34). The underlying pathophysiology of AKI in endurance exercise is multifactorial and several mechanisms and risk factors have been proposed: as previously mentioned, the increased blood flow to the muscles leads to vasoconstriction in the splanchnic region, which ultimately leads to decreased renal blood flow, where the resultant ischemia, when extremely prolonged, as in ultramarathons, can be associated with glomerular and tubular damage; rhabdomyolysis, which frequently occurs with extreme endurance exercise, is a main factor for the release of myoglobin in the bloodstream, which can have a direct toxic effect on the tubules or obstruct them mechanically; dehydration from racing in extreme heat and humidity can exacerbate the effect of renal vasoconstriction; NSAIDs are probably the most important risk factor specifically in ultramarathons, where their usage between participants is highly prevalent, and pathophysiologically act by diminishing the intrinsic glomerular production of prostaglandins, which are the main mechanism for maintaining adequate kidney blood flow in the conditions of endurance exercise (32,

33). Concerning the asymptomatic nature of AKI, medical coverage at races should focus on the accompanying risk factors, their prevention and the education of athletes on early signs of rhabdomyolysis and diminished kidney function. Alertness about the environmental risk factors and their effects should also be of consideration.

Exertional rhabdomyolysis

Rhabdomyolysis is literally defined as dissolution of striated muscle. It is caused by the breakdown and necrosis of muscle tissue and the release of intracellular content into the blood stream (35). When the cause of muscle damage is strenuous physical activity it is defined as exertional rhabdomyolysis (ER) (36). The underlying pathophysiology of rhabdomyolysis has been investigated thoroughly and a satisfactory explanation has been established. During rest the muscle cell homeostasis is largely directed by the action of the Na-K-ATP-ase pump and the Na-Ca exchanger, which secure a high intracellular concentration of potassium and low of sodium and calcium. At the onset of physical activity when muscle contractions are needed, there is a surge in Ca concentration in the sarcoplasm which along with ATP, actively act in the process of actin-myosin linking and thus, contraction (37). The main mechanism that leads to rhabdomyolysis is damage to the sarcolemma and the direct underlying cause is the increased intracellular concentration of Ca ions which activate proteolytic enzymes, ultimately leading to myocyte disintegration (35). Sarcolemmal injury can be multifactorial and the most important factors are depletion of ATP and direct destruction through trauma or toxins. The specificity of ER to ultramarathons is associated with both of these factors – the extreme energy expenditure can lead to ATP depletion in certain muscle groups, whereas

muscle damage can occur in part because of the multi-hour duration of races or also due to repetitive eccentric contractions, which are typical for downhill running in races with multiple elevation changes (38, 39). Additionally, environmental factors like heat and humidity, and the resulting dehydration, can compound the effect of physical activity. The result of ER is the release of intracellular myocytic proteins like creatine kinase (CK), lactate dehydrogenase (LDH), aspartate and alanine transaminase (AST, ALT), myoglobin, alongside with intracellular ions, most importantly potassium and phosphorus. The increased levels of these proteins and ions in the blood are the main diagnostic laboratory markers (40). The clinical presentation of ER is a complicated problem, where in most patients only asymptomatic elevation of CK, LDH, and myoglobin levels is observed, in 10% is present the triad of muscle pain, weakness and dark-coloured urine, and in a small subset there can be severe complications like acute kidney injury, compartmental syndrome and cardiovascular abnormalities (35, 40). Generally the data on the prevalence of ER in ultrarunners is incomplete, owing to the high amount of asymptomatic cases, which leads to the conclusion that severe cases as a result of ultra endurance racing are not frequent (38, 41). However, medical coverage at races should be attentive to the symptomatic athletes, as the consequences of ER can be life-threatening. Treatment depends on the severity of symptoms: in mild cases, rest and oral rehydration in an outpatient setting are sufficient; in symptomatic athletes or when levels of CK are five times above the upper reference value, hospitalization should be considered, where in the appropriate setting it is easier to perform venous rehydration and tracking of CK and electrolyte levels (42).

Musculoskeletal injuries

Injuries to muscles, tendons and cartilage are a typical part of most sports and ultramarathon runners are not spared. What's more, running is associated more often with such types of damage, because of the great physical load that it exerts on the body – the continuous ‘pounding’ of the feet on the earth. Logically, injuries affect almost exclusively the lower limb, with the ankle and knee most susceptible. There is a clear difference between experienced and novice runners, where in the latter injuries occur more often due to the combination of worse running technique and economy and weaker musculoskeletal apparatus (43). By mechanism injuries in running are overuse injuries and often include mechanical damage to tissues with subsequent inflammatory changes. The most prevalent injuries reported due to ultramarathon running are the following: femoropatellar syndrome in the knee; Achilles tendinitis and peritendinitis of the extensor muscle tendons in the anterior of the ankle (also termed ‘ultramarathoner’s ankle’); medial tibial stress syndrome (shin splints) and exercise-induced compartmental syndrome in the lower leg (2, 44). The most widely used method for treatment is the RICE (rest, ice, compression, elevation) protocol, although the severity of the injury may merit a consultation with physical therapy and sports medicine specialists for more specific treatment, including surgery (44).

5. Conclusion

Ultra-endurance exercise, particularly ultramarathon running, has grown exponentially in popularity in the last twenty years. The growing number of participants in such events, has made available a large and still growing group of recreational and professional athletes, available for the scientific

exploration of the physiologic and patho-physiologic effects of this extreme endurance feat. Our review compiled a sample of the most recent studies to show that although there seem to be no particular physiologic adaptations specific to ultra-endurance exercise, there are some clear medical complications that are exclusively related to this sport, namely – hyponatremia, exercise-induced gastrointestinal distress and rhabdomyolysis. Nevertheless, this review is by no means as comprehensive and detailed as the topic suggests and further investigation on the long-term effects, adaptations and complications of the sport will be needed, especially in Bulgarian athletes.

References

1. <https://en.wikipedia.org/wiki/Pheidippides>.
2. Knechtle B, Nikolaidis PT. Physiology and Pathophysiology in Ultra-Marathon Running. *Front Physiol*. 2018 Jun 1;9:634.
3. <https://racecalendar.bg/>.
4. McArdle WD, Katch FI, Katch VL. Exercise Physiology: Nutrition, Energy, and Human Performance. Wolters Kluwer Health/Lippincott Williams & Wilkins; 2015. 1028 p.
5. Tiller NB. Pulmonary and Respiratory Muscle Function in Response to Marathon and Ultra-Marathon Running: A Review. *Sports Med*. 2019 Jul;49(7):1031–41.
6. Martínez-Navarro I, Sánchez-Gómez JM, Aparicio I, Priego-Quesada JI, Pérez-Soriano P, Collado E, et al. Effect of mountain ultramarathon distance competition on biochemical variables, respiratory and lower-limb fatigue. Boulosa D, editor. *PLOS ONE*. 2020 Sep 11;15(9):e0238846.
7. Scheer V, Tiller NB, Doutreleau S, Khodaei M, Knechtle B, Pasternak A, et al. Potential Long-Term Health Problems Associated with Ultra-Endurance Running: A Narrative Review. *Sports Med*. 2022 Apr;52(4):725–40.
8. Tiller NB, Stewart GM, Illidi CR, Levine BD. Exercise Is Medicine? The Cardiorespiratory Implications of Ultra-marathon. *Curr Sports Med Rep*. 2020 Aug;19(8):290–7.
9. Mahler DA, Loke J. The Physiology of Marathon Running. *Phys Sportsmed*. 1985 Jan;13(1):84–97.
10. Thompson PD. Cardiovascular Adaptations to Marathon Running: The Marathoner's Heart. *Sports Med*. 2007;37(4):444–7.
11. Żebrowska A, Waśkiewicz Z, Nikolaidis PT, Mikołajczyk R, Kawecki D, Rosemann T, et al. Acute Responses of Novel Cardiac Biomarkers to a 24-h Ultra-Marathon. *J Clin Med*. 2019 Jan 8;8(1):57.
12. Scott JM, Esch BTA, Shave R, Warburton DER, Gaze D, George K. Cardiovascular Consequences of Completing a 160-km Ultramarathon. *Med Sci Sports Exerc*. 2009 Jan;41(1):25–33.
13. Davies CT, Thompson MW. Physiological responses to prolonged exercise in ultramarathon athletes. *J Appl Physiol*. 1986 Aug 1;61(2):611–7.
14. Bird SR, Linden M, Hawley JA. Acute changes to biomarkers as a consequence of prolonged strenuous running. *Ann Clin Biochem Int J Lab Med*. 2014 Mar;51(2):137–50.
15. Koop J. Training Essentials for Ultrarunning: How to Train Smarter, Race Faster, and Maximize Your Ultramarathon Performance. VeloPress; 2016. 378 p.
16. Ndahimana D, Kim EK. Measurement Methods for Physical Activity and Energy Expenditure: a Review. *Clin Nutr Res*. 2017;6(2):68.
17. Alghannam AF, Ghaith MM, Alhussain MH. Regulation of Energy Substrate Metabolism in Endurance Exercise. *Int J Environ Res Public Health*. 2021 Jan;18(9):4963.
18. Urdampilleta A, Arribalzaga S, Viribay A, Castañeda-Babarro A, Seco-Calvo J, Mielgo-Ayuso J. Effects of 120 vs. 60 and 90 g/h Carbohydrate Intake during a Trail Marathon on Neuromuscular Function and High Intensity Run Capacity Recovery. *Nutrients*. 2020 Jul;12(7):2094.
19. Costa RJS, Knechtle B, Tarnopolsky M, Hoffman MD. Nutrition for Ultramarathon Running: Trail, Track, and Road. *Int J Sport Nutr Exerc Metab*. 2019 Mar 1;29(2):130–40.
20. Tiller NB, Roberts JD, Beasley L, Chapman S, Pinto JM, Smith L, et al. International Society of Sports Nutrition Position Stand: nutritional considerations for single-stage ultra-marathon training and racing. *J Int Soc Sports Nutr*. 2019 Jan 15;16(1):50.
21. Frizzell RT, Lang GH, Lowance DC, Lathan SR. Hyponatremia and Ultramarathon Running.
22. Sanchez LD, Corwell B, Berkoff D. Medical problems of marathon runners. *Am J Emerg Med*. 2006 Sep;24(5):608–15.
23. Baldwin KM, Fitts RH, Booth FW, Winder WW, Holloszy JO. Depletion of muscle and liver glycogen during exercise. *Pflug Arch*. 1975 Sep 1;354(3):203–12.
24. Gonzalez JT, Fuchs CJ, Betts JA, van Loon LJC. Liver glycogen metabolism during and after prolonged endurance-type exercise. *Am J Physiol-Endocrinol Metab*. 2016 Sep;311(3):E543–53.
25. Hew-Butler T, Loi V, Pani A, Rosner MH. Exercise-Associated Hyponatremia: 2017 Update. *Front Med [Internet]*. 2017 [cited 2023 Apr 17];4. Available from: www.frontiersin.org/articles/10.3389/fmed.2017.00021
26. Stuempfle KJ, Hoffman MD, Hew-Butler T. Association of Gastrointestinal Distress in Ultramarathoners With Race Diet. *Int J Sport Nutr Exerc Metab*. 2013 Apr 1;23(2):103–9.

27. Costa RJS, Snipe RMJ, Kitic CM, Gibson PR. Systematic review: exercise-induced gastrointestinal syndrome—implications for health and intestinal disease. *Aliment Pharmacol Ther.* 2017;46(3):246–65.
28. Stuempfle KJ, Hoffman MD. Gastrointestinal distress is common during a 161-km ultramarathon. *J Sports Sci.* 2015 Oct 21;33(17):1814–21.
29. Rontoyannis G, Skoulis T, Pavlou K. Energy balance in ultramarathon running. *Am J Clin Nutr.* 1989 Jun 1;49:976–9.
30. ter Steege RWF, Kolkman JJ. Review article: the pathophysiology and management of gastrointestinal symptoms during physical exercise, and the role of splanchnic blood flow. *Aliment Pharmacol Ther.* 2012;35(5):516–28.
31. Kellum JA, Romagnani P, Ashuntantang G, Ronco C, Zarbock A, Anders HJ. Acute kidney injury. *Nat Rev Dis Primer.* 2021 Jul 15;7(1):1–17.
32. Tidmas V, Brazier J, Bottoms L, Muniz D, Desai T, Hawkins J, et al. Ultra-Endurance Participation and Acute Kidney Injury: A Narrative Review. *Int J Environ Res Public Health.* 2022 Dec 15;19(24):16887.
33. Hodgson L, Walter E, Venn R, Galloway R, Pitsiladis Y, Sardat F, et al. Acute kidney injury associated with endurance events—is it a cause for concern? A systematic review. *BMJ Open Sport Exerc Med.* 2017 May;3(1):e000093.
34. Poussel M, Touzé C, Allado E, Frimat L, Hily O, Thilly N, et al. Ultramarathon and Renal Function: Does Exercise-Induced Acute Kidney Injury Really Exist in Common Conditions? *Front Sports Act Living* [Internet]. 2020 [cited 2023 Apr 24];1. Available from: <https://www.frontiersin.org/articles/10.3389/fspor.2019.00071>
35. Cabral BMI, Edding SN, Portocarrero JP, Lerma EV. Rhabdomyolysis. *Dis Mon.* 2020 Aug;66(8):101015.
36. Rawson ES, Clarkson PM, Tarnopolsky MA. Perspectives on Exertional Rhabdomyolysis. *Sports Med.* 2017 Mar 1;47(1):33–49.
37. Torres PA, Helmstetter JA, Kaye AM, Kaye AD. Rhabdomyolysis: Pathogenesis, Diagnosis, and Treatment. *Ochsner J.* 2015;15(1):58–69.
38. Skenderi KP, Kavouras SA, Anastasiou CA, Yiannakouris N, Matalas AL. Exertional Rhabdomyolysis during a 246-km Continuous Running Race. *Med Sci Sports Exerc.* 2006 Jun;38(6):1054–7.
39. Eston RG, Mickleborough J, Baltzopoulos V. Eccentric activation and muscle damage: biomechanical and physiological considerations during downhill running. *Br J Sports Med.* 1995 Jun 1;29(2):89–94.
40. Patel DR, Gyamfi R, Torres A. Exertional Rhabdomyolysis and Acute Kidney Injury. *Phys Sportsmed.* 2009 Apr;37(1):71–9.
41. Bruso JR, Hoffman MD, Rogers IR, Lee L, Towle G, Hew-Butler T. Rhabdomyolysis and Hyponatremia: A Cluster of Five Cases at the 161-km 2009 Western States Endurance Run. *Wilderness Environ Med.* 2010 Dec 1;21(4):303–8.
42. Tietze DC, Borchers J. Exertional Rhabdomyolysis in the Athlete: A Clinical Review. *Sports Health Multidiscip Approach.* 2014 Jul;6(4):336–9.
43. Stöggl T, Wunsch T. Biomechanics of Marathon Running. In: Zinner C, Sperlich B, editors. *Marathon Running: Physiology, Psychology, Nutrition and Training Aspects* [Internet]. Cham: Springer International Publishing; 2016 [cited 2023 May 3]. p. 13–45. Available from: https://doi.org/10.1007/978-3-319-29728-6_2
44. Spittler J, Oberle L. Current Trends in Ultramarathon Running. *Curr Sports Med Rep.* 2019 Nov;18(11):387–93.

Corresponding author

Nikolay Mandadzhiev
 Belitsa 15, Plovdiv, Bulgaria
 E-mail: nikmann16@gmail.com
 Tel.: +359882283323

Signs of Early and Established Rheumatoid Arthritis and Their Treatment Outcomes

Maritza Chtere¹, Abdulkadir Farah Abdi¹, Vaishnav Krishna¹, Plamen Todorov²

1. Faculty of Medicine, Medical University of Plovdiv

2. Department of Internal Medicine Propaedeutic and Rheumatology, Faculty of Medicine, Medical University of Plovdiv

Introduction: Rheumatoid arthritis is a progressive autoimmune disease, affecting primarily the peripheral synovial joints, characterized by inflammation and pannus formation, that results in pain, structural deformities, and disability. The distinction between early and established RA is based on the duration of symptoms and signs; early RA has a duration of less than 3 months and established RA with a duration of more than 3 months. **Objective:** This paper explores the differences and similarities between early and established RA, discusses the diagnostic methods, and evaluates the efficacy of early and late treatment. **Material and methods:** The investigators did a systematic search of publications in PubMed based on keywords which were predetermined and related to the objective of this review. Sources which only contained an abstract were removed. The investigators conducted the initial search and verification of the sources, and papers which fit the requirements were selected. **Results and discussion:** Early treatment is associated with less structural joint damage and decreased disability. On the other hand, without timely treatment, there is a high possibility of developing permanent structural damages and extraarticular spread of the disease. Thorough knowledge and understanding of the early symptoms of RA permits an adequate and timely treatment of the disease. **Conclusion:** Early RA diagnosis leads to a better prognosis. Symptoms of established RA are easier to recognize and more easily diagnosed, however they are associated with a worse outcome.

Keywords: Rheumatoid arthritis, treatment outcomes, RA stages, early symptoms

Introduction

Rheumatoid arthritis is a progressive autoimmune disease primarily of the joints characterized by inflammation of the articular and extra-articular structures resulting in pain, disability, and increased mortality^[1]. The most common are the knees, wrists, and ankles. Rheumatoid arthritis is estimated to affect about 1% of the population and to be twice as common in women than in men. The onset of RA is between 30–50 years of age, and it is thought to be a combination of genetic and environmental factors^[2]. The onset of the disease, the severity of symptoms, and the joints affected varies depending on the presence or absence of certain genetic factors interacting with various environmental elements. The delineation between the stages of RA is complex and there is still no formal consensus about the terms “early RA” (ERA) and “established RA” (EsRA). Some studies provide a specific time period for the two terms, others just use general terms, such as “early disease”, and other

studies differentiate based on specific clinical or laboratory findings (joints affected, level of erosion, level of CRP or RF). There are currently no pathognomonic tests for rheumatoid arthritis and the diagnosis is primarily clinical. The main clinical assessment tool used to diagnose RA and differentiate it from other inflammatory arthritis is the EULAR criteria^[3]. The treatment of RA should begin at the onset of the clinical symptoms in order to prevent extensive tissue damage. RA treatment options include pharmacological (DMARDs, NSAIDs, corticosteroids, biologics) and non-pharmacological (smoking cessation, nutrition, physical therapy, and surgery) methods.^[2]

Purpose

This paper will discuss the differences and similarities between early and established rheumatoid arthritis. It will explore the diagnostic methods for early RA and evaluate the efficacy of early and late treatment methods.

Material and methods

Investigators systematically searched relevant databases such as PubMed using predetermined keywords related to rheumatoid arthritis, including early signs, outcomes, early and late stages, and spotting early signs. Inclusion and exclusion criteria were used to narrow down the target papers. The inclusion criteria contained papers which fit the requirements and the exclusion criteria included studies that only contained abstracts, case reports, letters to editors, editorials or required payment or subscription. Full text screening was then conducted by reviewing the articles in detail. Relevant data was extracted for the objectives, and conflicts between reviewers were resolved with the assistance of a supervisor. Duplicate papers were eliminated from the review.

Results

When beginning the analysis of the results, it is vital to highlight the fact that there is no formal definition of “early RA”. Of the 25 papers which were analyzed, 6 of them had no specific definition for early RA, 12 of them used generalized terms like early and established RA, and 7 of them gave a definition and a specific time frame. Two papers define early RA as symptoms lasting less than 3 months, 3 describe it as less than 2 years, and 2 describe it as less than one year. Established RA is more clearly defined. Most rheumatologists agree that established RA is associated with pain in the joints, reduced functionality, and in some cases substantial structural changes^[4].

Rheumatoid arthritis is a multifaceted condition characterized by heterogeneous clinical manifestations and resemblance to other types of connective tissue disorders, rendering its diagnosis challenging. There are no pathognomonic symptoms of RA, therefore the diagnosis consists of the clinical findings,

laboratory results, and diagnostic imaging. 12 of the total 25 papers found do not discuss diagnostic methods. In the other 13 papers, the most commonly mentioned diagnostic methods are the 2010 ACR/EULAR criteria (mentioned 14 times), laboratory tests (mentioned 7 times), and diagnostic imaging (mentioned 3 times). All papers discussing diagnosis of rheumatoid arthritis used a combination of diagnostic methods, rather than relying on a single criterion for classification. In addition to the ACR/EULAR criteria, there were 7 papers which discussed using laboratory analysis for diagnosis of RA. In all 7 papers, the main serological factors mentioned were ESR, CRP, RF, and anti-CCP. In 3 of the papers, there were other tests mentioned such as testing for liver/kidney function, WBC count, CBC, immunoglobulins, and complement levels.

In the 25 papers on treatment and medical therapy, 22 listed the main medications used, including DMARDs (conventional and biological), NSAIDs, corticosteroids, and biologic therapies. Only 1 paper did not mention any medication, and 2 papers had no specific medication mentioned.

DMARDs

17 of the 25 papers mentioned DMARDs as a treatment used for RA, with conventional DMARDs types such as methotrexate, gold, sulfasalazine, hydroxychloroquine being prescribed. 8 papers mentioned methotrexate (MTX) as the main conventional DMARD used for RA. Sulfasalazine was mentioned in 8 papers, often in combination with other DMARDs. 2 of the papers specified that sulfasalazine was the most frequent initial DMARD used. Conventional DMARDs hydroxychloroquine and leflunomide were mentioned in 6 and 5 papers respectively, but their use as first line therapy was not specified.

In 14 of the papers, DMARDs were either the first line medication or recommended as an initial treatment for RA after diagnosis. In 7 of those papers, methotrexate was the first line treatment, whereas in 1 of the papers, it was a second line treatment. According to ACR and EULAR guidelines, if methotrexate is contraindicated then leflunomide or sulfasalazine are used as the first line treatment^[2]. In paper 6, it was shown that sulfasalazine is the most frequent initial DMARDs used, with methotrexate monotherapy being the next choice^[6].

Biologics/Biological DMARDs

These include agents such as TNF alpha inhibitors (Infliximab, etanercept and adalimumab), IL-1 inhibitors, B-cell and T-cell depletors. 21 of the papers discuss the use of biologics and their specific types. 17 papers mentioned TNF-alpha inhibitors/antagonists as a treatment option and the remaining 4 papers do not mention this medication specifically. Paper 7 states that the first biologics used in practice were the TNF-inhibitors^[7]. Paper 8 states that TNF-alpha inhibiting agents were the initial class of biologics or biological DMARDs, with Infliximab being the first TNF-inhibitors for RA treatment^[8]. In terms of other types of biological medication or biological DMARDs, Tocilizumab (IL-6 inhibitor) and Anakinra (IL-1 inhibitor) were mentioned twice, and Rituximab (B cell depletor) was mentioned 3 times.

Other medications

Various treatments were mentioned in the papers, including JAK inhibitors, corticosteroids (glucocorticoids), and NSAIDs. Two papers discussed the use of JAK inhibitors in combination with biologics or DMARDs. Corticosteroids were mentioned in 11 papers, with glucocorticoids specified as the corticosteroid used in 4 of those

papers and mentioned as a first-line treatment in 1 paper. NSAIDs were mentioned in 4 papers, with 1 paper considering them as a first-line treatment. Steroids were also discussed as treatment options in 2 papers.

Surgery

Surgery for rheumatoid arthritis is a last resort mentioned only once in the 25 papers. The type of surgery is determined by patient needs and may include tenosynovectomy, radiosynovectomy, or osteotomy.

Outcomes regarding early and established RA

In 12 papers, references were made to different treatment types used in early RA and their outcomes regarding clinical and radiographic progression, disease activity, and remission. Only 2 papers reported on treatments and outcomes for both early and established RA. 8 papers discussed treatment's positive impact on radiographic progression and outcomes. In paper 1, a meta-analysis of 12 studies found that DMARD treatment within 2 years of diagnosis resulted in 33% less radiographic progression compared to later treatment, with benefits lasting up to 5 years^[1]. Natural remission occurs in 10% of early RA cases. 6 papers reported on post-treatment remission rates, with 1 paper reporting a higher rate of remission and the others noting patients achieving remission with various indices.

DAS28 (disease activity score) and HAQ (health assessment questionnaire) indices were mentioned in 4 papers. ERA showed a greater decrease in DAS28 value (0.9 ± 1.5) than established RA (0.2 ± 1.0)^[23]. Early treatment groups had lower HAQ DI scores in paper 11^[11]. 75.2% of ERA patients reached HAQ score below 0.5 with MTX in paper 13.^[13]

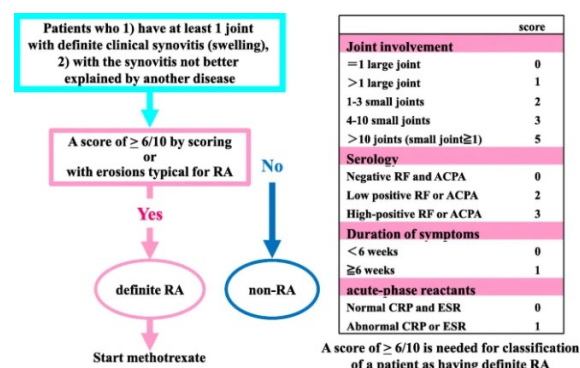
Other indicators used to assess the outcomes after treatment included functional capacity, progression of erosion, EULAR response, and disability. In 2 of the papers, the progression of erosion was reduced following treatment. Functional capacity was also found to be improved after early treatment^[2]. In terms of established RA, paper 6 showed that established RA patients showed better control of disease activity and reduction of progression of erosion in initial combination DMARD therapy. Similar outcomes were found for early rheumatoid arthritis patients. Paper 23 highlights that in the therapeutic management of ERA and EsRA, there was a greater decrease in DAS28 value in ERA.^[23]

Discussion

There is no formal time frame for the terms “early RA” or “established RA”, despite the use of these in many of the studies reviewed. The problem with applying a time frame is that the onset and development of the disease varies greatly among patients, and the clinical symptoms, such as joint pain, disability, and erosion, occur at different rates and may not develop in some cases.

Early detection and treatment of RA is vital, as with any disease. However, the difficulty lies in diagnosing the condition because of the challenge in identifying relevant markers. Another difficulty arises when attempting to distinguish RA from other inflammatory conditions. Given the absence of pathognomonic tests for RA, a diagnostic strategy that encompasses clinical symptoms, laboratory assessments, and diagnostic imaging becomes quite essential. The main way in which RA is diagnosed is by the ACR/EULAR criteria. These criteria were designed to differentiate RA from other forms of arthritis and allow for prompt treatment. Patients are scored based on 4 criteria

(arthritis, serological tests, disease duration, and acute-phase reaction). Scoring 6 or more points out of 10, results in a classification of definite rheumatoid arthritis.^[3]



[3]

* Rheumatoid arthritis classification criteria published by the ACR/EULAR in 2010

The ACR/EULAR criteria is reliable in diagnosing RA because they consider clinical symptoms, laboratory analysis, and duration of symptoms. However, a weakness is that they do not distinguish between early RA and established RA. Its only function is to diagnose RA and to differentiate it from other inflammatory disorders. The second weakness is in the serological markers. Two of the markers used are CRP and ESR. These two markers are not specific to RA and can be elevated in any number of other diseases. The two other markers are RF and ACPA. These two markers are more sensitive and specific for RA; however, RF can still be elevated in patients with other connective tissue diseases such as systemic lupus and scleroderma. Despite this, if both RF and ACPA are positive, the sensitivity and specificity of the diagnosis is increased substantially.

In EsRA, the primary challenge is controlling disease activity and preventing joint damage. EsRA is often associated with more severe symptoms and joint damage, and treatment can be more complicated as patients may have already developed drug resistance and may require more aggressive

treatment strategies. Treatment options for EsRA include DMARDs, NSAIDs, corticosteroids, and surgery in severe cases. DMARDs are the mainstay of treatment but more aggressive approaches may be necessary. NSAIDs manage pain and inflammation, while corticosteroids may be used in higher doses for longer periods. Joint replacement surgery may be required for severe cases.

The main objective of initial treatment is to reduce pain and inflammation, using fast-acting drugs like nonsteroidal anti-inflammatory drugs (NSAIDs). These include Aspirin, Naprosyn, Advil, Motrin, and Lodine. Aspirin, one of the oldest NSAIDs, is effective at high doses for RA due to its prostaglandin inhibition properties^[14]. Co-administration of steroids in conjunction with DMARDs or biological therapies during the early stages of rheumatoid arthritis (RA) may confer a greater propensity for remission induction and control of radiological progression when compared to DMARD monotherapy^[1].

The objective of second-line treatment, achieved through the use of DMARDs, is to slow or halt joint destruction, deformity progression and promote remission. DMARDs are considered slow-acting as they may take weeks to months to take effect^[14]. Early DMARD therapy is crucial and cost-effective when started promptly. Monotherapy, combination DMARDs, and biologic therapies all show better efficacy in early disease compared to established RA^[1]. However, combining 3 DMARDs is more effective than single DMARD therapy in reducing radiographic erosions in early RA. Current evidence supports the use of conventional synthetic DMARD combinations over MTX monotherapy. Although biological and synthetic DMARD combinations have shown greater efficacy than MTX alone, they come

with increased side effects and costs. TNF is a protein that triggers joint inflammation. Biologic drugs like Enbrel, Remicade, Humira, Simponi, and Cimzia inhibit TNF, preventing inflammation by stopping cell recruitment, leading to rapid symptom relief. They are recommended when other second-line treatments are ineffective^[14].

The progression of disease activity is measured by the DAS28 and HAQ criteria. While these are both used as the main methods for measuring disease progression, the DAS criteria is more reliable. The DAS28 criteria is a more objective way of measuring the progression of disease, through swollen joints, general health, and laboratory measures of acute inflammation including ESR and CRP. While the HAQ criteria is a more subjective measure, relying on patient self-assessment of the difficulties of doing specific everyday tasks (eating, dressing, walking, etc).

Surgery is a final option for treating RA, reserved for cases where joint destruction leads to intractable pain and functional decline after nonsurgical methods have failed. At this stage, the disease is considered “end-stage”. The aim of surgery is to relieve pain and restore joint function, with the specific procedure chosen based on the patient's individual needs.^[14]

In terms of the accuracy and validity of this paper, there are a few weaknesses that need to be mentioned. One weakness is the limited sample size of only 2 papers directly comparing early vs established RA. Few papers directly compare early and established RA due to challenges in diagnosing RA as a whole, and defining a specific time period for “early RA”. Additionally, the recognition of symptoms and diagnosis of arthritis in the earlier stages pose further difficulties. Early stages of RA may be asymptomatic, and by the time clinical symptoms appear,

significant inflammation and damage may have already occurred. This leads to a delay in diagnosis which in turn leads to a delay in the starting of treatment in RA. Finally, there were many inaccessible articles due to paywalls, subscriptions, or institutional access requirements. These may have been relevant to the discussion and could have altered the results of the paper. It is also important to note that due to the strict requirements and criteria set by the researchers, many papers had to be excluded from the analysis leaving few studies to actively use for the discussion.

Conclusion

Rheumatoid arthritis is a complex disease with a relatively unknown etiology. It is further complicated by differing symptoms, different age of onset, and different progression of the disease in every person. This paper aimed to analyze differences between “early RA” and “established RA”, discuss diagnostic methods, and evaluate treatment approaches. The line differentiating early, and established arthritis is still the subject of much disagreement within the medical community, and the factors which need to be considered are many and varied. The diagnosis is also multifaceted and requires a combination of clinical symptoms, laboratory analysis, and diagnostic imaging. The researchers found that while the identification and diagnosis of RA can be challenging, it is also vital for medical professionals to recognize the signs as early as possible and to begin treatment. Identification of RA and its treatment in earlier stages can affect disease course, prevent the development of joint erosions, or retard progression of erosive disease. In summary, there is no way to effectively emphasize the importance of recognizing the early signs of systemic inflammation and being able to place a diagnosis of early rheumatoid arthritis.

Bibliography

1. Heidari B. Rheumatoid arthritis: Early diagnosis and treatment outcomes [Internet]. Caspian journal of internal medicine. U.S. National Library of Medicine; 2011 [cited 2023Apr8]. Available from: www.ncbi.nlm.nih.gov/pmc/articles/PMC3766928/
2. Chauhan K, Jandu JS, Brent LH, et al. Rheumatoid Arthritis. [Updated 2023 Jan 10]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK441999/>
3. Tanaka Y. Rheumatoid arthritis - inflammation and regeneration [Internet]. BioMed Central. BioMed Central; 2020 [cited 2023Apr8]. Available from: www.inflammregen.biomedcentral.com/articles/10.1186/s41232-020-00133-8
4. Stack RJ, Sahni M, Mallen CD, Raza K. Symptom complexes at the earliest phases of rheumatoid arthritis: A synthesis of the qualitative literature [Internet]. Arthritis care & research. U.S. National Library of Medicine; 2013 [cited 2023Apr9]. Available from: www.ncbi.nlm.nih.gov/pmc/articles/PMC4030621/
5. Rindfleisch JA, Muller D. Diagnosis and management of rheumatoid arthritis [Internet]. American Family Physician. 2005 [cited 2023Apr9]. Available from: www.aafp.org/pubs/afp/issues/2005/0915/p1037.html
6. Raza K, Buckley CE, Salmon M, Buckley CD. Treating very early rheumatoid arthritis [Internet]. Best practice & research. Clinical rheumatology. U.S. National Library of Medicine; 2006 [cited 2023Apr8]. Available from: www.ncbi.nlm.nih.gov/pmc/articles/PMC3145120/
7. Scott DL. Early rheumatoid arthritis [Internet]. Academic.oup.com. British Medical Bulletin ; 2007 [cited 2023Apr8]. Available from: <https://academic.oup.com/bmb/article/81-82/1/97/283483>
8. Aletaha D, Eberl G, Nell VPK, Machold KP, Smolen JS. Practical progress in realisation of early diagnosis and treatment of patients with suspected rheumatoid arthritis: Results from two matched questionnaires within three years [Internet]. Annals of the rheumatic diseases. U.S. National Library of Medicine; 2002 [cited 2023Apr9]. Available from: www.ncbi.nlm.nih.gov/pmc/articles/PMC1754154/
9. Hua C, Daien CI, Combe B, Landewe R. Diagnosis, prognosis and classification of early arthritis: Results of a systematic review informing the 2016 update of the EULAR recommendations for the management of early arthritis [Internet]. RMD Open. BMJ Specialist Journals; 2017 [cited 2023Apr9]. Available from: <https://rmdopen.bmj.com/content/3/1/e000406>
10. Donahue KE, Gartlehner G, Schulman ER, et al. Drug Therapy for Early Rheumatoid Arthritis: A Systematic Review Update [Internet]. Rockville (MD): Agency for Healthcare Research and Quality (US); 2018 Jul. (Comparative Effectiveness Review, No. 211.) Available from: <https://www.ncbi.nlm.nih.gov/books/NBK524950/>

11. Hua L, Du H, Ying M, Wu H, Fan J, Shi X. Efficacy and safety of low-dose glucocorticoids combined... : Medicine [Internet]. LWW. 2020 [cited 2023Apr9]. Available from: www.journals.lww.com/md-journal/Fulltext/2020/07020/Efficacy_and_safety_of_low_dose_glucocorticoids.47.aspx
12. Davis JM, Matteson EL. My treatment approach to rheumatoid arthritis [Internet]. Mayo Clinic proceedings. U.S. National Library of Medicine; 2012 [cited 2023Apr9]. Available from: www.ncbi.nlm.nih.gov/pmc/articles/PMC3538478/
13. Bosello S, Fedele AL, Peluso G, Gremese E, Tolusso B, Ferraccioli G. Very early rheumatoid arthritis is the major predictor of major outcomes: Clinical ACR remission and Radiographic non-progression [Internet]. Annals of the Rheumatic Diseases. BMJ Publishing Group Ltd; 2011 [cited 2023Apr9]. Available from: <https://ard.bmj.com/content/70/7/1292>
14. Bullock J, Rizvi SAA, Saleh AM, Ahmed SS, Do DP, Ansari RA, et al. Rheumatoid arthritis: A brief overview of the treatment [Internet]. Medical principles and practice : international journal of the Kuwait University, Health Science Centre. U.S. National Library of Medicine; 2018 [cited 2023Apr9]. Available from: [/www.ncbi.nlm.nih.gov/pmc/articles/PMC6422329/](http://www.ncbi.nlm.nih.gov/pmc/articles/PMC6422329/)
15. Sudol-Szopińska I, Jans L, Teh J. Rheumatoid arthritis: What do MRI and Ultrasound Show [Internet]. Journal of ultrasonography. U.S. National Library of Medicine; 2017 [cited 2023Apr8]. Available from: www.ncbi.nlm.nih.gov/pmc/articles/PMC5392548/
16. Burgers LE, Raza K, Annette H van der Helm - van Mil. Window of opportunity in rheumatoid arthritis – definitions and supporting evidence: From old to New Perspectives [Internet]. RMD Open. BMJ Specialist Journals; 2019 [cited 2023Apr8]. Available from: <https://rmdopen.bmj.com/content/5/1/e000870>
17. Gwinnutt J, Symmons D, MacGregor A, Chipping J, Marshall T, Lunt M, et al. Twenty-year outcome and association between ... - Wiley Online Library [Internet]. Wiley Online Library . 2017 [cited 2023Apr9]. Available from: www.onlinelibrary.wiley.com/doi/10.1002/art.40090
18. Bosello S, Fedele AL, Peluso G, Gremese E, Tolusso B, Ferraccioli G. Very early rheumatoid arthritis is the major predictor of major outcomes: Clinical ACR remission and Radiographic non-progression [Internet]. Annals of the rheumatic diseases. U.S. National Library of Medicine; 2011 [cited 2023Apr9]. Available from: www.ncbi.nlm.nih.gov/pmc/articles/PMC3103665/
19. Guo Q, Wang Y, Xu D, Nossent J, Pavlos NJ, Xu J. Rheumatoid arthritis: Pathological mechanisms and modern pharmacologic therapies [Internet]. Bone research. U.S. National Library of Medicine; 2018 [cited 2023Apr9]. Available from: www.ncbi.nlm.nih.gov/pmc/articles/PMC5920070/
20. Choy E. Understanding the dynamics: Pathways involved in the pathogenesis of rheumatoid arthritis [Internet]. OUP Academic. Oxford University Press; 2012 [cited 2023Apr9]. Available from: https://academic.oup.com/rheumatology/article/51/suppl_5/v3/1787104
21. Innala L, Berglin E, Möller B, Ljung L, Smedby T, Södergren A, et al. Age at onset determines severity and choice of treatment in early rheumatoid arthritis: A prospective study - arthritis research & therapy [Internet]. SpringerLink. BioMed Central; 2014 [cited 2023Apr9]. Available from: <https://link.springer.com/article/10.1186/ar4540>
22. Suresh E. Diagnosis of early rheumatoid arthritis: What the non-specialist needs to know [Internet]. Journal of the Royal Society of Medicine. U.S. National Library of Medicine; 2004 [cited 2023Apr9]. Available from: www.ncbi.nlm.nih.gov/pmc/articles/PMC1079582/
23. Castañeda S, Navarro F, Fernández-Carballido C, Tornero C, Marced E, Corteguera M. Differences in the management of early and established rheumatoid arthritis: Reumatología Clínica [Internet]. Reumatología Clínica (English Edition). Elsevier; 2011 [cited 2023Apr9]. Available from: <https://www.reumatologiaclinica.org/en-differences-in-management-early-established-articulo-S2173574311700375>
24. Nestoriuc Y, Orav EJ, Liang MH, Horne R, Barsky AJ. Prediction of nonspecific side effects in rheumatoid arthritis patients by beliefs about medicines [Internet]. Arthritis care & research. U.S. National Library of Medicine; 2010 [cited 2023Apr9]. Available from: www.ncbi.nlm.nih.gov/pmc/articles/PMC2940323/
25. Ma SN, Zaman Huri H, Yahya F. Drug-related problems in patients with rheumatoid arthritis [Internet]. Therapeutics and clinical risk management. U.S. National Library of Medicine; 2019 [cited 2023Apr9]. Available from: www.ncbi.nlm.nih.gov/pmc/articles/PMC6432894/

Corresponding author

email: abdukadirabdi@gmail.com

III. Pharmacy and Pharmacotherapy Session

Ropinirol-based Delivery Systems for Nose-to-brain Administration – Review Article

Yoana Tchoukova¹, Bissera Pilicheva²

1. Faculty of Pharmacy, Medical University of Plovdiv

2. Department of Pharmaceutical Sciences, Faculty of Pharmacy, Medical University of Plovdiv

The administration of drugs through the nasal passage (Nasal Drug delivery systems) is emerging as an effective and reliable pathway to deliver therapeutic agents widely used in the treatment of neurodegenerative disorders such as Parkinson's and Alzheimer's diseases. It represents a noninvasive pathway to reach the brain and cerebrospinal fluid, successfully bypassing the blood-brain barrier (BBB), which is an effective barrier to the diffusion of large and polar molecules, such as some drugs, from the blood stream into the central nervous system (CNS). Ropinirole is a dopamine agonist used in the treatment of Parkinson's disease and restless legs syndrome (RLS). It is typically taken orally, but this method of administration is associated with nausea, vomiting and extensive first pass effect. The medicine, delivered via a nasal form can provide improved bioavailability and reduced side effects compared to the traditional oral administration. The aim of this review is to summarize the data published in the scientific literature regarding the nasal administration of ropinirole by different types of delivery systems. Several types of nasal delivery systems have been explored, including intranasal suspensions, solutions, and gels. These delivery forms contain ropinirole nano- and microparticles, which offer several potential advantages over traditional oral administration, including controlled release of the drug, better bioavailability and faster onset of action. However, they also present unique challenges related to drug stability, dosage accuracy, and mucociliary clearance. Nasal administration of ropinirole nano- and microparticles solutions is a promising option for patients with Parkinson's disease and restless legs syndrome. Further research is needed regarding the delivery systems and to determine the frequency of administration.

Nasal route as an alternative to conventional drug delivery paths

Nasal drug delivery systems are gaining popularity as a potential solution to the challenges associated with the oral administration of drugs, particularly for neurodegenerative disorders such as Alzheimer's and Parkinson's diseases, which require direct access of the drug to the brain. These disorders are characterized by the loss of specific neurons in the brain, leading to cognitive and motor dysfunction.

Traditional oral administration of drugs for these conditions often faces challenges such as poor blood-brain barrier (BBB) penetration, extensive first-pass effect, and low bioavailability. The successful treatment of

central nervous system (CNS) diseases requires quick and effective delivery of drugs into the brain by fast onset of the desired drug concentration in the systemic circulation and availability of the drug for a prolonged time.

The more conventional techniques for administering drugs are often not effective in delivering certain therapeutic agents to the brain. The BBB limits the transfer of drugs from the circulatory system to the central nervous system. While the BBB protects the brain and spinal cord from a range of pathogens, it also acts as a significant obstacle to many xenobiotics used in treatments, and the transport of any molecule into the brain via the circulatory system is highly regulated.

Another challenge in oral drug delivery is the first-pass metabolism. It occurs when a drug is absorbed through the gastrointestinal tract (GIT) and enters the portal circulation, which takes the drug to the liver. There, the drug undergoes metabolism, resulting in a reduced amount of drug that reaches the blood and the intended target, respectively, thereby compromising its efficacy.

An additional problem with orally administered drugs is undesired systemic effects. These can occur once the drug is in the systemic circulation, where it can interact with other tissues and organs, thus leading to side effects.

Nasal drug delivery offers several advantages, including improved drug uptake and transport, bypassing the blood-brain barrier, enhanced therapeutic effects, and reduced systemic toxicity. The Nose to brain method explores the physiological connection between the nose and cerebrospinal fluid (CSF) through the olfactory and

trigeminal nerves to administer drugs to the brain (Fig. 1).

The olfactory neural pathways begin in an area located on the roof of the nasal cavity. Olfactory neurons are dispersed among epithelial cells found in the location. When drugs are applied to the olfactory epithelium, they can be transported to the central nervous system through olfactory neurons, either through paracellular or transcellular transport. The trigeminal nerve also ends in the olfactory bulbs in the brain, transmitting sensory information from the nasal cavity to the central nervous system.

Even though the nasal route of drug delivery has numerous advantages, there are still factors like enzyme activity and mucociliary clearance (MC) that can reduce drug absorption and bioavailability. Enzymes in the nasal cavity, such as cytochrome P450, and aminopeptidase, can degrade drugs, and nasal mucociliary clearance limits drug uptake.

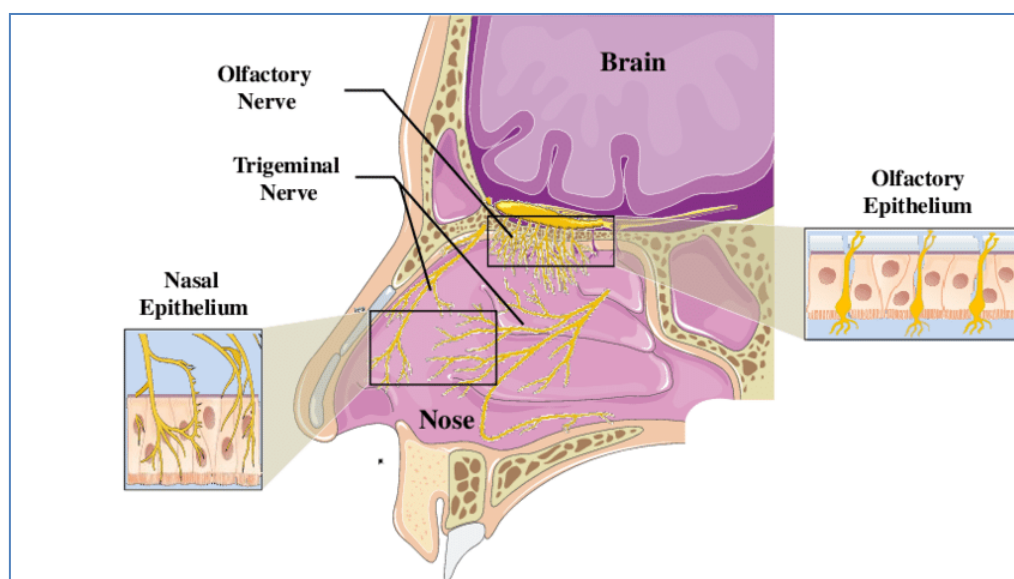


Figure 1. Olfactory and Trigeminal nerve position in the nasal cavity, Nose-to-brain peptide delivery – The potential of nanotechnology – Scientific Figure on ResearchGate. Available from: www.researchgate.net/figure/Olfactory-and-Trigeminal-nerve-position-in-the-nasal-cavity_fig3_320863158 [accessed 19 Mar 2023]

Ropinirole in the treatment of diseases of the central nervous system

Parkinson's disease (PD) is a chronic neurodegenerative condition that results in the reduction of dopamine (DA) and degradation of neurons in the brain. The disease is characterized mainly by motor dysfunction symptoms such as tremors, rigidity, bradykinesia, and postural disturbances. Currently available medications improve symptoms and reduce physical disability in patients, but these effects usually last only a few years before the disability progresses.

Ropinirole is a drug commonly used in the treatment of neurodegenerative disorders, including Parkinson's disease. It is a non-ergoline D2/D3 dopamine agonist. Dopamine agonists act directly on the dopamine receptors and mimic dopamine's effect. Although the exact mechanism of action of ropinirole is not yet known, it is thought to be due to stimulation of D2-type receptors in the brain. In addition to Parkinson's disease, ropinirole is also used to treat restless leg syndrome and other movement disorders. Generally, the drug is well-tolerated. Some common side effects have been noted, including nausea, dizziness, sleep disorders, and GIT discomfort. However, they are linked mainly to the oral administration of the medication. These issues may be addressed by using alternative routes of drug administration such as the nasal delivery.

The ideal drugs for nasal drug delivery for Parkinson's disease should have properties such as good lipophilic solubility and low molecular weight. In addition, drugs should have neuroprotective effects, to promote neuronal survival and function.

Ropinirole-loaded dosage forms for nasal administration

Ropinirole nasal drug formulations can be designed as gels, emulsions, solutions and

others, for optimal drug delivery. Ropinirole nasal solutions are typically designed to be sprayed into the nostrils and can provide rapid symptom relief. Ropinirole nasal gels and emulsions, on the other hand, can offer sustained drug release and enhanced drug stability.

Advantages of thermoreversible in situ nasal gels include their ability to provide a sustained release of medication and form a more viscous formulation enduring MC, thanks to their mucoadhesive properties.

Thermoreversible solutions are in a liquid state at low temperatures but transform into a gel when exposed to higher temperatures – optimal being 31–34°C which is close to the physiological temperature of the human nasal cavity. The close contact between the mucoadhesive polymer and biological tissue wets the polymer, thus leading to its swelling and bonding with mucosa.

Thermoreversible in situ nasal gel of ropinirole was prepared using PF 127 as the thermoreversible polymer. Gelling time, gelling temperature, gel strength and adhesive force were evaluated. Ex vivo studies were carried out on fresh sheep nasal mucosa. Histological evaluation of the treated sheep nasal mucosa with plain drug solution and the In situ nasal gel revealed that the gel has a protective effect on the mucosa, whereas plain ropinirole resulted in slight cellular damage.

Ex vivo diffusion of several batches of gels was found to be between 56 to 100% in a span of 5 h. A decrease in the diffusion of drug was evident with an increase in the concentration of PF 127.

Nanoparticles have gained significant attention as a promising platform for drug delivery due to their unique size, shape and surface characteristics. They have a size range of 1–1000 nanometers and can be used as carriers to enhance the bioavailability of

drugs. Once administered through the nose, nanoparticles can take different pathways based on their size. If their size is between 10 and 300 nm, drugs are delivered straight to the brain by the olfactory pathway.

Ropinirole-loaded solid lipid nanoparticles (SLNs) were created using the double emulsion method, which is used for the encapsulation of highly hydrophilic drugs such as ropinirole. Glycerol monostearate (GMS) and soya lecithin were used as carrier and stabilizer respectively.

Different ratios of GMS/Soya lecithin were prepared and was studied their effect on particle size. (Table 1)

As the ratio of GMS to Soya lecithin decreased, the particle size of SLNs also

decreased. F3 has been found to be the formulation with the highest drug entrapment and optimal particle size.

Drug release of the particles, as well as pure ropinirole solution, was tested in vitro using a diffusion cell and a dialysis membrane soaked in water and with buffer solution. Ex-vivo permeation studies were also carried out – a diffusion cell was used with the sheep nasal mucosa as the permeation barrier.

Ex-vivo drug release of particles over 24 h was 53.75%. When tested with the pure ropinirole solution, ex-vivo drug release was 88.15% within 6 h and 93.66 % by 24 h. (Fig. 2)

Table 1. Composition of the nanoparticles, with differences in the GMS/Soya lecithin ratio (Priya, Sneh & Koland, Marina & Kumari, Suchetha. (2015). Formulation and characterization of ropinirole hydrochloride loaded solid lipid nanoparticles. International Journal of Pharmacy and Pharmaceutical Sciences. 7. 85-89.)

Effect of GMS: soya lecithin ratio on particle size			
Formulation code	GMS/soya lecithin ratio (W/W)	Particle size (nm)	PDI
F1	90:10	867±6.75	0.753±0.016
F2	85:15	571.6±6.27	0.685±0.005
F3	80:20	462.2±4.55	0.547±0.013
F4	75:25	433.1±5.95	0.288±0.006

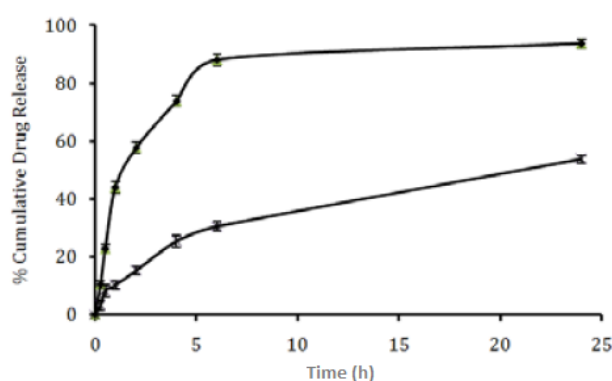


Figure 2. Ex-vivo release profile of drug formulations SLNs (below) and pure ropinirole solution (above) over 24 h period (Priya, Sneh & Koland, Marina & Kumari, Suchetha. (2015). Formulation and characterization of ropinirole hydrochloride loaded solid lipid nanoparticles. International Journal of Pharmacy and Pharmaceutical Sciences. 7. 85-89.)

Conclusion

Both in vitro and ex vivo release experiments showed that Ropinirole-loaded solid lipid nanoparticles and in-situ gel had a prolonged release compared to pure drug solution. SLNs have shown impressive advantage compared to gels in regards to percentage of drug release over the course of the first 5–12 h after administration. Therefore, these nasal drug administration formulations could be a promising platform for enhancing the bioavailability of Ropinirole.

References

1. Pardeshi CV, Belgamwar VS. Direct nose to brain drug delivery via integrated nerve pathways bypassing the blood-brain barrier: an excellent platform for brain targeting. *Expert Opin Drug Deliv*. 2013 Jul;10(7):957-72. doi: 10.1517/17425247.2013.790887. Epub 2013 Apr 16. PMID: 23586809.
2. Misra A, Kher G. Drug delivery systems from nose to brain. *Curr Pharm Biotechnol*. 2012 Sep; 13(12):2355-79. doi: 10.2174/138920112803341752. PMID: 23016642.
3. Pahwa R, Lyons KE, Hauser RA. Ropinirole therapy for Parkinson's disease. *Expert Rev Neurother*. 2004 Jul;4(4):581-8. doi: 10.1586/14737175.4.4.581. PMID: 15853577.
4. Boyuklieva, R.; Pilicheva, B. Micro- and Nanosized Carriers for Nose-to-Brain Drug Delivery in Neurodegenerative Disorders. *Biomedicines* 2022, 10, 1706. <https://doi.org/10.3390/biomedicines10071706>
5. Khan, A.R.; Liu, M.; Khan, M.W.; Zhai, G. Progress in brain targeting drug delivery system by nasal route. *J. Control Release*. 2017, 268, 364–389
6. Su Y, Sun B, Gao X, Dong X, Fu L, Zhang Y, Li Z, Wang Y, Jiang H, Han B. Intranasal Delivery of Targeted Nanoparticles Loaded With miR-132 to Brain for the Treatment of Neurodegenerative Diseases. *Front Pharmacol*. 2020 Aug 6;11:1165. doi: 10.3389/fphar.2020.01165. PMID: 32848773; PMCID: PMC7424054.
7. Priya, Sneha & Kolland, Marina & Kumari, Suchetha. (2015). Formulation and characterization of ropinirole hydrochloride loaded solid lipid nanoparticles. *International Journal of Pharmacy and Pharmaceutical Sciences*. 7. 85-89.
8. Khan S, Patil K, Bobade N, Yeole P, Gaikwad R. Formulation of intranasal mucoadhesive temperature-mediated in situ gel containing ropinirole and evaluation of brain targeting efficiency in rats. *J Drug Target*. 2010 Apr;18(3):223-34. doi: 10.3109/10611860903386938. PMID: 20030503.
9. Monica Rao, Deepak Kumar Agrawal & Chaitanya Shirsath (2016): THERMOREVERSIBLE MUCOADHESIVE IN SITU NASAL GEL FOR TREATMENT OF PARKINSON'S DISEASE, *Drug Development and Industrial Pharmacy*, DOI: 10.1080/03639045.2016.1225754

Corresponding author

Yoana Tchoukova
Faculty of Pharmacy
Medical University of Plovdiv
Plovdiv, Bulgaria
20301071@mu-plovdiv.bg

“Green Synthesis” of Silver and Iron Nanoparticles – Preparation, Characterization and Photothermal Potential

Nikola Kostadinov¹, Vladislav Velchev¹, Tsenka Grancharova^{2,4}, Stanislava Simeonova^{3,4}
Supervisors: Plamen Zagorchev^{2,4}

1. Faculty of Medicine, Medical University of Plovdiv

2. Department of Medical Physics and Biophysics, Faculty of Pharmacy, Medical University of Plovdiv

3. Department of Pharmaceutical Sciences, Faculty of Pharmacy, Medical University of Plovdiv

4. Research Institute at Medical University of Plovdiv

Introduction: Preparation of nanoparticles by the “green synthesis” has the advantage of using environmentally friendly and biodegradable materials. The used plants provide new and unique properties to the synthesized nanoparticles due to the presence of bioactive compounds in them. *Uncaria tomentosa* and *Camellia sinensis* have been the subjects of numerous studies due to their antioxidant, anti-inflammatory and immunomodulatory effects. Metal and metal oxide-based nanoparticles are widely used in cancer therapy as drug delivery carriers, diagnostic agents, and mediators of innovative therapies. **Aim:** Preparation, characterization and photothermal investigation of silver and iron nanoparticles obtained by the “green synthesis” by *Uncaria tomentosa* and *Camellia sinensis*. **Materials and methods:** *Uncaria tomentosa*, *Camellia sinensis*, AgNO₃ and FeNO₃ were used to synthesize the nanoparticles. The resulting nanoparticles were characterized by electron microscopy and dynamic light scattering. An infrared laser was used for the photothermal studies, and temperature changes were recorded with a thermal camera. **Results and Discussion:** Nanoparticles were successfully prepared by „green synthesis“. At the beginning of the fabrication, the color of the extracts changed, which is an indicator of preparation of nanoparticles. This was later confirmed by electron microscopy and dynamic light scattering. The thermal results indicated their potential application in photothermal therapy. **Conclusion:** *Uncaria tomentosa* and *Camellia sinensis* are suitable reducing agents in green synthesis. The resulting silver and iron nanoparticles have the potential to mediate photothermal therapy.

Keywords: nanoparticles, green synthesis, nanomedicine, photothermal therapy, laser therapy

1. Introduction

Nanotechnology as a field of study focuses on the synthesis, characterization, and practical applications of nanomaterials across various scientific disciplines. Nanoparticles, with their unique properties, have been extensively utilized in medicine and pharmacology for the diagnosis and treatment of tumors and other diseases. Due to their surface properties, nanoparticles exhibit higher reactivity to macromolecules such as proteins, antibodies, nucleic acids, and more^{1,2}. Therefore, they can be designed to target specific tissues accurately, resulting in a much lower effective dose of medication, thereby minimizing side effects.

Traditional methods of creating nanoparticles are often expensive, require specialized equipment and can have a negative impact on the environment. In contrast, “green

synthesis” involves the use of environmentally friendly materials to produce nanoparticles³. One of the notable advantages of green synthesis is its simplicity. The process involves using plant extracts as reducing agents, eliminating the need for harmful chemicals or toxic solvents typically used in conventional synthesis methods. Moreover, plant phytochemicals not only act as reducing agents but also contribute to the therapeutic potential of the synthesized nanoparticles.

Uncaria tomentosa (also known as cat’s claw) has antioxidant, antimicrobial, immunomodulating and anti-inflammatory properties. Due to its content of oxindole alkaloids, the plant possesses potential anti-tumor properties⁴. Extracts from *U. tomentosa* demonstrate antiproliferative effects against various cancer cell lines. Similarly,

Camelia sinensis, known as green tea, has been found to lower the risk of cancer and exhibit efficacy in inhibiting tumor growth⁵. It is associated with cancer prevention due to its ability to modulate genes involved in the initiation, promotion, and progression of tumors.

Photothermal therapy is a type of cancer treatment that uses light energy to generate heat and destroy cancer cells. This therapy works by using a photosensitive agent, usually nanoparticles⁶, to absorb light energy and convert it into heat. Cancer cells are more heat-sensitive compared to healthy cells due to disorganized tumor blood vessels, which reduced heat dissipation. Additionally, photothermal therapy has fewer side effects than traditional cancer treatments, and it can be used in combination with other therapies to enhance their effectiveness⁷.

The photothermal performance of nanoparticles depend on their conversion efficiency. Conversion efficiency is a measure of how efficiently the absorbed laser energy is converted into heat and is great for inorganic nanoparticles such as silver and iron. Bimetallic nanoparticles are of growing interest⁸ because they can exhibit a variety of properties with synergistic effect when two different metals are combined.

The aim of our research was preparation, characterization and photothermal investigation of bimetallic nanoparticles composed of silver (Ag) and iron (Fe) obtained by the “green synthesis” by *Uncaria tomentosa* and *Camellia sinensis*.

2. Materials and methods

2.1. Synthesis of Ag-Fe nanoparticles

Uncaria tomentosa and *Camellia sinensis* water extracts were prepared at room temperature or at 80°C, for 60 minutes. The

synthesis of Ag-Fe bimetallic nanoparticles was performed by mixing 0.1 M AgNO₃ and 0.1 M FeNO₃ in a solution, which was then heated to 60°C. The resulting mixture was added to a plant extract in a 1:1 proportion, stirred, and heated at 80°C for 1 hour. The formation of the nanoparticles was monitored and confirmed by a change of the solution color to black. The reaction was centrifuged, and the supernatant was discharged. The product was then washed with ethanol and left to dry.

2.2. Characterization of nanoparticles

TEM images were performed by a Talos® F200C G2 TEM transmission electron microscope. The hydrodynamic particle size distribution was determined by using a Microtrac® Nanotrac particle size and zeta potential analyzer. Elemental composition of the synthesized bimetallic nanoparticles was determined using energy-dispersive X-ray spectroscopy (EDS). Fourier transform infrared analysis was used for functional group detection.

2.3. Thermal measurements of samples

Suspensions of Ag-Fe nanoparticles in distilled water were prepared in concentrations of 10 mg/ml, 25 mg/ml, and 50 mg/ml. The samples were placed into the 2.5-ml eppendorf conical tubes and irradiated with 808 nm infrared laser. All studies were obtained at room temperature and were conducted triplicate. We used distilled water as a control. For tissue phantom samples a porcine muscle tissue was homogenized by stirrer and mixed with Ag-Fe nanoparticles in the same concentration – 10 mg/ml, 25 mg/ml, and 50 mg/ml tissue. The control was blended porcine muscle alone. Changes in temperatures were measured by an infrared camera (FLIR Systems).

3. Results and discussion

3.1. Synthesis and characterization of Ag-Fe nanoparticles

The Ag-Fe bimetallic nanoparticles were successfully synthesized. TEM revealed that all the nanoparticles are nearly spherical with an average core size in the range of approximately 16–33 nm (Fig. 1A and 1B). The minimal hydrodynamic diameter was measured to be 60 nm for green tea-based nanoparticles and 50 nm for cat's claw-based nanoparticles. Additionally, both TEM and DLS analysis revealed the formation of aggregates.

EDS confirmed the presence of Ag and Fe ions in the nanoparticles. Interestingly, the

ratio of Ag to Fe ions was found to be different in the nanoparticles synthesized using green tea and cat claw. In the case of green tea-based nanoparticles, Ag signals dominated, while in the case of cat claw-based nanoparticles, both Ag and Fe ions had approximately equal ratios. The presence of C and O peaks could be due to the phytochemicals present on the surface of the Ag-Fe nanoparticles.

FTIR identified IR bands corresponding to hydroxy groups stretching (O–H) within polyphenols, C–H stretching vibration of methyl groups (2946 cm^{-1} and 2873 cm^{-1}), C–O stretching of ester groups (1290) and C–O stretching of primary alcohols (1080) (Fig. 2A and 2B).

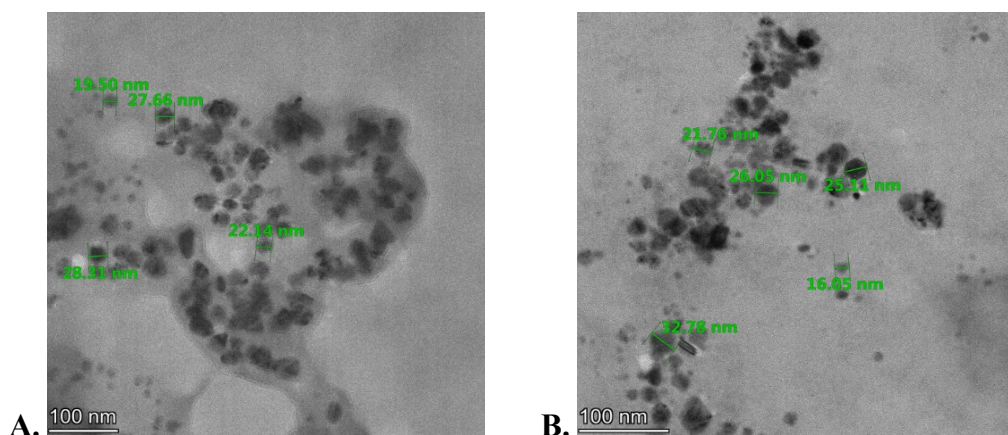


Figure 1. Characterization of nanoparticles. A) TEM of *Camellia sinensis* Ag-Fe Nps. B) TEM of *Uncaria tomentosa* Ag-Fe Nps.

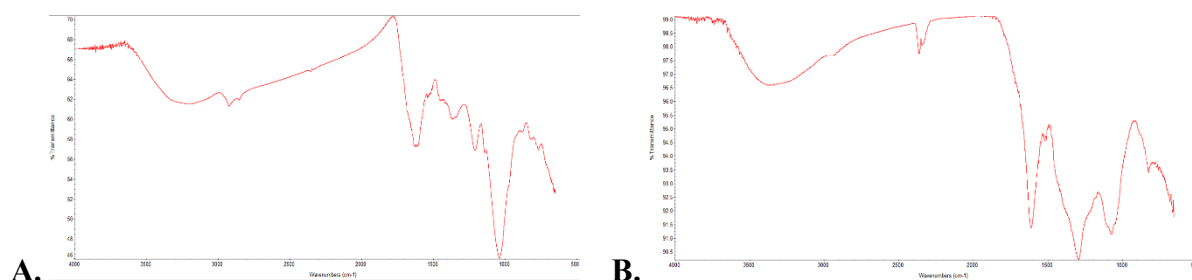


Figure 2. Characterization of functional groups. A) FTIR of *Camellia sinensis* Ag-Fe Nps. B) FTIR of *Uncaria tomentosa* Ag-Fe Nps.

3.2. Thermal performance of nanoparticles

We investigated the thermal properties of 4 samples – two were synthesized using *Camellia sinensis* (one at room temperature and one at 80°C) and two were synthesized using *Uncaria tomentosa* under the same conditions. Upon laser irradiation, all nanoparticle suspensions exhibited a significant increase in temperature, sufficient for achieving hyperthermia (Fig. 3A). Notably, the nanoparticles synthesized using green tea extract at room temperature demonstrated the highest performance, with a temperature increase of 26.7°C.

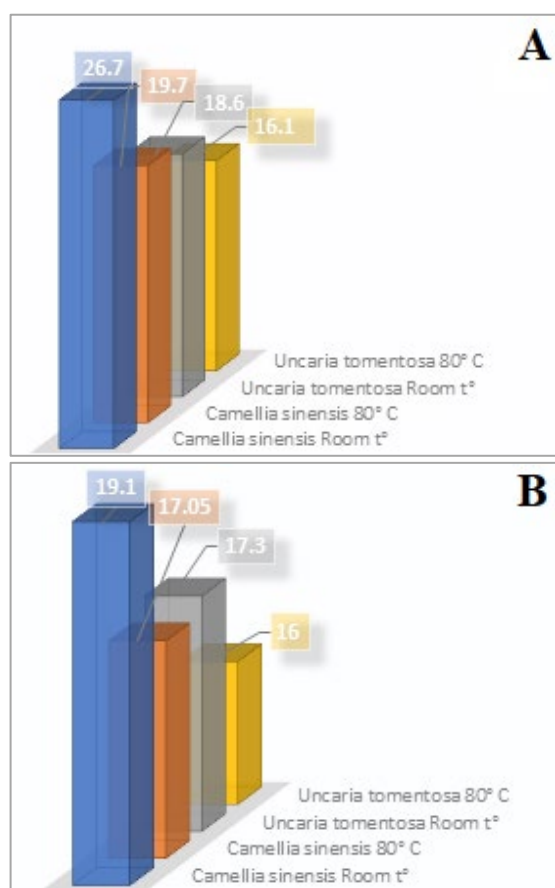


Figure 3. Thermal measurements. A) Water suspension of Ag-Fe Nps. B) Tissue phantom with Ag-Fe Nps.

We also tested the temperature change in tissue phantom (Fig. 3B). In this experiment we used blended porcine muscle tissue instead of distilled water, in order to mimic the body environment. Results were lower than

these of nanoparticle suspensions, but sufficient for hyperthermia. Again, the nanoparticles synthesized using green tea extract at room temperature demonstrated the highest performance.

4. Conclusion

The “green synthesis” is a promising method for the synthesis of bimetallic nanoparticles. All the Ag-Fe nanoparticles produced in this study exhibited potential for application in photothermal therapy. The *Camellia sinensis* Ag-Fe nanoparticles synthesized with plant extraction at room temperature showed the highest photothermal performance.

Acknowledgments

This work was funded by National Science Fund of Bulgaria, Project No. KP-06-H63/3.

Reference

1. Mohanraj VJ, Chen Y. Nanoparticles – A Review. Tropical Journal of Pharmaceutical Research, 2006; 5 (1): 561-573.
2. S Anu Mary Ealia and M P Saravanakumar. A review on the classification, characterisation, synthesis of nanoparticles and their application. 2017. IOP Conf. Ser.: Mater. Sci. Eng. 263 032019
3. Muhammad Rafique, Iqra Sadaf, M. Shahid Rafique & M. Bilal Tahir (2017). A review on green synthesis of silver nanoparticles and their applications, Artificial Cells, Nanomedicine, and Biotechnology, 45:7, 1272-1291
4. Batiha, G.E.-S.; Magdy Beshbishy, A.; Wasef, L.; Elewa, Y.H.A.; Abd El-Hack, M.E.; Taha, A.E.; Al-Sagheer, A.A.; Devkota, H.P.; Tufarelli, V. *Uncaria tomentosa* (Willd. ex Schult.) DC.: A Review on Chemical Constituents and Biological Activities. Appl. Sci. 2020, 10, 2668.
5. Arshad H. Rahmani, Fahad M. Al shabrimi, Khaled S. Allemailem, Salah M. Aly, Masood A. Khan, "Implications of Green Tea and Its Constituents in the Prevention of Cancer via the Modulation of Cell Signalling Pathway", BioMed Research International, vol. 2015,
6. Cherukuri P, Glazer ES, Curley SA. Targeted hyperthermia using metal nanoparticles. Adv Drug Deliv Rev. 2010 Mar 8;62(3):339-45.
7. Li C, Cheng Y, Li D, An Q, Zhang W, Zhang Y, et al. Antitumor Applications of Photothermal Agents and Photothermal Synergistic Therapies. International Journal of Molecular Sciences 2022;23:7909.
8. Haseena M, Saffiya H, Moganavelli S, Bimetallic nanoparticles as suitable nanocarriers in cancer therapy, Scientific African, Volume 20, 2023

Novel Beta-lactam Antibiotics in the Treatment of Resistant Infections

Geniya Jerry¹, Akanksha Prasad¹, Hristina Zlatanova²

1. Faculty of Medicine, Medical University of Plovdiv

2. Department of Pharmacology and Clinical Pharmacology, Medical University of Plovdiv

Antibiotic resistance is a massive public health concern that necessitates the development of new antibiotics. One such development is novel beta-lactam/beta-lactamase inhibitor combinations (BLBLIs). The presence of lactamases, enzymes that degrade the beta-lactam rings and render antibiotics ineffective, causes bacterial resistance to beta-lactam antibiotics. However, BLBLIs inhibit these enzymes, enabling the antibiotic to take effect. Examples include Ceftazidime-avibactam, Ceftolozane-tazobactam, Meropenem – vaborbactam, among several others. These options are for treating multidrug-resistant (MDR) *Pseudomonas aeruginosa* and Enterobacterales. Ceftazidime-avibactam is used to treat Enterobacterales that produce extended-spectrum-lactamase (ESBL), *Klebsiella pneumoniae* carbapenemase (KPC), AmpC, as well as carbapenem-resistant *Pseudomonas aeruginosa*. Ceftolozane-tazobactam is also a carbapenem-resistant *P. aeruginosa* (non-carbapenemase producing) treatment option with some activity against ESBL-producing Enterobacterales. Meropenem-vaborbactam has emerged as a treatment option for Enterobacterales producing ESBL, KPC, or AmpC, with anti-P activity. Beta-lactamase inhibitors are classified as beta-lactam compounds (tazobactam) or non-beta-lactam compounds (avibactam and vaborbactam); these three agents are part of the combinations included in this review. The first combination of Beta-Lactam/Beta-Lactamase inhibitors used over a long period is amoxicillin and clavulanic acid. Compared to amoxicillin-clavulanic acid, the new BLBLIs have different content ratios. For example, Ceftolozane-Tazobactam has a 2:1 ratio; Ceftazidime-avibactam has a 4:1 ratio. Lower MIC (minimum inhibitory concentration) values indicate that less drug is required to inhibit the organism's growth; therefore, drugs with lower MIC scores are more effective antimicrobial agents. New approaches to developing and expanding the effectiveness of Beta-lactamase inhibitors are currently in progress.

Keywords: Antibiotic resistance, BLBLI, Minimum inhibitory concentration

Introduction

Antibiotic resistance is a growing problem worldwide, leading to 1.27 million deaths globally, surpassing HIV/AIDS or malaria-related deaths.¹ This issue requires immediate attention, and developing new antibiotics is necessary to tackle antibiotic resistance rates. One approach to addressing antibiotic resistance is the development of novel beta-lactam antibiotics, a class of agents that target bacteria by interfering with their cell wall synthesis.² The beta-lactam/beta-lactamase inhibitor (BL/BLI) combinations prevent the degradation of beta-lactam antibiotics by beta-lactamases, which are enzymes produced by bacteria. By inhibiting the action of beta-lactamases, the BL/BLI combinations enable beta-lactam antibiotics to exert their antibacterial effect. This review will discuss novel BL/BLIs to treat resistant infections. One such agent is

ceftazidime-avibactam, which combines a third-generation cephalosporin (ceftazidime) with a beta-lactamase inhibitor (avibactam). Ceftazidime-avibactam has been approved by the EMA (European Medicines Agency)³ to treat complicated urinary tract infections (cUTIs), complicated intra-abdominal infections (cIAIs), hospital-acquired pneumonia (including ventilator-associated pneumonia), and infections caused by aerobic Gram-negative organisms. Another novel combination is meropenem-vaborbactam – a combination of the carbapenem antibiotic meropenem and the beta-lactamase inhibitor vaborbactam.⁴ It was approved by EMA in 2018 based on several clinical trials.⁵ It is indicated for the treatment of complicated urinary tract infections, including pyelonephritis, caused by susceptible Gram-negative bacteria in adult patients. Ceftolozane-tazobactam is an antibiotic combination that has

been approved by EMA for the treatment of cUTIs and cIAIs in adults. Ceftolozane-tazobactam consists of two active ingredients: ceftolozane, a cephalosporin antibiotic, and tazobactam, a beta-lactamase inhibitor.⁶

Studies conducted by public and private organizations reveal the alarming rise of β -lactam resistance among Gram-negative bacteria in both hospital and community settings. The primary mechanism of antibiotic resistance in these bacteria is the production of β -lactamases, enzymes that deactivate β -lactam antibiotics by breaking down their amide bond. Despite being identified for over 70 years, β -lactamases remain a significant scientific challenge. Two classification systems categorize β -lactamases. The Ambler classification system, introduced 30 years ago, divides β -lactamases into four classes based on their amino acid sequences.⁷ In contrast, the Bush-Medeiros-Jacoby classification system groups them according to functional properties. This review focuses on the Ambler classification system.

Class A enzymes include plasmid-mediated and chromosomally-encoded β -lactamases with broad-spectrum, extended-spectrum, and carbapenemase activity. Class B enzymes are metallo- β -lactamases that hydrolyze penicillins, cephalosporins, and carbapenems, such as NDM-1 found in *Klebsiella pneumoniae* and *Escherichia coli*. Class C enzymes are cephalosporinases that are chromosomally encoded or plasmid-mediated. Class D enzymes, or oxacillinases, have a substrate preference for oxacillin and rapidly expand with enzymes that hydrolyze extended-spectrum cephalosporins and carbapenems.⁸

Currently, three commercially available β -lactamase inhibitors exist: clavulanic acid, sulbactam, and tazobactam. These inhibitors have a standard β -lactam structure and are active against most class A β -lactamases but

have limited effect on class C and are essentially inactive against class B and most class D enzymes.

Developing novel BL/BLI antibiotics is a fundamental approach to antibiotic resistance. Much research has been conducted on the class of beta-lactam antibiotics that show positive results against treating several drug resistance infections using MRSA,⁹ however, more research and development of novel beta-lactam antibiotics will be crucial to fighting antibiotic resistance.

Methods

PubMed was searched for articles published between 2010 and 2023 using the keywords “novel beta-lactam antibiotics”, “ceftazidime-avibactam”, “ceftolozane-tazobactam”, “meropenem-vaborbactam”, “mechanism of action”, “clinical trials” and “efficacy”. Data on the mechanism of action and in vitro activity of these antibiotics against resistant bacteria and resistance mechanisms were extracted.¹⁰

Results and discussion

The authors found that ceftazidime-avibactam, ceftolozane-tazobactam, and meropenem-vaborbactam effectively treat infections caused by multidrug-resistant Gram-negative bacteria, a growing concern in healthcare due to limited treatment options. Ceftazidime-avibactam effectively treats complicated urinary tract infections, complicated intra-abdominal infections, and hospital-acquired pneumonia caused by Gram-negative bacteria, including those that produce beta-lactamases such as extended-spectrum beta-lactamases (ESBLs) and *Klebsiella pneumoniae* carbapenemase (KPC).¹¹ The efficacy and safety of ceftazidime-avibactam have been evaluated in several clinical trials. In a phase 3 trial, the combination was found to be non-inferior to

meropenem in treating cIAIs. Another phase 3 trial demonstrated the non-inferiority of ceftazidime-avibactam to doripenem in treating cUTIs. Additionally, the combination is effective in the treatment of hospital-acquired pneumonia caused by Gram-negative bacteria.

Ceftolozane-tazobactam has demonstrated efficacy in treating complicated urinary tract infections and complicated intra-abdominal infections caused by Gram-negative bacteria, including those that produce ESBLs and AmpC beta-lactamases. Tazobactam is effective against many beta-lactamase enzymes, including classes A and C. Ceftolozane-tazobactam is effective in clinical trials for treating complicated intra-abdominal infections and complicated urinary tract infections. In a phase 3 trial, ceftolozane-tazobactam was found to be non-inferior to meropenem for the treatment of cIAI. In another phase 3 trial, ceftolozane-tazobactam was found to be non-inferior to levofloxacin plus metronidazole for the treatment of cUTI.¹² Ceftolozane-tazobactam has also been studied to treat ventilator-associated bacterial pneumonia (VABP). In a phase 3 trial, ceftolozane-tazobactam was found to be non-inferior to meropenem for treating VABP caused by Gram-negative bacteria.

Meropenem-vaborbactam was approved by the Food and Drug Agency in 2017 for treating complicated urinary tract infections, complicated intra-abdominal infections, and hospital-acquired pneumonia caused by Gram-negative bacteria, including those that produce KPC.¹³ One of the advantages of meropenem-vaborbactam over other BL/BLI combinations is its spectrum of activity against MDR *P. aeruginosa*, which is often resistant to other antibiotics commonly used to treat Gram-negative infections.

Avibactam, one of the recently developed BLIs, and clavulanic acid, the oldest BLI in

use, are both used in combination with certain antibiotics to enhance their activity against a broader range of bacteria, but there are specific differences in their ability to affect various types of β -lactamases.¹⁴ Clavulanic acid is a class A and C β -lactamase inhibitor; it is active against many beta-lactamases, including those produced by some strains of *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, and others Gram-negative bacteria. However, it has a narrow spectrum of inhibition and is ineffective against carbapenemases or metallo- β -lactamases.¹⁵ On the other hand, avibactam is an extensive spectrum beta-lactamase inhibitor that has been shown to function against a broader range of beta-lactamases, in particular, some classes A, class C, and class D.¹⁶ Avibactam can also inhibit some carbapenemases and certain metallo- β -lactamases resistant to other beta-lactamase inhibitors. Another difference is the mechanism of action. Clavulanic acid is a competitive inhibitor of beta-lactamases, meaning it competes with the antibiotic for binding to the enzyme's active site. In contrast, avibactam is a non-suicidal beta-lactamase inhibitor that forms a covalent bond with the enzyme, which can prevent it from being inactivated by the beta-lactamase.¹⁷ Finally, avibactam has a better safety profile than clavulanic acid. Avibactam has been associated with fewer adverse events and has a lower potential for drug interactions than clavulanic acid. Overall, avibactam is a newer and more advanced beta-lactamase inhibitor than clavulanic acid, with a broader spectrum of activity, a unique mechanism of action, and a better safety profile. This makes it a more powerful tool for combating antibiotic resistance caused by beta-lactamase-producing bacteria. However, it is essential to note that the choice of beta-lactamase inhibitor depends on the type of beta-lactamase

produced by the bacterial strain causing the infection and the antibiotic used in combination.¹⁸

The β -lactamase inhibitors are formulated with a partner-lactam based on two primary considerations: i) the inhibitor's ability to inhibit β -lactamases that are capable of hydrolyzing the β -lactam and ii) similarities in pharmacokinetic properties (such as elimination half-lives, metabolic pathways, and biodistribution) to guarantee that the β -lactam structural integrity will be safeguarded over a predetermined amount of time.¹⁹ The pathogen, bacterial burden at the site of infection, the β -lactamase involved, the β -lactamase transcription level(s), the involvement of another resistance mechanism, the potency of the inhibitor, the potency of the β -lactam, the pharmacokinetics/biodistribution of the β -lactamase inhibitor and the pharmacokinetics/biodistribution of the β -lactam are all critical factors.²⁰ Most BL/BLIs that are commercially available are provided in fixed-dose ratio combinations, except amoxicillin-clavulanic acid, which is available in a variety of β -lactam-to-inhibitor ratios. BL/BLIs' dose ratios are partly determined by their *in vivo* and *in vitro* antimicrobial efficacy. For instance, ceftolozane-tazobactam has a 2:1 dose ratio of ceftolozane to tazobactam because this ratio had minimum inhibitory concentrations (MICs) against ESBL-producing strains that were significantly lower than those of the 8:1 or 4:1 dose ratios.⁶ Studies on the survival of infected mice treated with various ratios of ceftazidime-avibactam supported the commercial 4:1 ratio.²¹ Even though these commercial formulations have demonstrated high rates of efficacy in clinical trials, in some clinical scenarios (such as when enzyme hyperproduction or severe [high inoculum] infections are encountered), it may be necessary to allow for some flexibility in the

pairing and dosing of the individual components of the combination. The plan of independent inhibitors could likewise work with convenient pairings to suit one-of-a-kind clinical requirements (e.g., promptly matching aztreonam with avibactam against disconnects associated with holding onto class A and B β -lactamases).

There is ongoing debate regarding the most effective method for BL/BLI susceptibility testing. Though concentrations vary, a fixed ratio of β -lactam to inhibitor is used for susceptibility testing of older combinations, such as amoxicillin-clavulanic acid and ampicillin-sulbactam. The 2:1 fixed dose ratio used in susceptibility testing for ampicillin-sulbactam reflects the 2:1 dose ratio used in all commercial formulations.²² This susceptibility testing arrangement is expected to reflect the concentration ratios that can be achieved *in vivo* due to the similarities in pharmacokinetics between ampicillin and sulbactam. The same 2:1 ratio is used for *in vitro* susceptibility testing of clavulanic acid.

Piperacillin-tazobactam and ceftazidime-avibactam are examples of combinations in which various parent-lactam concentrations are used alongside a fixed inhibitor concentration of 4 mg/l.²³ A static inhibitor concentration is assumed to be sufficient, even though the concentrations of β -lactam mimic the dynamic concentrations observed *in vivo*. In this scheme, it is generally anticipated that the inhibitor concentration will have little effect on MIC above the 4 mg/l threshold. However, the fixed concentration approach may not accurately reflect the inhibitor's impact on MIC reduction and, ultimately, *in vivo* efficacy if *in vivo* concentrations above this value are associated with additional MIC reductions (also known as the nonsaturable effect).²⁴

Determining the driver of efficacy is a relatively novel and complicated process for

lactamase inhibitors. The method that has been utilized the most frequently entails determining a PK-PD (pharmacokinetic-pharmacodynamic) index to characterize the effect that the β -lactamase inhibitor has on the target enzyme in all four of the BL/BLIs that have been approved most recently.²⁵

Conclusion

The emergence of resistant bacterial infections has become a significant public health concern worldwide. Developing novel BL/BLIs such as ceftazidime-avibactam, ceftolozane-tazobactam, and meropenem-vaborbactam, has shown promising results in treating these resistant infections. These antibiotics have demonstrated high efficacy and safety profiles and are effective against many resistant bacteria, including carbapenem-resistant ones. However, it is essential to note that the overuse and misuse of antibiotics can lead to further resistance. It is crucial to use these antibiotics judiciously and in conjunction with other infection control measures, such as proper hygiene practices and vaccination, to reduce the spread of resistant infections.

References

1. Thompson T. The staggering death toll of drug-resistant bacteria. *Nature* 2022 Jan 31. doi: 10.1038/d41586-022-00228-x.
2. Pandey N, Cascella M. Beta Lactam Antibiotics. 2022 Sep 26. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. PMID: 31424895.
3. EMA. Zavicefta. 2022. [Internet]. Available at: <https://www.ema.europa.eu/en/medicines/human/EPAR/zavicefta> (Accessed: April 27, 2023).
4. Antimicrobial prescribing: Meropenem with vaborbactam (2022). [Internet]. Available at: <https://www.nice.org.uk/advice/es21/evidence/evidence-review-pdf-6965792605> (Accessed: April 28, 2023).
5. EMA. Vaborem. 2022. [Internet]. Available at: <https://www.ema.europa.eu/en/medicines/human/EPAR/vaborem> (Accessed: 11 May 2023).
6. Zhanel GG, Chung P, Adam H, et al. Ceftolozane/tazobactam: a novel cephalosporin/ β -lactamase inhibitor combination with activity against multidrug-resistant gram-negative bacilli. *Drugs* 2014;74(1):31-51.
7. Sawa T, Kooguchi K, Moriyama K. Molecular diversity of extended-spectrum β -lactamases and carbapenemases, and antimicrobial resistance. *J Intensive Care* 2020;8:13.
8. Halat DH, Sarkis DK, Moubareck CA. Carbapenem-Resistant, Gram-Negative Bacilli: The State of the Art. In: *Antibiotic Resistance*, Eds. Kon K, Rai M. Academic Press 2016; pp. 93-119, ISBN 9780128036426.
9. Beta-lactams and Methicillin-Resistant *Staphylococcus aureus* - Laboratory Continuing Education. [Internet]. Available at: www.labce.com/spg1142268_beta_lactams_and_methicillin_resistant_staphylococ.aspx#:~:text=MRSA%20is%20resistant%20to%20the, longer%20the%20drug%20of%20choice. (Accessed: 11 May 2023).
10. Alby K, Miller MB. Mechanisms and Detection of Antimicrobial Resistance. In: *Principles and Practice of Pediatric Infectious Diseases* (6th ed), Eds. Long SS. Elsevier 2023; pp.1506-16, ISBN 9780323756082.
11. Akpaka PE, Vaillant A, Wilson C, et al. Extended Spectrum Beta-Lactamase (ESBL) Produced by Gram-Negative Bacteria in Trinidad and Tobago. *Int J Microbiol* 2021;2021:5582755.
12. Hori T, Owusu YB, Sun D. US FDA-Approved Antibiotics During the 21st Century. In: *Encyclopedia of Infection and Immunity*. Ed. Rezaei N. Elsevier 2022; pp. 556-85, ISBN 9780323903035.
13. Charlton CL, Babady E, Ginocchio CC, et al. Practical Guidance for Clinical Microbiology Laboratories: Viruses Causing Acute Respiratory Tract Infections. *Clin Microbiol Rev* 2018;32(1):e00042-18.
14. Bush K, Bradford PA. β -Lactams and β -Lactamase Inhibitors: An Overview. *Cold Spring Harb Perspect Med* 2016;6(8):a025247.
15. Then R. Beta-Lactamase*. In: *xPharm: The Comprehensive Pharmacology Reference*, Eds. Enna SJ, Bylund DB. Elsevier 2007; pp. 1-9, ISBN 9780080552323.
16. Lahiri SD, Johnstone MR, Ross PL, et al. Avibactam and class C β -lactamases: mechanism of inhibition, conservation of the binding pocket, and implications for resistance. *Antimicrob Agents Chemother* 2014;58(10):5704-13.
17. Falcone M, Paterson D. Spotlight on ceftazidime/avibactam: a new option for MDR Gram-negative infections. *J Antimicrob Chemother* 2016; 71(10):2713-22.
18. Carcione D, Siracusa C, Sulejmani A, et al. Old and New Beta-Lactamase Inhibitors: Molecular Structure, Mechanism of Action, and Clinical Use. *Antibiotics (Basel)* 2021;10(8):995.
19. Smith PW, Zuccotto F, Bates RH, et al. Pharmacokinetics of β -Lactam Antibiotics: Clues from the Past To Help Discover Long-Acting Oral Drugs in the Future. *ACS Infect Dis* 2018;4(10):1439-47.

20. Gutiérrez-Gutiérrez B, Pérez-Galera S, Salamanca E, et al. A Multinational, Preregistered Cohort Study of β -Lactam/ β -Lactamase Inhibitor Combinations for Treatment of Bloodstream Infections Due to Extended-Spectrum- β -Lactamase-Producing Enterobacteriaceae. *Antimicrob Agents Chemother* 2016; 60(7):4159-69.
21. Daikos GL, da Cunha CA, Rossolini GM, et al. Review of Ceftazidime-Avibactam for the Treatment of Infections Caused by *Pseudomonas aeruginosa*. *Antibiotics (Basel)* 2021;10(9):1126.
22. Abodakpi H, Wanger A, Tam VH. What the Clinical Microbiologist Should Know About Pharmacokinetics/Pharmacodynamics in the Era of Emerging Multidrug Resistance: Focusing on β -Lactam/ β -Lactamase Inhibitor Combinations. *Clin Lab Med* 2019;39(3):473-85.
23. EMA. Assessment report: Zerbaxa. 2019. [Internet] Available at: https://www.ema.europa.eu/en/documents/variation-report/zerbaxa-h-c-3772-ii-0020-epar-assessment-report-variation_en.pdf (Accessed: 11 May 2023).
24. Kowalska-Krochmal B, Dudek-Wicher R. The Minimum Inhibitory Concentration of Antibiotics: Methods, Interpretation, Clinical Relevance. *Pathogens* 2021;10(2):165.
25. Principe L, Lupia T, Andriani L, et al. Microbiological, Clinical, and PK/PD Features of the New Anti-Gram-Negative Antibiotics: β -Lactam/ β -Lactamase Inhibitors in Combination and Cefiderocol—An All-Inclusive Guide for Clinicians. *Pharmaceuticals (Basel)* 2022;15(4):463.

Pectin-based Drug Formulations for Gastrointestinal Targeted Delivery

Plamen Simeonov¹, Paolina Lukova², Bissera Pilicheva^{1,3}

1. Department of Pharmaceutical Sciences, Faculty of Pharmacy, Medical University of Plovdiv

2. Department of Pharmacognosy and Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University of Plovdiv

3. Research Institute at Medical University of Plovdiv

Introduction: Mucoadhesive oral drug delivery systems have always been of a great interest for pharmaceutical scientists due to their numerous advantages, compared to the conventional dosage forms, such as longer retention time, extended drug release, improved therapeutic outcome, etc. All these advantages make opportunities for the investigation of polymers that have appropriate parameters to be used as mucoadhesive carriers. **Aim:** The aim of this study is to overview the mucoadhesive properties of different pectin derivatives and to evaluate their potential role in the formulation of oral drug delivery systems. **Results and discussion:** Pectin is a biopolymer extracted from citrus peel and apple pomace. It is a linear polysaccharide, composed of d-galacturonic residues with 1–4 linkage and carboxyl groups. Based on the degree of esterification (DE), pectins are classified as high methoxyl (DE > 50%) and low methoxyl (DE < 50) derivatives. The latter group is an object of increased scientific research due to its properties to form gel structures, which can be used as a micro- or nanosized delivery system. **Conclusion:** Overall, pectin is a promising polymer for the development of mucoadhesive drug formulations, intended for drug targeting to the gastrointestinal tract. Its ability to form different types of drug delivery systems makes it a suitable carrier for various drug substances.

Keywords: pectin, polysaccharides, gastrointestinal tract, drug delivery

Introduction

Mucoadhesion can be defined as the ability of natural or synthetic macromolecules to adhere for an extended period of time to the mucus that covers biological tissues [1]. The attachment and bonding of mucoadhesive polymers to the mucous surface depend mainly on the strength and degree of interaction between the functional groups of the polymer and the mucin – the main component of the mucus. Mucin is an anionic polyelectrolyte at a neutral pH. Due to the presence of both hydrophilic (glycosylated) and hydrophobic (non-glycosylated) units in its structure, it possesses relatively strong adhesive properties, forming mainly hydrogen bonds and participating in hydrophobic and electrostatic interactions. Most mucoadhesive polymers have hydrophilic structure, which contains a large number of polar functional groups. These groups allow the polymer not only to physically attach to the

mucous surface, but also to subsequently form chemical bonds [2–4].

Mucoadhesion is of particular importance for the development of polymer drug formulations for oral, buccal, nasal, ophthalmic, vaginal and rectal administration. The use of mucoadhesive polymers allows localization of the drug substance to the desired site of absorption. Due to the adhesion, an extended contact time between the administered formulation and the targeted biological tissue can be achieved, which can significantly increase the drug bioavailability. With mucoadhesive drug delivery systems, the frequency of the dosage form administration can be reduced, which can lead to better patient compliance [5].

The formulation of mucoadhesive drug delivery systems depends mainly on the properties of the polymers used. Most natural polysaccharides, such as pectin, chitosan, alginate, cellulose, etc., possess very good

mucoadhesive properties. They can interact with the mucous surface through non-covalent chemical bonds – mostly hydrogen, van der Waals or ionic bonds, which ensure the effective attachment of the polymer to the targeted tissue [6]. Therefore, polysaccharides have been outlined as promising drug carriers that can successfully guide drug substances to desired mucous surfaces in the body, including the gastrointestinal tract.

Aim

The aim of the study is to review the mucoadhesive properties of different pectin derivatives and to evaluate their potential role in oral drug delivery systems.

Material and methods

The review article is based on literature found in the databases of PubMed, Web of science and Science Direct. The performed survey was within the year interval 2000–2023, and 25 references were selected according to their relevance to the topic of research.

Results and discussion

Pectin is an anionic polymer isolated mainly from citrus fruits and apples. It is a non-toxic natural polysaccharide, often used in food industry as a viscosity-enhancing or gelling agent [7, 8]. The chemical structure of pectin consists mainly of α -1,4-linked residues of D-galacturonic acid, partially esterified with methanol or acetylated. Rhamnose residues can also be found in the main chain of pectin, while other neutral monosaccharides such as arabinose, galactose, xylose etc. can be in the side chains of the polymer [9]. Pectin polymers differ in their content of uronic acids and neutral sugars, in the type of glycosidic bonds, the degree of esterification, amide content and molecular weight. Depending on their chemical structure, they are classified into two large groups: acid pectin polysaccharides – homogalacturonan (HG), xylogalacturonan (XHG), apiogalacturonan (AHG), rhamnogalacturonan I (RG I) and rhamnogalacturonan II (RG II) and neutral pectin polysaccharides – galactans, arabinans, arabinogalactans (AG) [10].

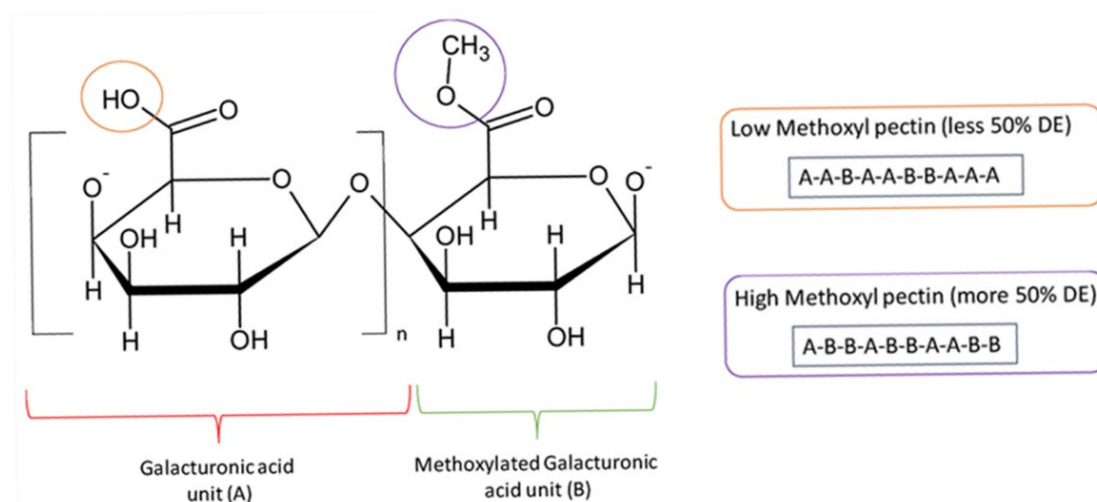


Figure 1. Chemical structure of pectin (with low and high methoxylation).

The ratio between esterified and free acid groups in the structure of pectin determines its degree of esterification (DE), which is an important factor influencing various properties of the polysaccharide such as solubility and ability to form gels. Depending on the DE pectin can be classified as a polymer with a high degree of methoxylation (HM; DE > 50%), or a polymer with a low methoxylation (LM; DE < 50%) [11]. HM pectins form gels at high concentrations, while those with LM – only in the presence of divalent ions [12]. The polysaccharide is stable in an acidic environment, even at high temperatures. Its molecules form aggregates at low pH, which are unstable in neutral environments. Pectin is resistant to the protease enzymes and amylase found in the upper parts of the gastrointestinal tract (GIT), while it is degraded by microorganisms in the colon [13].

As a drug carrier pectin demonstrates good mucoadhesive properties, attaching easily to mucous surfaces in the body. Many studies report the successful development of drug-loaded formulations using this polymer, especially for drug targeting in the GIT [14, 15]. To increase its mucoadhesive properties, different modification of the polymer can be developed. One of the most commonly used modified derivatives of pectin is the thiolated form of the polysaccharide. Thiolated pectins are usually synthesized by esterification with thioglycolic acid. This process implements sulfhydryl residues in the molecule. Thiolated pectins interact with the intestinal mucosa with hydrogen and electrostatic bonds, as other pectins do and the presence of the sulfhydryl residues implements a third type bonds through formation of disulfide bridges. Due to the above-mentioned characteristics, thiolated pectins tend to show stronger adhesive properties, compared to the unmodified polymer [16]. Sharma et al., for example, have

successfully formulated metformin-loaded beads with thiolated pectin using gelation method with calcium chloride as a cross-linker. The beads were tested *ex vivo* on goat intestine for their mucoadhesive properties and were compared to formulations with unmodified pectin. The results showed that the beads produced from thiolated polysaccharide adhered to the intestine for more than 300 minutes, whereas the beads from unmodified pectin were washed away within 150 minutes [16].

Pectin microparticles

Due to its relatively easy particle-forming properties, pectin is commonly used as a polymer drug carrier to produce micro- and nano-sized drug delivery systems. Drug-loaded polymer microparticles are associated with numerous advantages such as large surface area, high stability and possibility for targeted and controlled drug release. They belong to the so called “multiple units” systems, which makes them a reliable drug delivery system, as each particle releases independently its drug load [17]. Pectin microparticles can be formulated using standard preparation methods like spray drying, emulsification technique, ionotropic gelation. Vaidya et al. have used emulsion dehydration technique to obtain pectin microspheres with conjugated metronidazole in the presence of N, N-dicyclohexyl carbodiimide. The formulated polymer structures showed high drug entrapment efficiency up to 94.52%, depending on the degree of substitution. The systems showed significantly prolonged drug release, as after 8 hours around 20% of the incorporated metronidazole was released, compared to the non-conjugated drug (94% of released drug). The performed biodistribution analysis showed that the microparticles primarily accumulated in the colon after oral administration [18]. In another study, Deshmukh et al.

prepared microparticles from amidated pectin loaded with sulfasalazine for treatment of inflammatory bowel disease via modified ionic gelation technique using CaCl_2 . The obtained microparticles again showed high encapsulation efficiency of 95%. Drug release was investigated *in vitro*, and the results showed greater release in rat cecal medium, compared to simulated gastric fluid [19].

Pectin nanoparticles

Nanotechnologies provide the opportunity to develop new formulations that permit slow and selective release of various drugs at specific locations in the gastrointestinal tract. Pectin nanoparticles have been extensively studied as drug delivery systems due to their various advantages such as controlled drug release, improved drug permeability and precise targeting [13]. For example, pectin was used as a nanocarrier for curcumin in the preparation of polyelectrolyte core-shell nanostructures with zein [20]. The core was made from hydrophobic zein in which amorphous curcumin was dissolved. The shell was made of pectin, which was negatively charged at pH 4. The particles were formulated by electrostatic interactions between anionic pectin and cationic zein. The core-shell nanoparticles showed mean diameter size of 250 nm and relatively narrow particle size distribution, as the polydispersity index (PDI) was 0.24. Drug loading efficiency was between 86.8% and 91.8%, as it slightly decreased with the increase of the

polymer concentration.

In another study, Zhang et al. managed to synthesize folic acid-modified pectin nanoparticles, which were dually crosslinked using Ca^{2+} and adipic dihydrazide [21]. The particles were loaded with 2,3-dimethylmaleic anhydride-modified insulin and drug release was investigated in simulated gastrointestinal fluids, as well as *in vivo* in diabetic rat models. The fabricated particles had a mean diameter between 102 and 270 nm, with an encapsulation efficiency of 99%. The particles were tested in three media – simulated gastric fluid (pH 1.2), simulated intestinal fluid (pH 6.8) and in neutral medium (pH 7.4) without enzymes. The nanoparticles showed initial release of 16% of the incorporated drug in acidic medium, whereas in pH 6.8 and pH 7.4, they demonstrated sustained release, fitting to the Korsmeyer-Peppas kinetic model. Furthermore, *in vivo* tests indicated a prolonged glucose blood level decrease, compared to the subcutaneous injections, which caused a sharp decrease of the glucose levels.

Pectin liposomes

Liposomes are another commonly used nanosized drug delivery systems, which can offer advantages such as improved solubility, enhanced drug stability and targeted delivery. A study by Gottesmann et al. demonstrated that amoxicillin-loaded liposomes can be stabilized through pectin coating, resulting in a mucoadhesive formulations for treatment of *Helicobacter pylori* (Figure 2) [22].

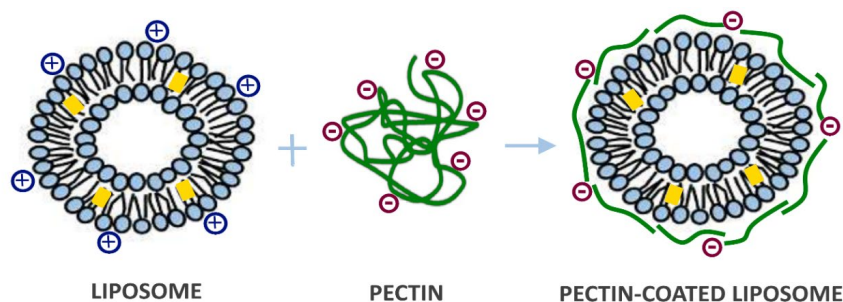


Figure 2. Cationic lipids containing liposomes coated with anionic pectin.

Liposomes were produced by thin film hydration method and then the obtained suspension was added dropwise to the pectin solution. The mucoadhesive characteristics of the coated liposomes were tested using soluble mucin from porcine stomach as well as mucus produced by HT29-MTX cells. Pectin coated liposomes showed significantly higher degree of mucoadhesion, compared to the uncoated ones. The stabilization effect of pectin was evident from the evaluated drug encapsulation efficiency – 83% for the coated liposomes compared to 66% for the uncoated structures. Although both formulations had an initial burst release, within the *in vitro* test the uncoated liposomes released 85% of the encapsulated drug, while the pectin-coated formulations released 75%. Fluorescence microscopic analysis was used to investigate the interaction between the liposomes and *H. pylori*. Pectin-coated liposomes showed high interaction with the bacterial cell wall, resulting in changed fluorescent signal, whereas the uncoated liposomes indicated no interaction, which was visible as two separated signals. The results complied with the consideration that pectin can inhibit the BabA and LPS-mediated adhesion of *H. pylori* to human stomach cells.

Pectin hydrogels

As already mentioned, pectin has good gel-forming properties and can be easily crosslinked in the presence of divalent ions or in combination with cationic polymers. There are many studies reporting the utilization of drug-loaded pectin hydrogels for treating different GIT-conditions [23–25]. Gautam et al., for example, prepared two types of pectin-based hydrogels for co-delivery of Ca^{2+} /Vitamin D₃ and Fe^{2+} /Vitamin C [24]. The gels were produced from pectin/PEG polymer blend matrix. The prepared drug delivery systems were analyzed for

entrapment efficiency, swelling properties, gel degradation as well as *in vitro* drug release profiles. Entrapment efficiency for vitamin D and vitamin C was evaluated using UV-Vis spectroscopy and metal ion content (Ca^{2+} and Fe^{2+}) was evaluated by ICP-emission spectrometer. The results showed an entrapment efficiency of 99.1% for vitamin D and 99.3% for vitamin C. Metal ion contents were 99.2% for Ca^{2+} and 99.6% for Fe^{2+} . The swelling index of the Ca^{2+} /Vitamin D hydrogels was measured to be 64% after 3 hours in simulated gastric fluid (SGF) and 130% after 6 hours in simulated intestinal fluid (SIF). The same measurement was conducted for the Fe^{2+} /Vitamin C hydrogels, which showed swelling index of 52% in SGF and 110% in SIF. The two hydrogels were compared to a blank pectin/PEG gel which showed 41% swelling index in SGF and 61% in SIF. Drug release studies indicated that 19% of vitamin D was released after 3 hours in SGF and 95% were released after 6 hours in SIF, whereas 19% of vitamin C were released after 3 hours in SGF and 91% in SIF, respectively.

In another study, Minhas et al. fabricated cross-linked pectin-co-poly(methacrylic acid) hydrogel for oral delivery of 5-fluorouracil (FU), intended for colon targeted delivery, which was tested *in vivo* in rabbit models [25]. The formulated systems showed 60% *in vitro* released drug at pH 1.2 and more than 90% at pH 7.4 after 12 hours. It was concluded that the results were due to the swelling behavior of the hydrogel which was significantly higher at pH 7.4.

Conclusions

As a natural, biocompatible, biodegradable, and non-toxic polymer, pectin has been widely used as an excipient in pharmaceutical technology. Its ability to form multiple types of drug delivery systems makes it a

suitable carrier for different groups of medicinal substances. Based on the various studies on pectin, it can be concluded that this polysaccharide is a promising polymer for the development of mucoadhesive dosage forms such as microspheres, nanoparticles, liposomes, hydrogels, etc., for targeted drug delivery to the gastrointestinal tract.

Acknowledgments

The publication is supported by the European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria project number BG-RRP-2.004-0007-C01.

References

1. Alawdi S, Solanki AB. Mucoadhesive drug delivery systems: A review of recent developments. *Journal of Scientific Research in Medical and Biological Sciences*. 2021 Feb 28;2(1):50-64.
2. Mansuri S, Kesharwani P, Jain K, Tekade RK, Jain NK. Mucoadhesion: A promising approach in drug delivery system. *Reactive and functional polymers*. 2016 Mar 1;100:151-72.
3. Alaei S, Omidian H. Mucoadhesion and mechanical assessment of oral films. *European Journal of Pharmaceutical Sciences*. 2021 Apr 1;159:105727.
4. Ashoor JA, Mohammed J, ALAayed M. The Principle of Mucoadhesion, Classifications of Mucoadhesive Polymers, Applications and Methods of Evaluation: A review Article. *kerbala journal of pharmaceutical sciences*. 2021;1(19).
5. Asati S, Jain S, Choubey A. Bioadhesive or mucoadhesive drug delivery system: a potential alternative to conventional therapy. *Journal of Drug Delivery and Therapeutics*. 2019 Nov 11;9(4-A):858-67.
6. Boddupalli BM, Mohammed ZN, Nath RA, Banji D. Mucoadhesive drug delivery system: An overview. *Journal of advanced pharmaceutical technology & research*. 2010 Oct;1(4):381.
7. Gawkowska D, Cybulska J, Zdunek A. Structure-related gelling of pectins and linking with other natural compounds: A review. *Polymers*. 2018 Jul 11;10(7):762.
8. Perez S, Rodríguez-Carvajal MA, Doco T. A complex plant cell wall polysaccharide: rhamnogalacturonan II. A structure in quest of a function. *Biochimie*. 2003 Jan 1;85(1-2):109-21.
9. Bonnín E, Garnier C, Ralet MC. Pectin-modifying enzymes and pectin-derived materials: applications and impacts. *Applied microbiology and biotechnology*. 2014 Jan;98:519-32.
10. Voragen AG, Coenen GJ, Verhoef RP, Schols HA. Pectin, a versatile polysaccharide present in plant cell walls. *Structural Chemistry*. 2009 Apr;20:263-75.
11. Esposito E, Cortesi R, Luca G, Nastruzzi C. Pectin-Based Microspheres: A Preformulatory Study. *Annals of the New York Academy of Sciences*. 2001 Nov;944(1):160-79.
12. Chen J, Liu W, Liu CM, Li T, Liang RH, Luo SJ. Pectin modifications: a review. *Critical reviews in food science and nutrition*. 2015 Oct 15;55(12):1684-98.
13. Mishra RK, Banthia AK, Majeed AB. Pectin based formulations for biomedical applications: a review. *Asian J Pharm Clin Res*. 2012;5(4):1-7.
14. Shukla S, Jain D, Verma K, Verma S. Pectin-based colon-specific drug delivery. *Chronicles of Young Scientists*. 2011 Apr 1;2(2):83.
15. Das S. Pectin based multi-particulate carriers for colon-specific delivery of therapeutic agents. *International Journal of Pharmaceutics*. 2021 Aug 10;605:120814.
16. Sharma R, Ahuja M. Thiolated pectin: Synthesis, characterization and evaluation as a mucoadhesive polymer. *Carbohydrate Polymers*. 2011 Jun 1;85(3):658-63.
17. Gutierrez-Alvarado K, Chacón-Cerdas R, Starbird-Perez R. Pectin Microspheres: Synthesis Methods, Properties, and Their Multidisciplinary Applications. *Chemistry*. 2022 Mar 1;4(1):121-36.
18. Vaidya A, Jain S, Agrawal RK, Jain SK. Pectin-metronidazole prodrug bearing microspheres for colon targeting. *Journal of Saudi Chemical Society*. 2015 May 1;19(3):257-64.
19. Deshmukh R, Harwansh RK, Paul SD, Shukla R. Controlled release of sulfasalazine loaded amidated pectin microparticles through Eudragit S 100 coated capsule for management of inflammatory bowel disease. *Journal of Drug Delivery Science and Technology*. 2020 Feb 1;55:101495.
20. Hu K, Huang X, Gao Y, Huang X, Xiao H, McClements DJ. Core-shell biopolymer nanoparticle delivery systems: Synthesis and characterization of curcumin fortified zein-pectin nanoparticles. *Food chemistry*. 2015 Sep 1;182:275-81.
21. Zhang F, Pei X, Peng X, Gou D, Fan X, Zheng X, Song C, Zhou Y, Cui S. Dual crosslinking of folic acid-modified pectin nanoparticles for enhanced oral insulin delivery. *Biomaterials Advances*. 2022 Apr 1;135:212746.
22. Gottesmann M, Goycoolea FM, Steinbacher T, Menogni T, Hensel A. Smart drug delivery against *Helicobacter pylori*: pectin-coated, mucoadhesive liposomes with antiadhesive activity and antibiotic cargo. *Applied Microbiology and Biotechnology*. 2020 Jul;104:5943-57.
23. Han SS, Ji SM, Park MJ, Suneetha M, Uthappa UT. Pectin Based Hydrogels for Drug Delivery Applications: A Mini Review. *Gels*. 2022 Dec;8(12):834.
24. Gautam M, Santhiya D. Pectin/PEG food grade hydrogel blend for the targeted oral co-delivery of

nutrients. Colloids and Surfaces A: Physicochemical and Engineering Aspects. 2019 Sep 20;577:637-44.
25. Minhas MU, Ahmad M, Anwar J, Khan S. Synthesis and characterization of biodegradable hydrogels for oral delivery of 5-fluorouracil targeted to colon: screening with preliminary in vivo studies. Advances in Polymer Technology. 2018 Feb;37(1):221-9.

Corresponding author

Plamen Simeonov
Department of Pharmaceutical Sciences
Faculty of Pharmacy
Medical University of Plovdiv
plamen.simeonov@mu-plovdiv.bg

Recent Advances in the Formulation of Polysaccharide-based Microparticles Using Jet Break-up and Microfluidic Techniques

Antonios Tsormpatzian¹, Paolina Lukova², Dimitar Penkov³, Plamen Katsarov^{3,4}

1. Faculty of Pharmacy, Medical University of Plovdiv

2. Department of Pharmacognosy and Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University of Plovdiv

3. Department of Pharmaceutical Sciences, Faculty of Pharmacy, Medical University of Plovdiv

4. Research Institute at Medical University of Plovdiv

Introduction: Polysaccharide-based microparticles, as drug-delivery systems, are developed in order to obtain controlled or targeted drug delivery, enhanced bioavailability and stability, reduced toxicity, improved efficacy and patient compliance. The choice of a preparation method depends on several factors, like the nature of the active substance, properties of the carrier, route of administration, etc. and it affects the final product characteristics – particle size, size distribution, surface morphology, encapsulation efficiency. **Aim:** Evaluation of the recent progress in the formulation of polysaccharide microparticles with focus on the novel preparation techniques, offering precise control of the particle size. **Materials and methods:** The survey includes literature within the year interval 2000–2023, searched according to its relevance to the topic in the databases of PubMed, Web of science and Science Direct. **Results and discussion:** The drive to obtain microcarriers with a small polydispersity index leads to the development of technological approaches for formulation of monodisperse particles. Precise control of the particles size and structure can be achieved through production methods like jet break-up and microfluidic technologies. These techniques involve injecting a polymer solution through a nozzle, forming a laminar jet, which is subsequently broken into microspheres or using microfluidic devices that allow a polymer solution to be emulsified in the form of microdroplets using microchannels or microporous membranes. **Conclusion:** Novel technologies allow the preparation of polymer microparticles with uniform diameter and morphology, which is necessary in order to relate these properties to the drug release behavior and other functionalities such as drug encapsulation and dissolution.

Keywords: polysaccharides, microparticles, jet break-up methods, microfluidic techniques

Introduction

Polymer drug-loaded microparticles are particulate drug delivery systems, which offer both therapeutic and technological advantages, such as modified and targeted (site-specific) drug delivery and release, enhanced drug bioavailability and stability, improved therapeutic efficacy and reduced toxicity, individual therapy, and better patient compliance [1, 2]. The size of the microparticles can vary from 1 to 1000 μm , their structure can be either homogenous or heterogeneous and they can have spherical or irregular shape (torus-shaped, bullet-shaped, cubic-shaped microparticle). There are different types of microstructures – core-shell, multiple cores, coated, multi-wall, etc., but microparticles are mainly classified as matrix systems (microspheres) or reservoir

systems (microcapsules) [3]. Although microparticles can be composed of various materials – natural, semi-synthetic or synthetic polymers, biopolymers (mainly polysaccharides and proteins) are preferred due to their biodegradable, biocompatibility, and non-toxicity [4, 5].

There are different methods for preparing polymer microparticles. The choice of production method depends on various factors, such as: the nature of the active pharmaceutical ingredient (API) and its characteristics, the properties of the polymer carrier, the route of administration, the type of the drug formulation. The preparation method should allow control of the particle size, high drug entrapment efficiency, high drug stability, high production yield, drug release with a desired rate at a desired site [6, 7].

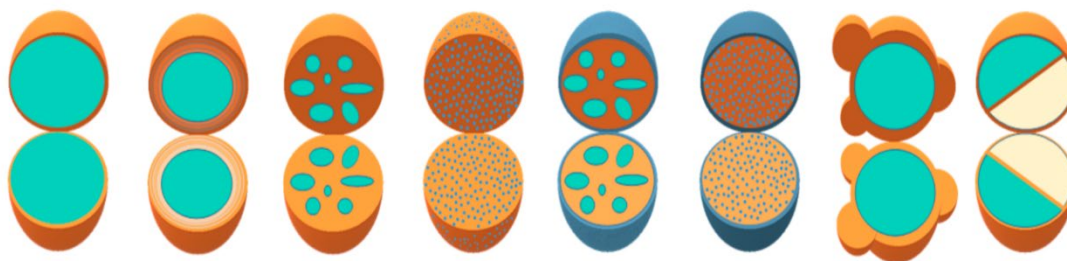


Figure 1. Different types of polymer microstructures [3].

Aim

The aim of the study is to evaluate the recent progress in the formulation of polysaccharide microparticles with focus on the novel preparation techniques like jet break-up methods and microfluidic technologies, which offer precise control of the particle size.

Material and methods

The review article is based on literature found in the databases of PubMed, Web of science and Science Direct. The performed survey was within the year interval 2000–2023, and 30 references were selected according to their relevance to the topic of research.

Results and discussion

Classical methods for microparticles preparation

Spray drying is one of the most used methods for preparation of polysaccharides microparticles. It is a relatively simple and easy to operate technique, which involves three main steps: a solution or dispersion of the polymer and the API is sprayed through a nozzle, micro-sized droplets are formed and the droplets are dried into solid particles [8]. Another classical particle preparation

technique is the emulsification solvent evaporation method – a solution of the polymer/API in organic solvent is emulsified in water, forming O/W emulsion. The organic solvent subsequently evaporates forming solid particles (Figure 2) [9]. Most polymers, which have anionic or cationic properties, can form microparticles when their solutions are mixed with solutions of oppositely charged materials. Electrostatic interactions occur, which lead to the formation of coacervates (complex coacervation method) [10].

Besides the advantages of the classical methods for production of microparticles, which have been thoroughly studied and the influence of their technological parameters on the particles' properties have been investigated in detail, these techniques also have some limitations. One of their disadvantages is the inability to obtain particles of a uniform size. Although the diameter of the resulting powder particles can be in the micro range, the obtained products usually have a very high polydispersity index. Size and particle size distribution are essential characteristics, which affect the stability, flowability, drug loading and drug release of the microparticles [11, 12]. Therefore, technological approaches for obtaining particles with uniform size are being developed.

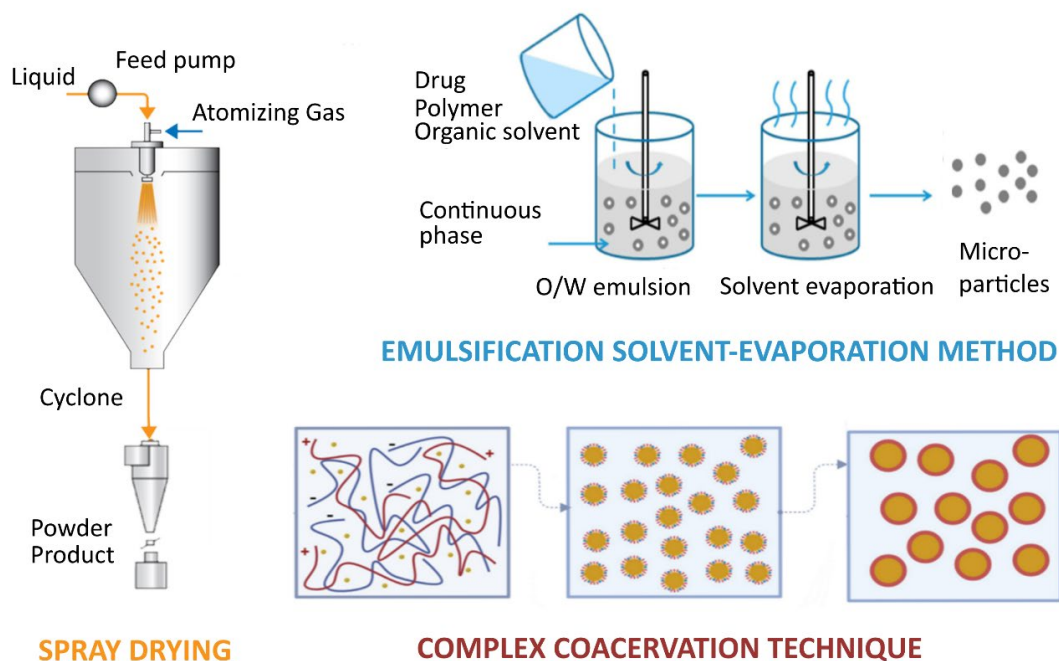


Figure 2. Preparation of polymer microparticles through spray drying, emulsification solvent-evaporation and complex coacervation techniques.

Jet break-up techniques

In recent years, as a result of the scientists' efforts to obtain monodisperse microparticles, new production methods are being created such as jet break-up and microfluidic techniques. In the jet break-up process, a polymeric solution with the API is pushed through a nozzle at a constant flow rate, forming a laminar jet. The jet is then broken into a chain of monodispersed droplets using different approaches [12]. Variants of this method include electrospraying, jet-cutting and jet vibration excitation.

The electrospraying technique uses electrostatic force to break the liquid sample into uniform droplets. There are two main types of electrospraying: dripping mode and jet mode. The first one applies low electric current (up to 4 kV) and the microparticles size is between 500–1000 μm . For the jet mode, a stable jet is formed, which is broken into droplets under higher electric current and the microparticles size is between 1–15 μm [13, 14].

Another option for jet break-up are the jet-cutting and jet vibration excitation techniques [15]. A dispersed jet is cut into uniform segments by means of a cutting tool consisting of several wires (Figure 3A) or the jet is broken into uniform segments by means of vibration excitation (Figure 3B).

Microfluidic microchannel techniques

Microfluidic techniques are another relatively new approach to creating uniform microdroplets that can subsequently be hardened into powder particles. In these methods, the dispersed phase (a polymer solution with the API) is pushed through a microchannel or micro-sized membrane and broken into droplets at the end of the channel or the membrane [17–22]. Microchannel devices with different geometry have been designed, as the most popular among them are: the terrace-like microchannel device, the flow focusing device and the T-junction microchannel device (Figure 4).

The terrace-like microchannels open to a terrace that descends to a well through which the continuous phase slowly passes. When the dispersed phase reaches the rim of the well, it flows over the rim into the well and interfacial forces contract the fluid to form droplets (Figure 4A) [12, 22, 23]. The flow focusing devices, on the other hand, are based on the principle of hydrodynamic concentration. The dispersed phase flows through a central microchannel, while a continuous medium is supplied by two side channels. In front of the central channel there is a small hole that allows a continuous medium to cut the flow of the dispersed phase, turning it into droplets of equal size

(Figure 4B) [24]. In the T-junction micro-channel device, the phase to be converted into drops is supplied by microchannels perpendicular to the central channel through which the dispersed medium flows (Figure 4C) [25].

Microfluidic microporous membrane techniques

In the microporous membrane technique, dispersed drops are generated at the membrane surface by pushing the dispersed (inner) phase through the membrane pores into the continuous (outer) phase under applied pressure of about 0.5–5 bars usually using nitrogen gas [27, 28].

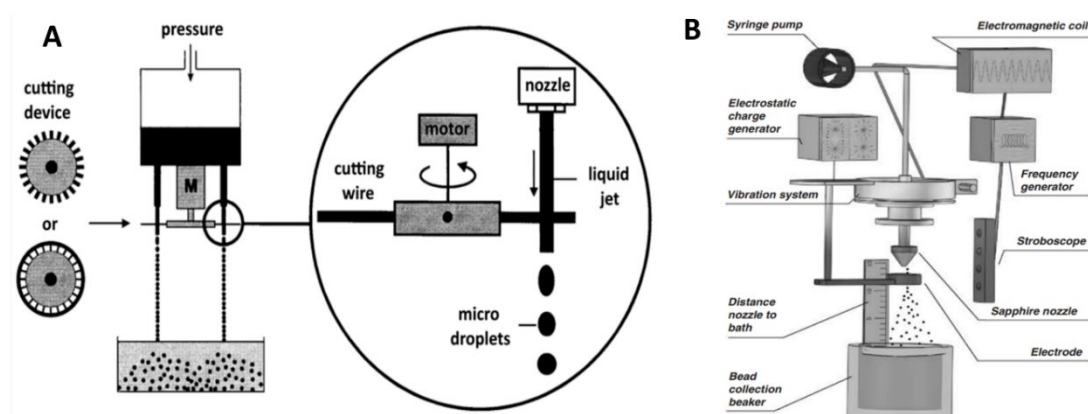


Figure 3. Illustrations for: A) jet-cutting and B) jet vibration excitation techniques for preparation of uniform microparticles [15, 16].

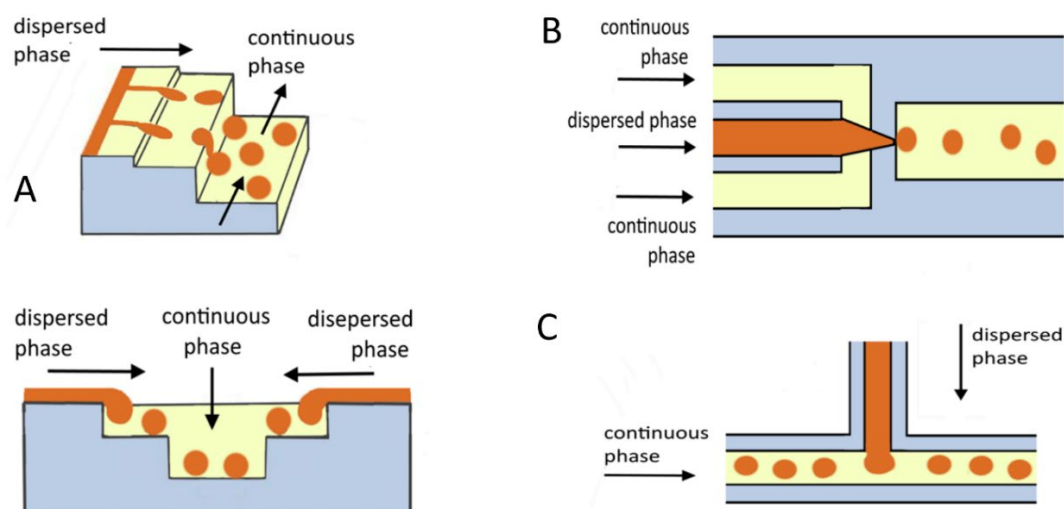


Figure 4. Illustrations for: A) terrace-like microchannel device, B) flow focusing device and C) T-junction microchannel device [26].

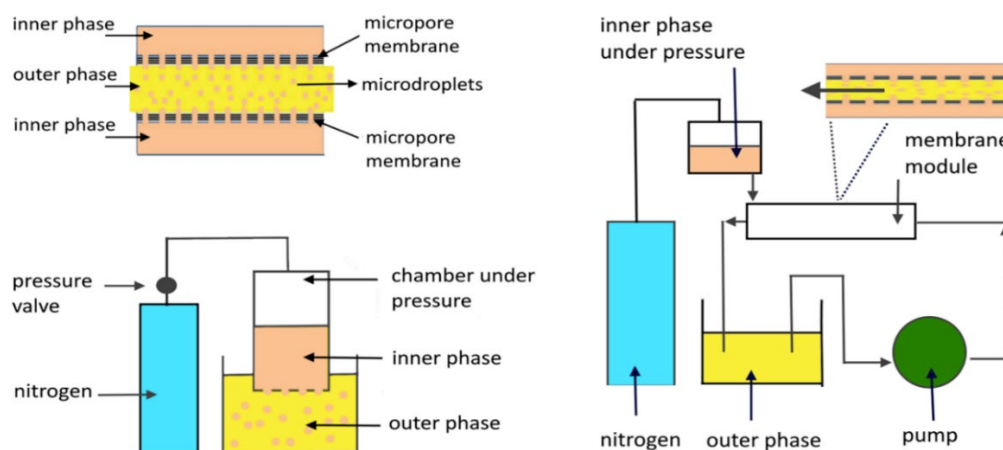


Figure 5. Microporous membrane emulsification techniques for preparation of polysaccharide-based microparticles.

The pore diameter of the membrane has the most significant influence on the size of the resulting microstructures. The droplet size can vary from 2 to 10 times the pore diameter, depending on the interfacial tension between the dispersed phase flow, the membrane surface and the dispersed medium. The membranes used, with a pore size of 0.49 to 40 μm , allow obtaining microparticles ranging in size from 10 μm to about 100 μm . For more efficient separation of the formulated droplets from the membrane and to reduce their fusion into larger structures, ultrasonic or vibrational membrane emulsification can be applied, in which the membrane performs vibrations at a certain frequency. In this way, the production rate can be increased and microspheres of 1–2 μm size can be obtained [29, 30].

Conclusions

The drive to obtain microcarriers with a small polydispersity index leads to the development of technological approaches for formulation of monodisperse particles such as jet break-up and microfluidic technologies. These methods allow precise control of the particles size and enable the preparation of polymer microparticles with uniform diameter and morphology, which is necessary

to relate these properties to the drug release behavior and other functionalities such as drug encapsulation and dissolution.

Acknowledgments

The publication is supported by the European Union-NextGenerationEU, through the National Recovery and Resilience Plan of the Republic of Bulgaria project number BG-RRP-2.004-0007-C01.

References

1. Yawalkar AN, Pawar MA, Vavia PR. Microspheres for targeted drug delivery-A review on recent applications. *Journal of Drug Delivery Science and Technology*. 2022 Aug 4;103659.
2. Raj H, Sharma S, Sharma A, Verma KK, Chaudhary A. A novel drug delivery system: review on microspheres. *Journal of Drug Delivery and Therapeutics*. 2021 Apr 15;11(2-S):156-61.
3. Lengyel M, Kállai-Szabó N, Antal V, Laki AJ, Antal I. Microparticles, microspheres, and microcapsules for advanced drug delivery. *Scientia Pharmaceutica*. 2019;87(3):20.
4. Barclay TG, Day CM, Petrovsky N, Garg S. Review of polysaccharide particle-based functional drug delivery. *Carbohydrate polymers*. 2019 Oct 1;221:94-112.
5. Jana P, Shyam M, Singh S, Jayaprakash V, Dev A. Biodegradable polymers in drug delivery and oral vaccination. *European Polymer Journal*. 2021 Jan 5;142:110155.
6. Procopio A, Lagreca E, Jamaledin R, La Manna S, Corrado B, Di Natale C, Onesto V. Recent fabrication methods to produce polymer-based drug delivery matrices (experimental and in Silico approaches). *Pharmaceutics*. 2022 Apr 15;14(4):872.

7. Galogahi FM, Zhu Y, An H, Nguyen NT. Core-shell microparticles: Generation approaches and applications. *Journal of Science: Advanced Materials and Devices*. 2020 Dec 1;5(4):417-35.
8. Baumann JM, Adam MS, Wood JD. Engineering advances in spray drying for pharmaceuticals. *Annual Review of Chemical and Biomolecular Engineering*. 2021 Jun 7;12:217-40.
9. Li M, Rouaud O, Poncelet D. Microencapsulation by solvent evaporation: State of the art for process engineering approaches. *International Journal of pharmaceuticals*. 2008 Nov 3;363(1-2):26-39.
10. Frenț OD, Duteanu N, Teusdea AC, Ciocan S, Vicaș L, Jurca T, Muresan M, Pallag A, Ianasi P, Marian E. Preparation and characterization of chitosan-alginate microspheres loaded with quercetin. *Polymers*. 2022 Jan 26;14(3):490.
11. Siepmann J, Faisant N, Akiki J, Richard J, Benoit JP. Effect of the size of biodegradable microparticles on drug release: experiment and theory. *Journal of Controlled Release*. 2004 Apr 16;96(1):123-34.
12. Tran VT, Benoît JP, Venier-Julienne MC. Why and how to prepare biodegradable, monodispersed, polymeric microparticles in the field of pharmacy?. *International journal of pharmaceuticals*. 2011 Apr 4;407(1-2):1-1.
13. Morais ÁI, Vieira EG, Afewerki S, Sousa RB, Honorio LM, Cambrussi AN, Santos JA, Bezerra RD, Furtini JA, Silva-Filho EC, Webster TJ. Fabrication of polymeric microparticles by electrospray: the impact of experimental parameters. *Journal of functional biomaterials*. 2020 Jan 15;11(1):4.
14. Wang J, Jansen JA, Yang F. Electrospraying: possibilities and challenges of engineering carriers for biomedical applications—a mini review. *Frontiers in Chemistry*. 2019 Apr 25;7:258.
15. Prüße U, Fox B, Kirchhoff M, Bruske F, Breford J, Vorlop KD. New process (jet cutting method) for the production of spherical beads from highly viscous polymer solutions. *Chemical engineering & technology*. 1998 Jan;21(1):29-33.
16. Mazzitelli S, Tosi A, Balestra C, Nastruzzi C, Luca G, Mancuso F, Calafiore R, Calvitti M. Production and characterization of alginate microcapsules produced by a vibrational encapsulation device. *Journal of biomaterials applications*. 2008 Sep;23(2):123-45.
17. Brzeziński M, Socka M, Kost B. Microfluidics for producing polylactide nanoparticles and microparticles and their drug delivery application. *Polymer International*. 2019 Jun;68(6):997-1014.
18. Moreira A, Carneiro J, Campos JB, Miranda JM. Production of hydrogel microparticles in microfluidic devices: a review. *Microfluidics and Nanofluidics*. 2021 Feb;25:1-24.
19. Rosellini E, Cascone MG. Microfluidic Fabrication of Natural Polymer-Based Scaffolds for Tissue Engineering Applications: A Review. *Biomimetics*. 2023 Feb 9;8(1):74.
20. Jo YK, Lee D. Biopolymer microparticles prepared by microfluidics for biomedical applications. *Small*. 2020 Mar;16(9):1903736.
21. He F, Zhang MJ, Wang W, Cai QW, Su YY, Liu Z, Faraj Y, Ju XJ, Xie R, Chu LY. Designable polymeric microparticles from droplet microfluidics for controlled drug release. *Advanced Materials Technologies*. 2019 Jun;4(6):1800687.
22. Xia H, Li J, Man J, Man L, Zhang S, Li J. Recent progress in preparation of functional microparticles based on microfluidic technique. *Materials Today Communications*. 2021 Dec 1;29:102740.
23. van Dijke K, Kobayashi I, Schroën K, Uemura K, Nakajima M, Boom R. Effect of viscosities of dispersed and continuous phases in microchannel oil-in-water emulsification. *Microfluidics and nanofluidics*. 2010 Jul;9:77-85.
24. Roberts CC, Rao RR, Loewenberg M, Brooks CF, Galambos P, Grillet AM, Nemer MB. Comparison of monodisperse droplet generation in flow-focusing devices with hydrophilic and hydrophobic surfaces. *Lab on a Chip*. 2012;12(8):1540-7.
25. Wang JT, Wang J, Han JJ. Fabrication of advanced particles and particle-based materials assisted by droplet-based microfluidics. *small*. 2011 Jul 4;7(13):1728-54.
26. Кацаров П, Лукова П. Полизахаридни микроносители за лекарствена доставка. 2023. Лакс Бук, Пловдив.
27. Veldhuis G, Gironès M, Bingham D. Monodisperse microspheres for parenteral drug delivery. *Drug Deliv Technol*. 2009;9(1):24-31.
28. Doan TV, Olivier JC. Preparation of rifampicin-loaded PLGA microspheres for lung delivery as aerosol by premix membrane homogenization. *International Journal of Pharmaceutics*. 2009 Dec 1;382(1-2):61-6.
29. Charcosset C, Fessi H. Preparation of nanoparticles with a membrane contactor. *Journal of Membrane Science*. 2005 Dec 1;266(1-2):115-20.
30. Vladisavljević GT, Williams RA. Recent developments in manufacturing emulsions and particulate products using membranes. *Advances in colloid and interface science*. 2005 Mar 17;113(1):1-20.

Corresponding author

Plamen Katsarov
Department of Pharmaceutical Sciences
Faculty of Pharmacy
Medical University of Plovdiv
plamen.katsarov@mu-plovdiv.bg

Computational Methods for *in silico* Prediction of Chemical Toxicity

Miglena Milusheva¹, Vera Gledacheva², Stoyanka Nikolova³, Spogami Khattak⁴

1. Department of Bioorganic Chemistry, Faculty of Pharmacy, Medical University of Plovdiv

2. Department of Medical Physics and Biophysics, Faculty of Pharmacy, Medical University of Plovdiv

3. Department of Organic Chemistry, Faculty of Chemistry, University of Plovdiv Paisii Hilendarski

4. Faculty of Medicine, Medical University of Plovdiv

Determining the toxicity of chemicals is necessary to identify their harmful effects on humans, animals, plants, or the environment. It is also one of the main steps in drug design. Animal models have been used for a long time for toxicity testing. To be effective as a drug, a molecule must reach its pharmacological target in the body, achieve an adequate concentration at the site of action, and persist in a bioactive form long enough for the expected biological events to occur. Many compounds failed as drugs because of their poor pharmacokinetics properties and limited bioavailability. The large number of newly synthesized experimental molecules, on one hand, and the quantitative limitations of tissue samples, together with the need to restrict animal testing, on the other hand, prevent systematic recourse to experiments. In this context, *in silico* computer models constitute a valid alternative and useful addition to biological experiments. Therefore, computational methods for estimating the toxicity of chemicals are considered useful. *In silico* toxicology is one type of toxicity assessment that uses computational methods to analyze, simulate, visualize, or predict the toxicity of chemicals. *In silico* toxicology aims to complement existing toxicity tests to predict toxicity, prioritize chemicals, guide toxicity tests, and minimize late-stage failures in drug design.

Keywords: drug design, synthesis, *in silico*, toxicity

Introduction

Chemical effects that are undesired or harmful are measured by their toxicity. Toxicology endpoints are specific categories of these negative effects and include traits like genotoxicity or carcinogenicity. They can be quantitative (e.g., LD50: lethal dose to 50% of tested individuals) or qualitative (e.g., binary, toxic or non-toxic, or ordinary, low, moderate, or high toxicity) [1]. Through acute or multiple exposure, toxicity tests seek to detect the negative effects that chemicals have on people, animals, plants, or the environment [2]. Route of exposure (e.g., oral, dermal, inhalation), the amount of the chemical (e.g. dose), frequency and duration of exposure, ADME properties (absorption, distribution, metabolism, and excretion/elimination), biological and chemical properties are a few of the factors that affect a substance's toxicity. Testing for toxicity has typically relied on animal models [3]. The development of high throughput screening, however, made *in vitro* toxicity tests

viable. To organize, analyze, model, simulate, depict, or predict the toxicity of substances, *in silico* toxicology (also known as computational toxicology) uses computational resources, such as methodologies, algorithms, software, data, etc. [4]. It is linked to *in silico* pharmacology, which examines the therapeutic benefits and drawbacks of medications using data from computer tools. In order to perhaps reduce the need for animal testing, decrease the cost and duration of toxicity tests, and improve toxicity prediction and safety evaluation, computational methods are intended to supplement *in vitro* and *in vivo* toxicity experiments. Additionally, a special benefit of computational approaches is their ability to predict the toxicity of substances even before they are synthesized. That would minimize the ecological effect of the overall drug-discovery process, minimize the carbon footprint, and constitute a valid alternative and useful addition to biological experiments.

Aim

Choosing suitable candidates for the synthesis of possible novel therapeutic molecules while utilizing the advantages of *in silico* methods was the main goal of the current research.

Materials and methods

Theoretical prediction of pharmacokinetic parameters (ADME):

Physicochemical properties, drug-likeness, and pharmacokinetic parameters such as ADME (absorption, distribution, metabolism, elimination) of the synthesized prodrugs were analyzed using SwissADME. It provides a predictive model for the pharmacokinetic profiling of a drug-like compound [5].

Theoretical prediction of toxicity:

For predicting acute as well as organ toxicity of the compounds, the ProToxII web tool was used. It predicts various toxicity endpoints, including acute toxicity and organ toxicities such as hepatotoxicity, cytotoxicity, carcinogenicity, mutagenicity, immunotoxicity, and toxicity targets. Toxicity class and LD50 values were also estimated [6, 7].

PASS online predictions:

PASS online (Prediction of Activity Spectra for Substances), a computer-based program, was used to screen biological activity of the compounds. The program predicts several thousand different biological activities based on the structural formula of a drug-like organic compound [8]. PASS has been used by many scientists for the discovery of new pharmaceutical agents in different therapeutic fields [9, 10].

Results and discussion

1,3-Disubstituted 3,4-dihydroisoquinolines (Figure 1) were chosen for the *in silico* calculations due to predicted by PASS online (Prediction of Activity Spectra for Substances) program spasmolytic activity, which is continuously relevant to our studies. Based on the previously reported amides [11], methyl, phenyl, 2-chlorophenyl, and benzyl-substituted 3,4-dihydroisoquinolines were planned to be synthesized *via* cyclization using the *Bischler-Napieralski* reaction.

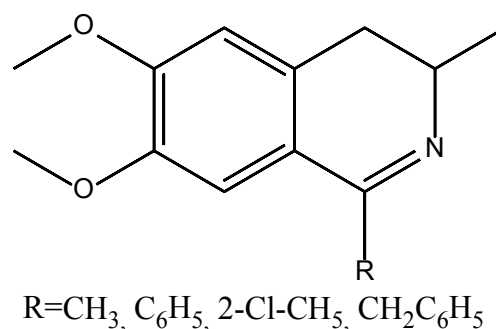


Figure 1. 1,3-Disubstituted 3,4-dihydroisoquinolines planned to be synthesized.

Before their synthesis, *in silico* methods to predict their biological activities were used. A molecule must reach its pharmacological target within the body, reach an appropriate concentration at the site of action, and stay in a bioactive form for a long enough period of time for the anticipated biological activities to take place in order for it to be effective as a medicine. Due to their poor pharmacokinetics and limited bioavailability, several substances failed as drugs. Systematic use of experiments is hindered by the abundance of recently synthesized experimental molecules, the quantitative constraints of tissue samples, and the requirement to limit animal testing, on the other hand. In this context, computer simulations are a viable substitute and a helpful addition to biological studies [12]. In our

calculations, the PASS Online Program was used. The percentage of potential activity is calculated using a comparison between the functional groups of the drug candidate and those currently on the market. The program predicted muscle relaxant and spasmolytic activity as papaverine-like structures for all the compounds.

In order to elucidate the ADME profile of the compounds free SwissADME web tools were used (Table 1), knowing that the blood-brain barrier (BBB) and gastrointestinal absorption are pharmacokinetics characteristics that play an essential role in the drug discovery process.

Molecular weight (MW) and octanol/water partition coefficient (XLOGP3) are the drug-likeness parameters, known as Lipinski's rule of five [13]. According to the rule, at least two parameters from four basic pharmacokinetic properties ($MW \leq 500$; $XLOGP3 \leq 5$; the number of hydrogen bond donors ≤ 5 ; and hydrogen bond acceptors ≤ 10.6) should be fulfilled for drug candidates. Bioavailability (BA) [14] reported an optimal range of distinct properties involving lipophilicity ($XLOGP3$: -0.7 to +5.0), size (MW : 150 to 500 g/mole), polarity (TPSA: 20 to 130 Å²), ESOL or estimated solubility (log S: not more than 6), saturation (Fraction Csp³ or fraction of carbons in the sp³ hybridization: not less than 0.25), and flexibility (RB: no more than 9). The ADME investigation shows that the compounds cross through the BBB and present good gastrointestinal absorption and could be considered as a drug candidate according to Lipinski's rule of five [13], an important step in a drug discovery process. The calculated TPSA values of the compounds are 30.82 Å², indicating good intestinal absorption and BBB penetration. The low number

of rotatable bonds (2, 3, and 4, Table 1) corresponds with sufficient oral bioavailability. P-glycoproteins are pharmacokinetic proteins functioning as xenobiotic neuroprotectors, drugs, etc. [15, 16]. According to the calculation, the studied compounds are not predicted as P-glycoprotein substrates. It is known that inhibition of cytochrome P450 (CYP) could lead to toxicity or lack of drug efficacy [17, 18]. According to the calculations, methyl-substituted dihydroisoquinoline is expected to inhibit the CYP1A2 isoform, while compounds having phenyl, benzyl, and 2-chlorophenyl can inhibit the CYP2C19, CYP2C9, CYP2D6, and CYP3A4 isoforms. The skin permeability factor log Kp is between -5.19 and -6.23. According to the calculations, the 2-chlorophenyl substituted compound has the lowest skin permeability with log Kp -5.19. The solubility parameter is very important for the pharmaceutical applications of the drug. All drugs were predicted by SwissADME to be water-soluble or moderately soluble in water. Values of the drug-likeness parameters of all compounds were found to remain within the BA, which confirmed that all the synthesized compounds behave as "drug-like" substances. All of the compounds scored BA of 0.55, thus demonstrating good oral bioavailability, according to Lipinski's rule [13]. Moreover, they have good synthetic accessibility (SA) scores, which are considerable parameters during the drug discovery processes. The calculated LD₅₀ for all the compounds is 480 mg/kg for all the compounds. Last but not least, all the substances, with the exception of 2-chlorophenyl-3,4-dihydroisoquinoline, were predicted by the pro-tox II to be mutagenic. Due to the lack of mutagenicity predicted, 2-chlorophenyl-3,4-dihydroisoquinoline was synthesized only.

Table 1. Predicted ADME parameters representing drug-likeness of the dihydroisoquinolines, depending on their substituent R to 1st position.

R	MW, g/mol	XLOG P3	TPSA, Å ²	ESOL log S	Fraction Csp ³	RB	BA Score	SA Score	LD ₅₀ , mg/kg
-CH ₃	219.28	1.98	30.82	-2.59	0.46	2	0.55	3.21	480
-C ₆ H ₅	281.35	3.64	30.82	-4.10	0.28	3	0.55	3.58	480
-CH ₂ C ₆ H ₅	295.38	3.58	30.82	-4.07	0.32	4	0.55	3.69	480
-2-Cl-C ₆ H ₅	315.79	4.27	30.82	-4.69	0.28	3	0.55	3.61	480

MW: Molecular weight, XLOGP3: Octanol/water partition coefficient, TPSA: Topological polar surface area, ESOL LogS: Estimated aqueous solubility, Fraction Csp³: Ratio of sp³ hybridized carbons over the total carbon count, RB: Rotatable bonds, BA: Bioavailability, SA: Synthetic accessibility, calculated LD₅₀ – lethal dose to 50% of animals.

Conclusion

To plan the synthesis of drug candidate molecules, we used three different computer programs. Based on the software predictions made, the target molecule for synthesis and future biological activity assessment was chosen. We found that the predicted compounds might have contractile activity, but they could be mutagenic. Due to the lack of predicted mutagenicity, 2-chlorophenyl-3,4-dihydroisoquinoline was obtained only. The advantages of the current research are that the calculations minimized the energy and chemicals utilized in medication development, hence reducing the carbon footprint, and decreasing the number of test animals needed for *ex vivo* or *in vivo* biological experiments.

In this context, *in silico* computer models constitute a valid alternative and useful addition to biological experiments, addressing ecological and ethical concerns, as well as economical costs.

Acknowledgments

This research is supported by Scientific Project No KP-06-M63/8 of the National Fund for Scientific Research in Bulgaria.

References

- Gollapudi B, Su SH, Li AA, Johnson G, Reiss R, Albertini RJ. Genotoxicity as a toxicologically relevant endpoint to inform risk assessment: A case study with ethylene oxide. *Environmental and Molecular*

Mutagenesis. 2020;61(9):852–71.

- Parasuraman S. Toxicological screening. *Journal of Pharmacology and Pharmacotherapeutics*. 2011;2(2):74.
- Giacomotto J, Ségalat L. High-throughput screening and small animal models, where are we? *British Journal of Pharmacology*. 2010;160(2):204–16.
- Raies AB, Bajic VB. *In silico* toxicology: computational methods for the prediction of chemical toxicity. *Wiley Interdisciplinary Reviews: Computational Molecular Science*. 2016;6(2):147–72.
- Isyaku Y, Uzairu A, Uba S. Computational studies of a series of 2-substituted phenyl-2-oxo-, 2-hydroxyl- and 2-acyloxyethylsulfonamides as potent anti-fungal agents. *Heliyon*. 2020;6(4):e03724.
- Banerjee P, Eckert AO, Schrey AK, Preissner R. ProTox-II: a webserver for the prediction of toxicity of chemicals. *Nucleic Acids Research*. 2018;46(W1):W257–63.
- Mazumder K, Md. Emran Hossain, Aktar A, Mohiuddin MW, Kishore Kumar Sarkar, Biswas B, et al. *In Silico* Analysis and Experimental Evaluation of Ester Prodrugs of Ketoprofen for Oral Delivery: With a View to Reduce Toxicity. *Processes*. 2021;9(12):2221–1.
- Anzali S, Barnickel G, Cezanne B, Krug M, Filimonov D, Poroikov V. Discriminating between Drugs and Nondrugs by Prediction of Activity Spectra for Substances (PASS). *Journal of Medicinal Chemistry*. 2001;44(15):2432–7.
- Mathew B, Suresh J, Anbazhagan S. Synthesis and PASS-assisted *in silico* approach of some novel 2-substituted benzimidazole bearing a pyrimidine-2, 4, 6(trione) system as mucomembranous protector. *Journal of Pharmacy & Bioallied Sciences*. 2013;5(1):39–43.
- Ekins S, Olechno J, Williams AJ. Dispensing Processes Impact Apparent Biological Activity as Determined by Computational and Statistical Analyses. de Brevem AG, editor. *PLoS ONE*. 2013;8(5):e62325.
- Milusheva M, Gledacheva V, Batmazyan M, Nikolova S, Stefanova I, Dimitrova D, et al. *Ex Vivo* and *In Vivo* Study of Some Isoquinoline Precursors. *Scientia Pharmaceutica*. 2022;90(2):37.
- Daina A, Michielin O, Zoete V. SwissADME: a

Free web Tool to Evaluate pharmacokinetics, drug-likeness and Medicinal Chemistry Friendliness of Small Molecules. Scientific Reports. 2017;7(1).

13. Lipinski CA, Lombardo F, Dominy BW, Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings 1PII of original article: S0169-409X(96)00423-1. The article was originally published in Advanced Drug Delivery Reviews 23 (1997) 3–25. 1. Advanced Drug Delivery Reviews. 2001;46(1-3):3–26.

14. Martin YC. A Bioavailability Score. Journal of Medicinal Chemistry. 2005;48(9):3164–70.

15. Pathan N, Shende P. Tailoring of P-glycoprotein for effective transportation of actives across blood-brain-barrier. Journal of Controlled Release. 2021; 335:398–407.

16. Testa B, Krämer Stefanie D. The Biochemistry of

Drug Metabolism - An Introduction. Chemistry & Biodiversity. 2008;5(11):2171–336.

17. Manikandan P, Nagini S. Cytochrome P450 Structure, Function and Clinical Significance: A Review. Current Drug Targets. 2018;19(1).

18. Jones BC, Rollison H, Johansson S, Kanebratt KP, Lambert C, Vishwanathan K, et al. Managing the Risk of CYP3A Induction in Drug Development: A Strategic Approach. Drug Metabolism and Disposition. 2016;45(1):35–41.

Corresponding author

Miglena Milusheva

Department of Bioorganic Chemistry

Medical University of Plovdiv

miglena.milusheva@mu-plovdiv.bg

Tel. 0883589339

Simultaneous Determination of Piracetam and Cinnarizine in Capsule Dosage form by Reversed Phase High Performance Liquid Chromatography

Deyan Stoilov, Lily Peikova, Boyka Tsvetkova

Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Medical University of Sofia

Introduction: An antihistaminic drug Cinnarizine in combination with nootropic drug Piracetam are often included in complex therapy of various type of disease as nausea, dizziness, inner ear syndrome and other brain disorders. **Aim:** The aim of this study is to develop simple, precise, accurate and specific reverse-phase high performance liquid chromatographic (RP-HPLC) method with isocratic elution mode for simultaneous determination of Piracetam and Cinnarizine in capsules. **Materials and methods:** The chromatographic separation was achieved on a LiChrosorb C18, 250 mm x 4.6 mm, 5 μ m column at a detector wavelength of 215 nm and a flow rate of 1.0 ml/min. Effect of composition of mobile phase and temperature on chromatographic behaviour of both analytes was studied. **Results:** This paper described the elaboration of simple and fast analytical procedure for assay of Piracetam and Cinnarizine in capsules by RP-HPLC. The method was validated for the parameters like specificity, linearity, precision, accuracy, limit of quantification and limit of detection. **Conclusion:** The chromatographic method developed can be applied for routine analysis of the active substances Piracetam and Cinnarizine in the investigated drug product.

Keywords: Piracetam, Cinnarizine, RP-HPLC, quality control

Introduction

Cinnarizine is a piperazine derivative with H1 blocking (antihistaminic) and calcium channel blocking activity. The drug is used for prevention and treatment of nausea and motion sickness [1, 2]. Piracetam (2-oxo-pyrrolidineacetamide) is a nootropic drug. Therapeutic applications of Piracetam include some conditions, mainly myoclonus, but is used off-label for a much wide range of applications like aging, alcoholism, Alzheimer's and senile dementia, depression and anxiety, schizophrenia, closed cranio-cerebral trauma, etc.

A combination of piracetam and cinnarizine is used in the treatment and prevention of cardiovascular disease such as stroke and to regulate blood pressure. Literature survey reveals that there are some RP-HPLC methods for the determination of these analytes in combination. These analytical procedures include gradient elution mode for simultaneous analysis of drugs of interest in the presence of their impurities [3, 4, 5, 7]. Ahmed MS et al. proposed UV-spectrophotometry for determination of piracetam and cinnarizine in binary mixture in bulk and pharmaceutical

dosage forms [6]. Fadia et al. described spectrophotodensitometry and spectrophotometry for determination of cinnarizine in mixture with piracetam [7].

Aim

The aim of this paper is to develop a specific, precise and accurate isocratic chromatographic method that could be applied in quality control for the simultaneous determination of piracetam and cinnarizine in pharmaceutical preparations. The proposed method was validated as per the International Conference on Harmonization (ICH) method validation guidelines [8].

Experimental

Chemicals and reagents

Working standards of piracetam RS (Purity 100.09) and cinnarizine RS (purity 99.81) were provided by (Sigma-Aldrich). The pharmaceutical preparation of combination of piracetam (400 mg) and cinnarizine (25 mg) was obtained commercially. LC-grade methanol and ortho-phosphoric acid were procured from Merck (Germany). All other chemical reagents were of analytical grade.

Instrumentation and chromatographic conditions

Chromatographic separation was performed on modular HPLC system LC-10A Shimadzu (Japan) arranged with a LC-10A pump, solvent degasser DGU-3A, Rheodyne injector, column oven CTO-10A, SPD-M10A diode array detector and communication bus module CBM-10A. Separation was achieved isocratically with a LiChrosorb C18, 250 mm x 4.6 mm, 5 μ m column eluted with a mixture of phosphate buffer (pH adjusted to 4.6 with ortho-phosphoric acid) and methanol (95:5 v/v) as the mobile phase at flow rate of 1.0 ml/min. Detection was carried out by absorbance at 215 nm. The analysis was carried out at an ambient temperature and injection volume was 20 μ l.

Preparation of standard solutions

For piracetam: 20 mg of piracetam was accurately weighed and transferred to a 20 ml volumetric flask and volume was made up to 20 ml with methanol (Stock solution A-1000 μ g/ml). From Stock solution A, 5.0 ml was taken into a 50 ml volumetric flask and volume was made up to 50 ml with methanol (Stock solution B-100 μ g/ml).

For cinnarizine: 20 mg of metoprolol was accurately weighed and transferred to a 20 ml volumetric flask and volume was made up to 20 ml with methanol (Stock solution C-1000 μ g/ml). From Stock solution C, 5.0 ml was taken into a 50 ml volumetric flask and volume was made up to 50 ml with methanol (Stock solution D-100 μ g/ml).

For mixed standard: From the stock solutions B and D dilutions of different concentration were made in the ratio 16:1 for both drugs.

Analysis of pharmaceutical preparation

The pharmaceutical preparation containing piracetam 400 mg and cinnarizine 25 mg was analyzed using this method. The content

of 20 capsules was taken and powdered. The powder equivalent to 100 mg of piracetam and 6.25 mg of cinnarizine was accurately weighed and transferred into a 25 ml volumetric flask, 10 ml of methanol was added and the content was ultrasonicated for 20 min. The volume was then diluted to the mark and mixed well. A small portion was withdrawn and filtered through a 0.22 μ m filter to ensure the absence of particulate matter. The filtered solution was appropriately diluted with methanol to produce a working standard solution 100% from calibration curves. The amount of drug present in the sample solution was determined using the calibration curves of standard drugs.

Results and discussion

The proposed method was validated as per ICH guidelines with respect to specificity, linearity, precision and accuracy.

Specificity

The specificity of the method was determined by checking the interference with the components from placebo. No interference was observed for any of the components like excipients of both drugs. The Fig. 1 showed typical chromatogram obtained from analysis of standard solution using the proposed method. The retention time observed – 3.06 min for piracetam and 6.97 min for cinnarizine permits a rapid assay, which is important for routine analysis.

The system suitability studies were carried out to determine theoretical plates, resolution and tailing factors. The results were given in table 1. The values obtained demonstrated the suitability of the system for the analysis of investigated drug combination, system suitability parameters may fall within $\pm 3\%$ standard deviation range during routine performance of the method.

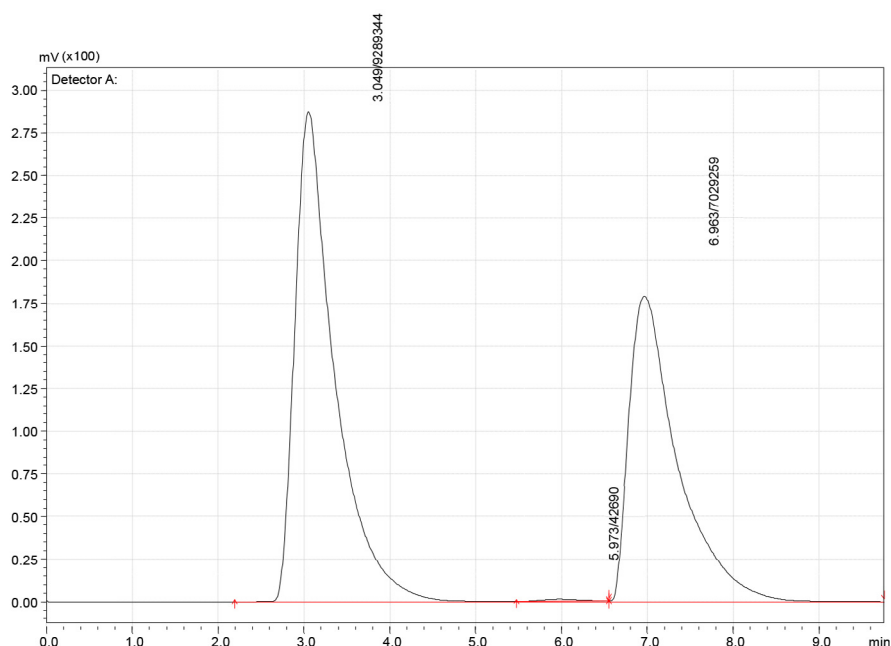


Figure 1. Chromatogram obtained from analysis of sample solution

Table 1. System suitability test parameters for piracetam and cinnarizine

Parameter	piracetam	cinnarizine
Retention time (min)	3.06	6.97
Resolution	2.68	-
Tailing factor	0.83	0.79
Theoretical plates	5641	7512

Linearity and calibration curves

To establish the linearity of analytical method, a series of dilution ranging from 80–400 µg/ml for piracetam and 5–25 µg/ml for cinnarizine was performed. All the solutions were filtered through 0.22 µm membrane filter prior to use and injected in chromatograph. A calibration curves were plotted between the mean peak areas vs.

respective concentrations. The corresponding linear regression equation was $y = 10658.x - 1250.6$ with square of correlation coefficient R^2 of 0.9999 for piracetam and $y = 17151.x - 1352.5$ with square of correlation coefficient R^2 of 0.9999 for cinnarizine, respectively.

Precision

The precision of the method was evaluated by performing six independent determinations of the test sample preparation and calculating RSD (%). The RSD values measured during assessment of precision was < 2.0% for both analytes, confirming the method is precise (Table 2).

Table 2. Precision of the method

piracetam		cinnarizine	
Amount claimed (mg/tablet)	Amount found (mg/tablet)	Amount claimed (mg/tablet)	Amount found (mg/tablet)
400.0	399.85	25.00	25.41
	399.47		24.63
	400.5		24.36
	399.85		24.87
	400.4		25.62
	400.1		25.29
Mean	400.03	Mean	25.03
S_d	0.385	S_d	0.489
%RSD	0.096	%RSD	1.953

Accuracy

Recovery studies were performed to validate the accuracy of developed method. To the preanalysed sample solution, a definite concentration of standard drug was added and then its recovery was analysed. The percent recovery for piracetam was found to be 99.24% and for cinnarizine it was 99.35% (Table 3).

Table 3. Statistical data for accuracy

Statistical data	piracetam	cinnarizine
% Recovery	99.24	99.35
SD	1.168	1.27
% RSD	1.177	1.278

Analysis of pharmaceutical preparation

The results from analysis of pharmaceutical formulation are shown in table 4.

Table 4. Results for assay of piracetam and aspirin in pharmaceutical preparation

Labeled amount (mg)		Observed amount $\pm$ SD		%RSD	
piracetam	cinnarizine	piracetam	cinnarizine	piracetam	cinnarizine
400	25	379.1 $\pm$ 0.45	24.78 $\pm$ 0.55	0.119	0.221

Conclusion

The newly developed HPLC method is specific, precise, accurate and rapid. The analytical procedure is suitable for quality control of pharmaceutical preparation containing piracetam and cinnarizine.

References

1. Maryadele O, editor. The Merck Index. 14th ed. Merck Research Laboratories: White House Station. 2006; 2308: 7482.
2. Seetman SC, editor. Martindale: The Complete Drug Reference. 37th ed. The Pharmaceutical Press: London. 2011; 623: 398, 1236.
3. El-Adl SM, El-sadek ME, Hasan MH. Exploring novel isocratic HPLC method for quantitative determination of cinnarizine and piracetam in their capsule preparations. Journal of Applied Pharmacy. 2013; 8 (3): 1000225.
4. El-Houssini OM, Zawilla NH, Mohammad MA. Development and validation of RP-HPLC method for the determination of cinnarizine/piracetam and cinnarizine/haeptaminol acefyllinate in presence of cinnarizine reported degradation products. Analytical Chemistry Insights. 2013; 8 (1): 99-106.

5. Navaneethan G, Karunakara K, Elango K. Stability indicating and simultaneous determination of cinnarizine and piracetam from capsule dosage form by reversed phase high performance liquid chromatography. Indian Journal of Chemical Technology. 2013; 20: 323-326.
6. Ahmed MS, Anes AM, Nabil A. Simultaneous spectrophotometric estimation of cinnarizine in binary mixture with piracetam in bulk and pharmaceutical dosage forms. World Journal of Pharmaceutical Research. 2020; 9 (8): 2099-2113.
7. Fadia H, Elzeany B, Darwish H. New methods for determination of cinnarizine in mixture with piracetam by spectrophotodensitometry, spectrophotometry, and liquid chromatography. Journal of AOAC International. 2005; 88 (6): 1666-1676.
8. International Conference on Harmonization 2005 ICH harmonized tripartite guideline Validation of analytical procedures: text and methodology Q2 (R1) ICH, Geneva, Nov; 2005

Corresponding author

Доц. Бойка Цветкова
1000 София, ул. „Дунав“ №2
Фармацевтичен факултет
+359879104012
btsvetkova@pharmfac.mu-sofia.bg

IV. Therapeutic Session

Continuous Positive Airway Pressure for Improvement of Respiratory Function in Infantile Spinal Muscular Atrophy: a Case Report

Akanksha Prasad¹, Geniya Jery¹, Katerina Gaberova²

1. Faculty of Medicine, Medical University of Plovdiv

2. Department of Paediatrics and Medical Genetics, Faculty of Medicine, Medical University of Plovdiv

Background: Infantile Spinal Muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder characterised by muscle weakness caused by alpha motor neuron degeneration. It is the current leading genetic cause of infantile death because of the deterioration of respiratory function. Non-invasive ventilation can reduce morbidity and mortality in these children and is an essential part of their healthcare. **Case presentation:** We present a case of a 1-year-old child with genetically proven infantile SMA complicated by chronic pneumonia. At 6 months of age, Risdiplam, an SMN2 mRNA splicing modifier therapy was initiated; nonetheless, at 10 months of age, pulmonary exacerbations had worsened from muscle weakness and chronic colonisation of *Pseudomonas aeruginosa* in the respiratory tract. At 11 months of age, she developed a total right lung atelectasis and severe respiratory failure. SMA paralyzes the expiratory intercostal muscles, making it challenging to exhale under continuous positive airway pressure (CPAP). As a result, bilevel positive airway pressure (BiPAP) is favoured over CPAP to accommodate this issue. However, in this case, CPAP was initiated instead of the standard BiPAP, which resulted in the improvement of respiratory failure, increased oxygenation, and decreased atelectasis. The patient was successfully discharged with continuous non-invasive ventilation at home. **Conclusion:** CPAP can improve respiratory function in patients with SMA type 1. It can be useful not only in the prevention of respiratory failure but also in the therapeutic approach of acute respiratory complications in these children.

Keywords: Non-invasive ventilation, SMA type 1, respiratory disease, CPAP, paediatrics

Introduction

SMA, an autosomal recessive neuro-degenerative disorder, is the leading genetic cause of infantile death with a prevalence rate of 1 in 11,000 births, making it the most widespread of its kind.¹ The condition manifests as proximal-to-distal muscle weakness and respiratory insufficiency due to the degeneration of alpha motor neurons in the spinal cord. The Survival of Motor Neuron 1 (SMN1) gene, located in the 5q13.2 region is vulnerable to genomic instability from high repetition. The gene can be mutated in the form of loss-of-function mutations, homozygous deletions at exon 7, and point mutations that converts SMN1 to SMN2. The SMA phenotypes provide a spectrum of disease severity. SMN1 and SMN2 genes both ubiquitously produce SMN protein, however,

SMN2 synthesises lower levels that only partially compensate for SMN1 loss. The disease severity is classified into five clinical subgroups based on SMN 2 gene expression, evaluated by age of onset, attainment of motor milestones, and death. Infantile SMA is the most severe form, with 80% having $\leq$ SMN2 copies², causing progressive respiratory decline and infant mortality.

Respiratory muscle weakness in SMA manifests a distinct pattern, with the expiratory intercostal muscles experiencing the most impact while the inspiratory diaphragm function is relatively intact. This results in severe ventilation restriction and frequent outbreaks of bronchitis and pneumonia. The gradual decline in spirometric values, worsened by scoliosis, leads to reduced pulmonary compliance, underdeveloped lungs, and

diminished coughing ability. Ultimately, respiratory failure develops, requiring ventilatory support to increase arterial oxygen saturation and reverse atelectasis.³

Case description

A 1-month-old infant was born in a depressed state due to severe perinatal asphyxia and signs of cerebral excitability. The delivery was a second normal pregnancy with extended forceps use. The infant presented with severe muscle hypotonia, tongue fasciculations, and hyporeflexia. Genetic testing revealed a diagnosis of infantile SMA with homozygous deletion of exons 7 and 8 in the SMN1- del gene, heterozygous deletion in exon 5 of the NAIP gene, and two copies of the SMN2 gene. Risdiplam was started at 6 months of age. However, at 10 months of age, the child developed respiratory compromise, indicated by abdominal diaphragmatic breathing, paralytic chest, vesicular breathing, and rales located in the right lung base. A microbiological examination and a chest X-ray confirmed respiratory involvement, Transtracheal secretion had chronic colonization strains of *Pseudomonas aeruginosa* and *Citrobacter koser* for which appropriate antibiotics were given.

The patient, who was intubated for respiratory assistance due to atelectasis, underwent an X-ray (Figure 1) which revealed right lung pneumonia and left lung emphysema. In the right lung, there was evidence of total atelectasis characterized by inhomogeneous spots, confluent shadows of the lung parenchyma and a right-shifted mediastinum.

Despite invasive respiratory support, the patient's atelectasis showed no improvement. However, using Continuous Positive Airway Pressure (CPAP) against the usual indication resulted in a reduction of atelectatic lung findings, as seen in Figure 2.

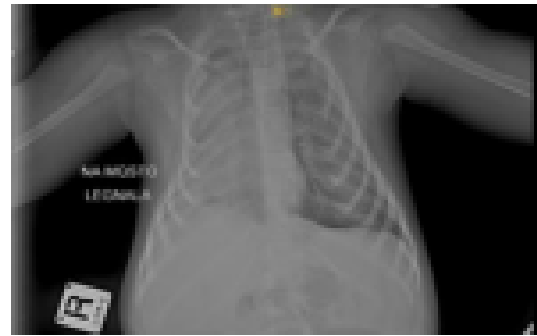


Figure 1.



Figure 2.

Discussion

Chronic respiratory care requires airway clearance, cough assist, and breathing assistance: invasive or non-invasive ventilation. These interventions are believed to extend survival.⁴ Non-invasive positive pressure ventilation (NPPV) has two modes: CPAP and biphasic positive airway pressure (BiPAP). CPAP is routinely used for treating obstructive sleep apnoea, whereas BiPAP is reserved for more severe cases of OSA that cannot be cured with CPAP. CPAP delivers a constant positive airway pressure, which increases the difficulty to exhale thereby increasing the carbon dioxide retention⁵. Whereas BiPAP cycles between delivering high and low-pressure during inspiration and expiration respectively, reducing the effort needed for exhalation. Given the existing exhalation paralysis in SMA1, BiPAP is the preferable technique. However, in this case

report, CPAP has been demonstrated to be successful in stabilising the respiratory state, as seen in the improvements depicted in Figure 2 following CPAP treatment. SMA1 patients, often known as non-sitters, exhibit early-onset symptoms and are at risk of respiratory failure. Therefore, respiratory therapy focuses on airway clearance and ventilation. The latest updated 2018⁶ consensus recommends NPPV for all symptomatic infants to prevent respiratory failure. However, CPAP is contraindicated to treat chronic respiratory failure and should only be used briefly to preserve the functional residual capacity.

In non-sitters' infants who are not synchronous with BiPAP and are not hypercapnic, it is recommended to initiate treatment with CPAP, with the intention of shifting to BiPAP as soon as possible. This is because extended CPAP usage can fatigue SMA patients and interfere with weaning. This approach is supported by evidence demonstrating the beneficial effects of BiPAP on pulmonary function, including minimising thoracic and sternal deformities and improve lung development in both non-sitters and sitters.⁷

Conclusion

In this case report, a 6-month-old infant with SMA1 was successfully treated with CPAP, which effectively alleviated their respiratory symptoms. Although BiPAP is the recommended regimen for respiratory support, the individualized approach taken in this case highlights the importance of considering the patient's needs. This provides evidence CPAP may be a beneficial alternative in select cases of SMA1. Overall, providing patients with personalised approach to respiratory therapy will reduce the risk of respiratory failure and related

complications. Respiratory support needs can vary among SMA patients, and a one-size-fits-all approach may not be appropriate.

References

1. Lefebvre S, Sarret C. Pathogenesis and therapeutic targets in spinal muscular atrophy (SMA). *Arch Pediatr.* 2020 Dec;27(7S):7S3-7S8. doi: 10.1016/S0929-693X(20)30269-4. PMID: 33357595.
2. Glascock J, Sampson J, Haidet-Phillips A, Connolly A, Darras B, Day J, Finkel R, Howell RR, Klinger K, Kuntz N, Prior T, Shieh PB, Crawford TO, Kerr D, Jarecki J. Treatment Algorithm for Infants Diagnosed with Spinal Muscular Atrophy through Newborn Screening. *J Neuromuscul Dis.* 2018;5(2):145-158. doi: 10.3233/JND 180304. PMID: 29614695; PMCID: PMC6004919.
3. LoMauro A, Aliverti A, Mastella C, Arnoldi MT, Banfi P, Baranello G. Spontaneous Breathing Pattern as Respiratory Functional Outcome in Children with Spinal Muscular Atrophy (SMA). *PLoS One.* 2016 Nov 7;11(11):e0165818. doi: 10.1371/journal.pone.0165818. PMID: 27820869; PMCID: PMC5098831.
4. Lemoine TJ, Swoboda KJ, Bratton SL, Holubkov R, Mundorff M, Srivastava R. Spinal muscular atrophy type 1: are proactive respiratory interventions associated with longer survival? *Pediatr Crit Care Med.* 2012 May;13(3):e161-5. doi: 10.1097/PCC.0b013e3182388ad1. PMID: 22198810; PMCID: PMC4334579.
5. Katayama M, Naritomi H, Nishio H, Watanabe T, Teramoto S, Kanda F, Hazama A. Long-term stabilization of respiratory conditions in patients with spinal muscular atrophy type 2 by continuous positive airway pressure: a report of two cases. *Kobe J Med Sci.* 2011 Dec 28;57(3):E98-105. PMID: 22971944.
6. Richard S, Finkel, Eugenio Mercuri, Oscar H. Meyer, Anita K. Simonds, Mary K. Schroth, Robert J. Graham, Janbernd Kirschner, Susan T. Iannaccone, Thomas O. Crawford, Simon Woods, Francesco Muntoni, Brunhilde Wirth, Jacqueline Montes, Marion Main, Elena S. Mazzone, Michael Vitale, Brian Snyder, Susana Quijano-Roy, Enrico Bertini, Rebecca Hurst Davis, Ying Qian, Thomas Sejersen. Diagnosis and management of spinal muscular atrophy: Part 2: Pulmonary and acute care; medications, supplements and immunizations; other organ systems; and ethics, *Neuromuscular Disorders*, Volume 28, Issue 3, 2018, Pages 197-207, ISSN 0960-8966
7. Wang CH, Finkel RS, Bertini ES, Schroth M, Simonds A, Wong B, Aloysius A, Morrison L, Main M, Crawford TO, Trela A; Participants of the International Conference on SMA Standard of Care. Consensus statement for standard of care in spinal muscular atrophy. *J Child Neurol.* 2007 Aug;22(8):1027-49. doi: 10.1177/0883073807305788. PMID: 17761659.

Acute Q Fever Pneumonia: a Case Report.

Daniela Milanova-Ilieva¹, Nina Dimitrova¹, Kostadin Ketelev^{2,3}

1. Faculty of Medicine, Medical University of Plovdiv

2. Medical Simulation Training Centre at Research Institute of Medical University of Plovdiv

3. Department of Paediatrics, University Hospital “St. George”, Plovdiv

Q fever is a zoonosis caused by the Gram-negative bacterium *Coxiella burnetii*. It is transmitted by inhalation of infected droplets, or in rare cases it could be a tick-borne infection. The acute Q fever presents with flu-like symptoms, atypical pneumonia or granulomatous hepatitis. The symptoms of Q fever pneumonia are dry cough, fever, chills, chest pain, fatigue. In rare cases the symptoms may include nausea, vomiting, diarrhea. This is a rare case of a tick-borne pneumonia caused by *Coxiella burnetii* in a 10-year-old boy. The child presents with a two-week history of subfebrile temperature and cough, as well as a history of a tick bite a month and a half before the symptoms appeared. An X-ray was performed, showing infiltrates, so a dual antibiotic treatment was prescribed. Despite the therapy, there was no significant improvement in the patient's condition, so he was admitted at the Clinic of Pediatrics “St. George” for further investigation. Several imaging tests were performed, the last one showing intraparenchymal lesion. No significant deviation in the microbiological and serological findings, except borderline IgM results for Q fever. Despite the treatment with several different antibiotics, the fever persisted up to over 39°C. The control CT showed progression of the lesion. Control Q fever serology was taken, showing fourfold increased values. This case illustrates the long way of reaching the diagnosis and although Q fever is an uncommon disease, it has to be taken into consideration in every child with a fever of unknown origin.

Keywords: *Q fever pneumonia, atypical pneumonia, Coxiella burnetii, fever of unknown origin*

Introduction

A 10-year-old boy presents with fever of unknown origin (FUO) to the clinic. With further investigations, a *Coxiella burnetii* infection, following a tick bite, was diagnosed. This microorganism causes Q fever. The Q fever can be an acute or chronic infection. The acute Q fever could present with flu-like symptoms, atypical pneumonia or granulomatous hepatitis.

Actual presentation of the case

History

Our patient is a 10-year-old boy, otherwise healthy. He is a full-term baby, with a birth weight 3200 g. The patient is fully immunised until the age of 7.

The symptoms started 2 weeks before admission to the hospital. The boy had an increased body temperature up to 38°C and a dry cough. There is a history of a tick bite 2 months ago, for which no prophylactic treatment was conducted. A chest X-ray was made on an outpatient basis, showing

pneumonia. He was treated with oral antibiotics – Cefixime and Azithromycin at home. On a control visit, despite the treatment, the patient had inflammatory constellations in his laboratory results, subfebrile temperature and malaise. The patient was referred to the Department of Paediatrics at the University Hospital of St George for further investigation.

Examination and laboratory results

The patient was admitted in a fair general condition. On auscultation, coarse breath sounds were heard.

The laboratory results showed an inflammatory activity and lymphopenia. Additional tests were made – rapid antigen test for COVID-19, HIV antibodies, serologies for Echinococcosis and Brucellosis. They showed no deviations in the reference interval. T-spotTB was negative. The serology for Q fever showed borderline IgM levels. *Staphylococcus haemolyticus* was isolated from the blood culture, as a probable contaminant. Later on, control serology for Q

fever showed fourfold increased values of IgM, confirming the diagnosis Q fever.

Diagnostic imaging

On admission and a week after, chest X-rays were made, showing no dynamics on the findings, regardless of the treatment.



Figure 1. Chest X-rays on admission and a week later

The echocardiography and the abdominal ultrasound showed no abnormal findings.

A chest CT scan was performed. The CT scan revealed intraparenchymal lesion with irregular borders in the left lung, near the left main bronchus. Adjacent to the lesion, there is a ground-glass appearance of the parenchyma.

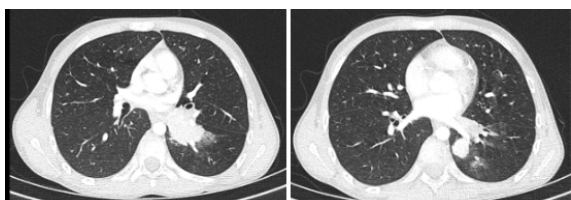


Figure 2. Chest CT scan

Peribronchial formation in the left lung.

Therapy

During the first days of the hospital stay, the patient was given Amikacin i.v. Due to persistence of the febrile episodes and no dynamics in the patient's condition, Clindamycin was started, and Amikacin was discontinued. Since the X-ray findings and the patient's symptoms persisted, dual antibiotic therapy was prescribed – Levofloxacin and

Teicoplanin following the discontinuation of the previous treatment. After obtaining the serology results, showing levels of IgM that are certain for the diagnosis of Q fever, we started Tygacil and Metronidazole. The patient had his first afebrile periods and started to recover.

Due to superimposed fungal infection, Difflican was given. At the time of hospital stay, the patient also received symptomatic treatment, intravenous infusions and Immunovenin i.v.

Discussion

Q fever is a zoonosis, caused by *Coxiella burnetii*, a strictly intracellular, gram-negative bacterium. Humans are usually infected by contaminated aerosols or in rare cases it could be a tick-borne infection. (1) Ingestion of high doses of *C. burnetii* via the digestive route is a rare alternative for acquiring infection. The incubation period is usually 1 to 3 weeks. (2) Although often asymptomatic, Q fever may manifest in humans as an acute disease or as a chronic disease (clinical evolution lasting longer than 6 months usually presenting as endocarditis). The acute Q fever manifests with flu-like symptoms, atypical pneumonia or granulomatous hepatitis. Acute Q fever can cause atypical pneumonia, with fever and non-productive cough. Chest radiographs usually show nonspecific findings, and auscultation is usually normal. Symptoms usually last between 1–10 weeks. Atypical pneumonia may be mistakenly diagnosed as a viral infection. (3)

Because of the fact that clinical manifestations of Q fever may be so variable, the disease remains often unrecognised because of poor surveillance of the disease. Specific diagnosis of Q fever is mainly based upon serology. The presence of a fourfold rise in phase II IgG or IgM antibodies within a

3-week to 6-week period confirms acute infection. (4)

The tetracyclines are still considered the drug of choice for acute Q fever in the elderly population. A potential alternative in this population could be macrolides. Compared with patients treated with tetracyclines, newer macrolides have shown equivalent clinical outcomes for Q fever pneumonia. Despite the fact that doxycycline is now considered safe for childhood infections, co-trimoxazole is still recommended for children under 8 years of age. Pregnant women are treated with co-trimoxazole. (5)

Conclusion

This is a 10-year-old child, who is admitted to the Pediatric clinic because of a two-week febrile period and signs of atypical pneumonia. Despite the long-term, broad-spectrum antimicrobial therapy, there was a persistence of the subfebrile and febrile episodes.

The patient presented with a FUO. For this reason, a series of imaging tests were performed, followed by serology testing which revealed fourfold increased titers of

the IgM antibodies, corresponding to Q fever. After initiating a treatment with tetracycline, which is used for acute Q fever infections, the patient started to improve his condition.

Although it is uncommon, every child with FUO should be evaluated for Q fever, despite the long way to diagnosis.

References

1. Tissot-Dupont H, Raoult D. Q fever. *Infect Dis Clin North Am.* 2008 Sep;22(3):505-14, ix. doi: 10.1016/j.idc.2008.03.002. PMID: 18755387.
2. Angelakis E, Raoult D. Q Fever. *Vet Microbiol.* 2010 Jan 27;140(3-4):297-309. doi: 10.1016/j.vetmic.2009.07.016. Epub 2009 Aug 8. PMID: 19875249.
3. Marrie TJ. Q fever pneumonia. *Infect Dis Clin North Am.* 2010 Mar;24(1):27-41. doi: 10.1016/j.idc.2009.10.004. PMID: 20171543.
4. España PP, Uranga A, Cillóniz C, Torres A. Q Fever (*Coxiella Burnetii*). *Semin Respir Crit Care Med.* 2020 Aug;41(4):509-521. doi: 10.1055/s-0040-1710594. Epub 2020 Jul 6. PMID: 32629489.
5. Gikas A, Kokkini S, Tsioutis C. Q fever: clinical manifestations and treatment. *Expert Rev Anti Infect Ther.* 2010 May;8(5):529-39. doi: 10.1586/eri.10.29. PMID: 20455682.

Corresponding author

Daniela Deyanova Milanova-Ilieva
33 Drin Str., 4000, Plovdiv
Tel: +359 885 962 670
mail: danmilano23@gmail.com

Acute Spondylodiscitis as a Debut of Infective Endocarditis

Ivan Kuchmov¹, Ivan Manolov², Bistra Dobрева-Yatseva²

1. Cardiology Clinic, University Hospital “St. George” LLC, Plovdiv

2. Cardiology Clinic, University Hospital “St. George” LLC, Plovdiv; First Department of Internal Diseases, Section of Cardiology, Faculty of Medicine, Medical University of Plovdiv

Despite technological progress and advances in medicine, infective endocarditis (IE) continues to be a challenge for physicians, due to difficulties in diagnosis and treatment and high in-hospital mortality. Atypical clinical presentation may delay diagnosis and appropriate treatment, increasing the risk of in-hospital complications and death. Cases of peripheral embolization with the appearance of spondylodiscitis are relatively rare – up to 8% and more – more often with *Staphylococcus aureus* infection. We present a case of acute spondylodiscitis as the onset of IE. A 68-year-old man, with acute difficulties in walking to the point of impossibility of independent movement, was hospitalized in the Clinic of Neurology of the UMBAL “St. George”. Established fever up to 38°C, inflammatory constellation with leukocytes – $22.53 \cdot 10^9$, CRP – 146 mg/l, anemia, newly diagnosed rough, holosystolic murmur of the aortic valve, Osler’s nodes on the fingers of the lower limbs. Consulted with a cardiologist. From the echocardiography – data on severe aortic stenosis (unknown until now), presence of aortic valve vegetations. Blood cultures – isolated *Staphylococcus aureus*. Transferred to Cardiology Clinic. From the performed magnetic resonance imaging (MRI) – evidence of spondylodiscitis in the lumbosacral department. A 6-week treatment followed, with a good result.

Keywords: *Infective Endocarditis, Spondylodiscitis, Magnetic Resonance Imaging (MRI)*

Introduction

Despite the technological and medical advances, infective endocarditis (IE) remains a diagnostic, treatment and a high-mortality challenge for the physicians. Atypical clinical manifestations could hinder the diagnostic process and adequate treatment, increasing the risk of treatment-related complications and fatal outcome.

Back pain is one of the most frequent musculoskeletal symptoms that 90% of the population will experience during their lifetime. The condition usually resolves quickly, usually within a week. In only 1% of the visits in the emergency department (ER) related to lumbar pain a serious condition is observed – vertebral fracture, cadua equina syndrome, infection.

Spondylodiscitis as a complication of IE is relatively rarely observed – in approximately 8% of the patients. It is more frequently associated with males above 69.7 ± 18 years of age, hypertensive patients (48% vs 34.5%; $p < 0.001$), diabetics, patients with autoimmune diseases (Carbone et al., 2020) and in *S. aureus* infection. Single case reports of association between IE and

spondylodiscitis have been reported since 1965. (Del Pace et al., 2021).

Case description

A 68-year-old male presents himself in the ER of “St. George” University Hospital LLC Plovdiv with acute abnormal gait to the inability of walking without assistance. He states reduced functional capacity and fever reaching 38.3°C dating back to a week. Arterial hypertension and type 2, non-insulin dependent diabetes on dietary treatment are reported as previous comorbidities.

The initial evaluation reveals fever up to 38°C. Normosthenic build. Chest wall symmetry with regular vesicular breathing, without any pathological auscultatory phenomenon. Rhythmic heart activity with a heart rate of 93 bpm, BP 130/70 mmHg, no pathological findings were reported. The abdomen was not tender, without hepatosplenomegaly. No lower limb edema was reported. Neurological status: quadri pyramidal syndrome, hyperreflective deep tendon reflexes, more noticeable on the right side. Babinsky, Hoffmann and Trömner were bilaterally positive. Quadriparetic syndrome, more noticeable for the lower right limb.

The patient was hospitalized in the Neurology clinic with the diagnosis “Intervertebral disc disorder with radiculopathy”.

From the conducted laboratory studies anemia: Hbg 121 g/l (140–180 g/l); RBC $4.22 \times 10^{12}/l$ ($4.5\text{--}6 \times 10^{12}/l$), Hct 0.378 l/l (0.40–0.54 l/l); and an inflammatory response: WBC $22.53 \times 10^9/l$ ($3.5\text{--}10.5 \times 10^9/l$), Sg $19.46 \times 10^9/l$ ($2.6\text{--}6.6 \times 10^9/l$), CRP 146 mg/l (0–10 mg/l), ESR 79 mm/h (2–25 mm/h) were noted. A chest radiogram was performed without any pathological findings. Nasal, throat and urine cultures were negative. A PCR test for detecting SARS-COV2 was negative. Three sets of hemocultures were acquired.

Spinal and cranial computed tomography (CT) were performed, revealing no cranial abnormalities. The spinal CT revealed a multilevel stenosis and degenerative changes. Treatment with Cefepime 1,0 g. b.i.d was initiated, without effect.

A rheumatological and cardiological consultations were performed in relation to the confirmed inflammatory response, anemic syndrome, persisting fever and the positive hemocultures with a *S. Aureus* isolate. A holosystolic Grade III murmur was observed on the right upper sternal border with a propagation to the carotid arteries. “Café au lait” skin pigmentation with subungual hemorrhages, Janeway’s and Osler’s nodes were noted.



Figure 1. Subungual hemorrhages and septic emboli



Figure 2. Janeway’s and Osler’s nodes

Thornsthoracic echocardiography was performed, revealing high-grade aortic stenosis with AV Vmax 4.63 m/s, AV Vmean 3.82 m/s, AV maxPG 85.77 mmHg, AV meanPG 61.99 mmHg and a vegetation on the right noncoronary leaflet with a cross-section of 0,8x1,3 cm. Symmetrical LVH with preserved EF were also reported.

A definite IE was confirmed based on the Dukes modified criteria, cited in the latest European Society of Cardiology Guidelines on Prevention, Diagnosis and Treatment of Infective Endocarditis (Habib et al., 2015). Confirmed were 2 big criteria (an isolate of a typical microorganism, causing IE, echocardiographic evidence of vegetation) and 4 minor criteria (degenerative valve disease, fever $\geq 38.0^{\circ}\text{C}$, vascular and immunological phenomena confirmed). The patient was transferred to the Clinic of Cardiology of “St. George” University Hospital LLC, Plovdiv. A spinal MRI was performed due to the persistent neurological symptoms, confirming spondylodiscitis at the Th.10–11 level as a complication of the IE.

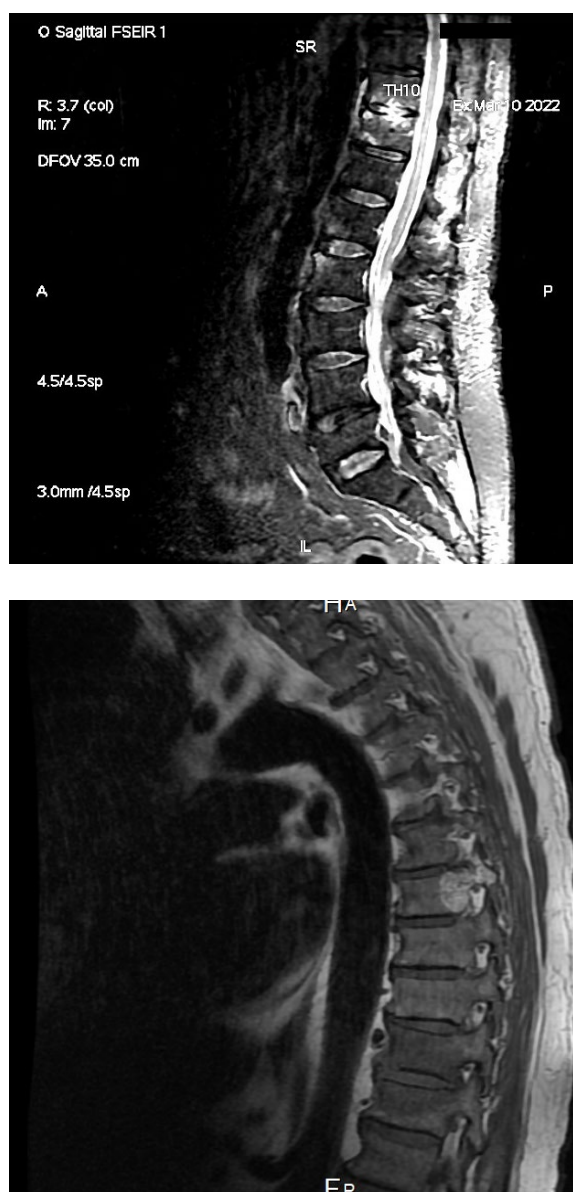


Figure 3. Spinal MRI. spondylodiscitis at the Th.10–11 level

The following six-week antibiotic treatment course was preformed: Vancomycin 2 x 1 g/daily for 14 days and 2 x 0.5 g/daily for 14 days; Gentamicin 2 x 80 mg/daily. For 7 days; Ciprofloxacin 2 x 200 mg/2 x 100 mg for 21 days; Norfloxacin 400 mg p.o for 14 days. Baclofen p.o. 2 x 10 mg; Symbiotic; Esomeprazole p.o. 2 x 20 mg; Torasemide p.o. 5 mg/daily ; Perindopril p.o 8 mg/daily. Enduring control of the fever and normalization of the inflammatory markers were recorded. An episode of atrial fibrillation was managed successfully with Amiodarone.

Serum creatinine levels elevation up to 403 $\mu\text{mol/L}$ imposed reduction of the dosages of antibiotics administered. Complete kidney function restoration was achieved 2 months post-treatment. A follow-up echocardiography did not find new vegetations and any new regurgitations. The ejection fraction was preserved.

Conclusions

Even with early diagnosis of IE and optimal conservative treatment initiation, considerable reduction of possible complications is not expected. In suspected spondylodiscitis, MRI should be the first-line imaging study, given the high sensitivity of the method. (Carbone et al. 2020). In patients with an implanted pacemaker, Cardiac resynchronization therapy (CRT) and implantable cardioverter-defibrillator (ICD) devices SPECT/CT and PET/CT should be considered.

Regarding the duration of antibiotic treatment of patients with IE and spondylodiscitis, no randomized clinical trials were performed and there is no consensus. Conservative therapy in spondylodiscitis in patients with IE should be expanded to a minimum of 6 weeks up to 3 months, or based on MRI, SPECT/CT, PET/CT. (Del Pace et al., 2021b)

Regardless of the problematic and often delayed even in modern times diagnosis of IE, the basic principles of the primary physical examinations – anamnesis and physical examinations, including cardiac auscultation remain essential in diagnostic decisions and further choice of imaging studies, necessary for a well-timed confirmation.

References

1. Calderaro, D., Gualandro, D. M., Yu, P. C., Marques, A. a. F., Puig, L. B., & Caramelli, B. (2008). Spondylodiscitis and infectious endocarditis: a round-trip to be considered. *Brazilian Journal of*

- Infectious Diseases*, 12(6), 544–545. <https://doi.org/10.1590/s1413-86702008000600021>
2. Carbone, A., Lieu, A., Mouhat, B., Santelli, F., Philip, M., Bohbot, Y., Tessonnier, L., Peugnet, F., D’Andrea, A., Cammilleri, S., Delpierre, Q., Gouriet, F., Camoin-Jau, L., Gun, M., Casalta, J., Riberi, A., Collart, F., Martel, H., Arregle, F., . . . Habib, G. (2020). Spondylodiscitis complicating infective endocarditis. *Heart*, 106(24), 1914–1918. <https://doi.org/10.1136/heartjnl-2019-316492>
 3. Chung, J., Chen, J., Tai, H. C., Huang, P., & Chen, W. (2014). Lower leg weakness as the presentation of infective endocarditis with septic emboli. *American Journal of Emergency Medicine*, 32(2), 191.e5–191.e8. <https://doi.org/10.1016/j.ajem.2013.08.048>
 4. Del Pace, S., Scheggi, V., Virgili, G., Cacioli, S., Olivotto, I., Zoppetti, N., Merilli, I., Ceschia, N., Andrei, V., Alterini, B., Stefano, P., & Marchionni, N. (2021b). Endocarditis with spondylodiscitis: clinical characteristics and prognosis. *BMC Cardiovascular Disorders*. <https://doi.org/10.1186/s12872-021-01991-x>
 5. Habib, G., Lancellotti, P., Antunes, M. J., Bon-figorni, M. G., Casalta, J., Erba, P. A., Miró, J. M., Mulder, B. J., Mas, P. T., & Zamorano, J. L. (2015). 2015 ESC Guidelines for the management of infective endocarditis. *European Heart Journal*, 36(44), 3075–3128. <https://doi.org/10.1093/eurheartj/ehv319>
 6. Morelli, S., Carmenini, E., Caporossi, A. P., G, A., Bernardo, M. E., & Gurgo, A. a. M. (2001). Spondylodiscitis and Infective Endocarditis. *Spine*, 26(5), 499–500. <https://doi.org/10.1097/00007632-200103010-00013>
 7. Ota, K., Onishi, N., Fujii, K., Nakamura, E., Oishi, Y., Oka, M., Ota, K., Sano, Y., Yokoyama, H., & Takasu, A. (2020). Tachyarrhythmia improved by management of low back pain in a patient with delayed diagnosis of infective spondylodiscitis: A case report. *SAGE Open Medical Case Reports*. <https://doi.org/10.1177/2050313x20952996>
 8. Skaf, G., Domloj, N. T., Fehlings, M. G., Bouclaous, C., Sabbagh, A. S., Kanafani, Z. A., & Kanj, S. S. (2010). Pyogenic spondylodiscitis: An overview. *Journal of Infection and Public Health*, 3(1), 5–16. <https://doi.org/10.1016/j.jiph.2010.01.001>
 9. Viezens, L., Dreimann, M., Strahl, A., Heuer, A., Koepke, L., Bay, B., Waldeyer, C., & Stangenberg, M. (2021). Spontaneous spondylodiscitis and endocarditis: interdisciplinary experience from a tertiary institutional case series and proposal of a treatment algorithm. *Neurosurgical Review*, 45(2), 1335–1342. <https://doi.org/10.1007/s10143-021-01640-z>

Corresponding author

Ivan Kuchmov
 ivkuchmov@gmail.com
 mob. +359 882277454

Reactivation of Hepatitis B after COVID-19 Infection – Case Presentation

Slaveya Chilova¹, Gabriela Raicheva^{1,2}, Veselin Popov^{1,3}, Victoria Trendafilova⁴, Ivanka Nenova^{1,2}

1. Department of Clinical Oncology, Medical University of Plovdiv

2. Clinic of Medical Oncology, UMHAT “Sv. Georgi”, Plovdiv

3. Clinic of Radiation Oncology, UMHAT “Sv. Georgi”, Plovdiv

4. Clinic of Infectious Diseases, UMHAT “Sv. Georgi”, Plovdiv

Introduction: Hepatitis B reactivation after SARS-CoV-2 infection is a subject of heated discussions. This complication is probably associated with COVID treatment. The higher risk is typical for patients with a wide range of immunosuppression causes. The subject of this presentation is a 77-year-old male with chronic lymphocytic leukemia. His 3 month course of chemoimmunotherapy came to an abrupt end due to severe COVID infection with respiratory failure. He was treated without anti SARS-CoV-2 antiviral drugs. Until then he was HbsAg negative, anti-HBc-positive, HBV DNA negative. Five months later he had jaundice with laboratory syndromes of cytotoxicity and cholestasis. The markers of an active HBV infection were positive – HBsAg-positive, anti HBc IgM-positive, anti HbeAg positive. He had liver failure with fatal outcome. **Discussion:** The literature outlines the unsearched interactions between both viruses (SARS-CoV-2 и HBV) as well as the necessity of monitoring and prophylaxis. **Conclusion:** The reactivation of HBV infection in potentially compromised patients after COVID infection leads to a bleak prognosis. The discussion about these patients with malignancies will contribute to the development of new treatment and prophylaxis approaches.

Keywords: Hepatitis B virus, reactivation, immunosuppression, COVID-19

Introduction

HBV reactivation as a result of SARS-CoV-2 infection is a subject of a debate.^{1,2} According to most theories, there is a direct connection between this process and COVID treatment, particularly the use of corticosteroids to combat the cytokine storm.^{1,3} Patients with malignancies who undergo anti-tumor treatment may experience immune deficiency in addition to the immunosuppression associated with corticosteroid use.

We present a case of a 77-year-old patient with chronic lymphocytic leukemia who was HbsAg and HBV DNA negative, anti-HBc-IgG positive. He had COVID-19. His HBV reactivation led to a fatal outcome.

He was diagnosed with chronic lymphocytic leukemia in December 2021. After ruling out active HBV replication, he underwent two courses of cytostatic monotherapy and one course with anti-CD20 antibody, which ended in February 2022. The patient was HbsAg negative, anti-HBc-IgG positive on 23.12.2021. HBV DNA test was negative on 28.01.2022.

Anti-tumor treatment ceased because of a severe COVID infection with a respiratory failure (March 2022). He was treated with Dexamethasone 8 mg/d, Levofloxacin, Bacteripim, anticoagulant, hepatoprotectors. His treatment plan did not include anti-SARS-CoV-2 antiviral drugs.

Jaundice and dark urine appeared at the end of September 2022. He was hospitalized. The treatment included Methylprednisolone, hepatoprotectors, vit. K, fresh frozen plasma, gastroprotectants, hemotransfusions, human albumin, Usofalk, an antibiotic and Transmethyl. A seroconversion was observed – **HbsAg positive, anti HBc IgM positive**, anti HCV-negative, **anti HbeAg positive**, anti HDV negative. (Table 1) His condition progressively worsened within the next 20 days with the development of a lethal liver failure.

Comorbidities were congestive heart failure, chronic ischemic heart disease, a condition after coronary angioplasty, chronic atrial fibrillation, non-insulin-dependent diabetes mellitus. He did not consume alcohol or take liver – toxic drugs.

Table 1. Dynamics of laboratory parameters:

Laboratory parameters	Initial 23.12.2021	COVID infection 18.03.2022	HBV reactivation 21–25.10.2022
Total bilirubin	33,5 µmol/l	43,7–45,2 µmol/l	283–581 µmol/l
Direct bilirubin	11 µmol/l	28,2–19,8 µmol/l	167–363 µmol/l
AST		22 IU/l	532–390–137 IU/l
ALT		16 IU/l	397–306–124 IU/l
LDH	777IU/l	349–554 IU/l	420–337 IU/l
HbsAg	-	-	positive
anti HBc-total	positive		positive
Anti HBe			positive
Anti-HCV	negative		negative
Total protein		65,6–61 g/l	58 g/l
albumin		36–29 g/l	30 g/l
Prothrombin time		66%	46–34%
SARS-CoV RNA		positive	negative
Anti-HDV			negative
Anti-HAV			negative
Anti-HEV-IgM			negative
HBV-DNA	undetected 28.01.2022		

Discussion

HBV reactivation is more common in patients with HbsAg positive, than in patients with HBsAg negative and anti-HBc positive.^{1,4} The interaction between both viruses are not clear.⁵ SARS-CoV-2 can cause liver damage with fast worsening of liver function and cytolysis laboratory tests on its own.⁶

COVID treatment includes two groups of drugs – directly influencing SARS-CoV-2 virus and aiming a treatment for the immune mediated damage.¹ Some authors associate the last group of drugs with a higher risk of HBV replication reinitiation in former cases.^{4,7} Dexamethasone 6–8 mg is recommended in the COVID treatment standards for respiratory failure. In practice this treatment is longer in order to win over the cytokine storm complications. Treatment of severe cases includes Remdesivir (viral RNA polymerase inhibitor) and Dexamethasone. Dexamethasone can be combined with monoclonal antibodies against IL 6 – receptor or

a Janus kinase inhibitor in specific indications.^{1,4,7}

Such cases have more severe inflammatory or necrotic processes in the liver in HBV reactivation. The causes are the SARS-CoV-2 direct impact on the liver cells, cytokines and liver ischemia. According to some authors HBV reactivation in these cases is potentially life threatening.^{1,8}

Our patient is significantly immunocompromised due to a hematological malignancy and anti-tumor treatment that included a one-time administration of anti-CD20 monoclonal antibody. Corticosteroid therapy is present in the treatment protocols of lymphomas and severe COVID infection.

What are the recommendations for monitoring and prophylaxis of patients with a higher risk of a HBV reactivation.^{1,9,10} According to the literature, it is recommended to test for HBV active infection markers in SARS-CoV-2 RNA-positive patients before initiating corticosteroid therapy.^{1,4} Our

patient also started anti-tumor treatment in the case of a negative test for HBV-DNA.

In the cases of HBsAg positive patients with COVID a prophylaxis of HBV reactivation with nucleoside analogues is highly recommended within the 6–12 months after the cessation of immunosuppressive therapy. According to the European Association for the Study of the Liver, a prophylaxis of HBsAg-negative anti-HBc-positive COVID patients (as in our case) is also recommended if there is a long immunosuppression.¹

Conclusion

The presented case highlights the issues faced by potentially vulnerable patients. HBV reactivation after a COVID infection has a poor prognosis. The discussion of these patients underscores the importance of the treatment and prophylaxis standards of immunocompromised patients.

References

1. Sagnelli C, Montella L, Grimaldi P. COVID-19 as Another Trigger for HBV Reactivation: Clinical Case and Review of Literature. *Pathogens*. 2022; 11(7): 816.
2. Yang Yu, Xingzhao Li, Taihu Wan. Effects of Hepatitis B Virus Infection on Patients with COVID-19: A Meta-Analysis. *Digestive Diseases and Sciences*, 2022 Sep 9; 1-17
3. Alqahtani S, But M. COVID-19 and hepatitis B infection *Antivir Ther*. 2020;25(8):389-397
4. Puri P. Acute Exacerbation of Chronic Hepatitis B: The Dilemma of Differentiation from Acute Viral Hepatitis B. *J. Clin. Exp. Hepatol*, 2013, 3, (4), 301–312
5. Anugwom CM, Aby ES, Debes JD. Inverse association between chronic hepatitis B infection and coronavirus disease 2019 (COVID-19): Immune exhaustion or coincidence? *Clin Infect Dis* 2021;72:180–182.
6. Wang Y, Liu S, Liu H et al. SARS-CoV-2 infection of the liver directly contributes to hepatic impairment in patients with COVID-19. *J Hepatol* 2020;73:807–816.
7. Yip TCF, Gill M, Lai-Hung G et al. Management of hepatitis B virus reactivation due to treatment of COVID-19. *Hepatol Int*. 2022 Apr; 16(2): 257–268.
8. Yang S, Wang S, Du M et al. Patients with COVID-19 and HBV Coinfection are at Risk of Poor Prognosis. *Infect. Dis Ther* 2022;11:1229–1242.
9. Liu J, Wang T, Cai Q et al. Longitudinal Changes of Liver Function and Hepatitis B Reactivation in COVID-19 Patients with Pre-existing Chronic HBV Infection. *Hepatol Res* 2020;50:1211–1221.
10. Zhang B, Huang W, Zhang S. Clinical Features and Outcomes of Coronavirus Disease 2019 (COVID-19) Patients With Chronic Hepatitis B Virus Infection. *Clin Gastroenterol Hepatol* 2020;18:2633–2637.

Corresponding author

Assoc.prof. Ivanka Nenova
Department of Clinical Oncology
Clinic of medical oncology
at UMBAL “St. George” EAD
MU-Plovdiv, Bul. “V. Aprilov”15A, 4002 Plovdiv
Tel. 0885612061
Email: iv_nenova@abv.bg

Cardiovascular Ochronosis – a Case Report

Evgenia Marinova¹, Spas Kitov², Zdravko Ivanov¹, Maria-Florans Kitova¹

1. Faculty of Medicine, Medical University of Plovdiv

2. First Department of Internal Medicine, Section of Cardiology, Faculty of Medicine, Medical University of Plovdiv

Introduction: Alkaptonuria (ochronosis) is an autosomal-recessive disorder affecting the metabolism of tyrosine and phenylalanine which results in accumulation of homogentisic acid (HGA) in connective tissues. The joints are most commonly affected while the most common cardiac injury is aortic valve stenosis. The focus of therapy is on reducing the symptoms. Aortic stenosis (AS) in alkaptonuria is treated with surgical aortic valve replacement (SAVR), however transcatheter aortic valve implantation (TAVI) procedures are increasing in number with excellent outcomes. **Case presentation:** We report a case of a 67-year old female with chronic back pain and large-joint arthralgia, who was recently diagnosed with alkaptonuria. Cardiovascular evaluation was performed due to dyspnea, retrosternal discomfort and fatigue during mild physical activity. She reported a long known heart murmur and intermittent dark-brown staining of her underwear since childhood. Comorbidities include arterial hypertension and atrial fibrillation (AF). Bilateral dark brown pigmentation of the sclera and both ear cartilages were visualized. A rough systolic murmur propagating towards the carotid arteries was auscultated. ECG confirmed AF and left ventricular hypertrophy. Cardiac ultrasonography showed severe AS, reduced global longitudinal strain and preserved ejection fraction. Along with the standard therapy the patient underwent TAVI with Boston Scientific prosthetics with a very good post-procedural outcome. **Discussion and conclusion:** There is limited information on the success rate of TAVI in patients with alkaptonuria. In the population the outcome of TAVI is generally good, however, the individual success in alkaptonuria patients depends on the severity of their heart valve damage and overall health.

Keywords: alkaptonuria, aortic stenosis, TAVI

Introduction

Alkaptonuria is a rare autosomal-recessive disorder that affects the metabolism of tyrosine and phenylalanine, resulting in the accumulation of homogentisic acid (HGA) in the body.¹⁻³ (fig. 1) HGA binds to fibrillar collagen in the extracellular matrix in the presence of mucopolysaccharides, leading to oxidative stress and tissue damage.³⁻⁴ One of the most frequent sites for pigment deposition is the aortic valve, as well as the intervertebral discs of the lumbar spine, the skin, the cartilage of the ear, and the eyes. Common clinical symptoms include dark urine, ochronotic arthropathy and spondylopathy, which manifest as joint pain. Dark-brown pigmentation of the conjunctiva, cornea, and sclera is also observed, although eyesight is not affected. The renal system effectively secretes HGA, therefore the clinical manifestation takes 3–4 decades.

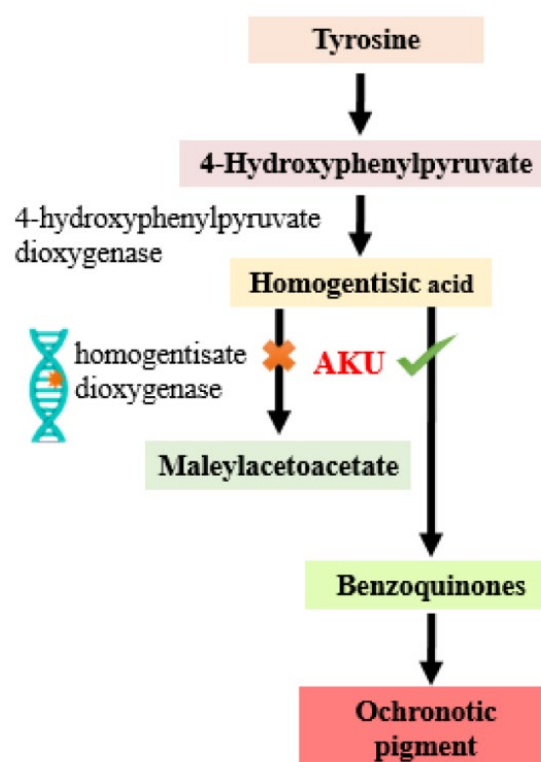


Figure 1. Metabolism of homogentisic acid

Prognosis of the disease depends on the intensity of heart injury as a result of the pigment deposition in the cardiac structures – coronary arteries, cardiac valves, and aorta. The most common cardiac pathology in patients with ochronosis is aortic valve stenosis.⁵ The diagnosis of ochronosis is complex and includes medical history, physical examination, laboratory testing of urine and blood, and imaging. Critical is the concentration of homogentisic acid in body fluids, which can be directly measured by gas chromatography or mass spectrometry analysis. Genetic testing with PCR is evaluating for HGD mutations. Imaging methods – X-ray, CT-scan and MRI prove typical changes in the skeletal bones. Arthroscopy and skin biopsy are very informative.⁶ Cardiovascular pathology is demonstrated in electrocardiography (ECG), echocardiography (EchoCG) and interventional coronarography.

Currently, there is no cure for alkaptonuria – management of the disease focuses on reducing symptoms and slowing down progression. It includes anti-inflammatory medications, pain management, and physical therapy. Low tyrosine and phenylalanine diet may also be recommended. Arthroplasty is commonly used, as joint damage is among the most severe manifestation. Aortic stenosis (AS) in alkaptonuria is treated with surgical aortic valve replacement (SAVR), however transcatheter aortic valve implantation (TAVI) procedures are increasing in number with excellent outcomes.⁷⁻⁹

Case presentation

We report a case of a 67-year-old female who complained of dyspnea, orthopnea, retrosternal discomfort and fatigue during mild physical activity. She also reported edema of the lower parts of both legs and chronic back

and joint pain for more than 30 years. There was dark staining of her underwear. Her comorbidities were interpreted as seronegative spondyloarthritis, arterial hypertension, and permanent atrial fibrillation (AF).

Upon physical examination, there was bilateral dark-brown pigmentation of the sclera and bluish-black discoloration of both ear cartilages. The patient's range of motion in the spine, shoulders, and knees was restricted. Muffled heart sounds and a rough systolic murmur propagating towards the carotid arteries with a punctum maximum on the aortic valve were auscultated.

Assessing all the symptoms and their duration, ochronosis was suspected. The patient was referred for testing of the concentration of HGA in her blood and urine and it was significantly elevated.

Cardiac findings

The ECG confirmed atrial fibrillation with left ventricular hypertrophy. EchoCG revealed left ventricular symmetrical concentric hypertrophy with a decreased ejection fraction of 45%, indicating chronic heart decompensation. The heart contractility was also decreased due to the persistent higher afterload, as well as the left ventricular longitudinal strain. Pathological pressure gradients were observed (fig. 2).

Management of the patient

The patient was presented at heart team and was assessed for TAVI procedure. Standard protocol was applied. The procedure underwent no complications, and the patient was discharged in improved general condition. The patient was followed up to the third month, at which point the valve had a normal gradient and the ejection fraction normalized, but the strain is still decreased due to irreversible heart damage.

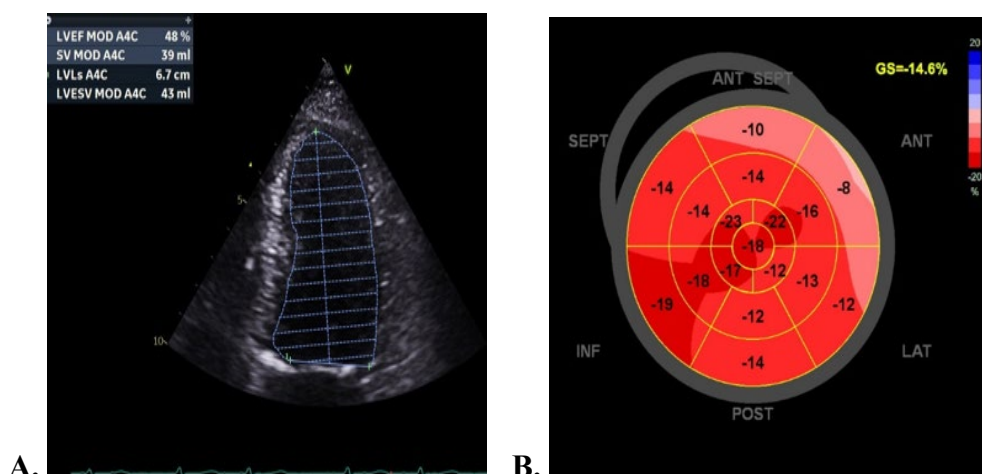


Figure 2. Echocardiography before the procedure 2A – ejection fraction by simpson's method; 2B – Left ventricular global longitudinal strain

Discussion and conclusion

There is limited information on the success rate of TAVI in patients with alkaptonuria. TAVI was chosen for this patient due to increased cases of suture dehiscence in patients with ochronosis, resulting from connective tissue insufficiency caused by ochronotic pigment deposition. Another reason for this decision is the more difficult recovery period after SAVR, as patients with this disorder usually have arthropathy, which disables them from movement, crucial for rehabilitation after SAVR. In the population the outcome of TAVI is generally good, however, the individual prognosis in alkaptonuria patients depends on the severity of their heart valve damage and overall health.¹⁰⁻¹³

References

- Phornphutkul C, Introne WJ, Perry MB, Bernardini I, Murphey MD, Fitzpatrick DL, Anderson PD, Huizing M, Anikster Y, Gerber LH, Gahl WA. Natural history of alkaptonuria. *N Engl J Med*. 2002 Dec 26;347(26):2111-21. doi: 10.1056/NEJMoa021736. PMID: 12501223.
- Zatkova A, Ranganath L, Kadasi L. Alkaptonuria: Current Perspectives. *Appl Clin Genet*. 2020 Jan 23;13:37-47. doi: 10.2147/TACG.S186773. PMID: 32158253; PMCID: PMC6986890.
- Van Offel JF, De Clerck LS, Francx LM, Stevens WJ. The clinical manifestations of ochronosis: a review. *Acta Clin Belg*. 1995;50(6):358-62. doi: 10.1080/17843286.1995.11718475. PMID: 8571731.
- Pettit SJ, Fisher M, Gallagher JA, Ranganath LR. Cardiovascular manifestations of Alkaptonuria. *J Inher Metab Dis*. 2011 Dec;34(6):1177-81. doi: 10.1007/s10545-011-9339-z. Epub 2011 Apr 20. PMID: 21506017.
- Selvakumar D, Sian K, Sugito S, Singh T. Ochronosis of the aortic valve. *J Thorac Dis*. 2018 May;10(5):E332-E334. doi: 10.21037/jtd.2018.05.16. PMID: 29997987; PMCID: PMC6006137.
- Kostova T, Batalov Z, Karalilova R, Batalov A. Ochronotic arthropathy in the context of spondyloarthritis differential diagnosis: a case-based review. *Rheumatol Int*. 2022 Dec;42(12):2277-2282. doi: 10.1007/s00296-022-05191-4. Epub 2022 Sep 2. PMID: 36053307.
- Ather N, Roberts WC. Cardiovascular ochronosis. *Cardiovasc Pathol*. 2020 Sep-Oct;48:107219. doi: 10.1016/j.carpath.2020.107219. Epub 2020 Mar 7. PMID: 32473412.
- Planinc M, Unic D, Baric D, Blazekovic R, Sribar A, Sutlic Z, Rudez I. The Dark Side of the Heart: Cardiovascular Manifestation of Ochronosis. *Ann Thorac Surg*. 2019 Oct;108(4):e257-e259. doi: 10.1016/j.athoracsur.2019.02.042. Epub 2019 Mar 26. PMID: 30926473.
- Ffolkes LV, Brull D, Krywawych S, Hayward M, Hughes SE. Aortic stenosis in cardiovascular ochronosis. *J Clin Pathol*. 2007 Jan;60(1):92-3. doi: 10.1136/jcp.2005.032417. PMID: 17213354; PMCID: PMC1860586.
- Kenny D, Ptacin MJ, Bamrah VS, Almagro U. Cardiovascular ochronosis: a case report and review of the medical literature. *Cardiology*. 1990;77(6):477-83. doi: 10.1159/000174641. PMID: 2073665.
- Taniguchi S, Shimada T, Miyanaga T. Minimally invasive endoscopic aortic valve replacement for alkaptonuria-associated severe aortic stenosis: a case report and literature review. *General Thoracic and*

Cardiovascular Surgery, 24 Sep 2020, 69(3):605-609
12. J.F. Van Offel L.S. De Clerck L.M. Francx W.J. Stevens Review articles The Clinical Manifestations Of Ochronosis: A Review Pages 358-362
13. Kenny D, Ptacin MJ, Bamrah VS, Almagro U. Cardiovascular ochronosis: a case report and review of the medical literature. Cardiology. 1990;77(6): 477-83.

Corresponding author

Evgenia Marinova
35 "Borislav" str., 4000, Plovdiv
+359885573079
marinova.evgenia15@gmail.com.

Optical Coherence Tomography in Patients with Mild Cognitive Impairment: Is Retinal Thickness an Early Predictable Marker for Neurodegeneration?

Vesela Ivancheva¹, Diana Marinova², Aleksandar Lyubenov³

1. Medical University of Varna

2. Medical University of Pleven

3. Heart and Brain Center of Excellence – Pleven

Scientific purpose: to compare the optical coherence tomography (OCT) measurements of retinal nerve fiber layer (RNFL) thickness and ganglion cell complex (GCC) in patients with mild cognitive impairment (MCI) and healthy controls. **Methods:** A total of forty eyes were recruited in this pilot study- twenty eyes of ten consecutive patients with early-stage cognitive disorder and 20 eyes of ten healthy controls of same age (58 ± 6 y.o.). Comprehensive standardized ophthalmic examinations included visual acuity, cycloplegic refraction, color vision and intraocular pressure. Optical coherence tomography was performed using 3D Topcon OCT; RNFL thickness and ganglion cell complex (GCC) was detected with the inbuild software. Mean values for the thickness of the peripapillary RNFL and ganglion cell complex (GCC) were calculated and compared between study groups. **Results:** Statistically significant differences between the RNFL thickness in each quadrant and ganglion cell complex (GCC) of MCI patients and the healthy controls were found. **Conclusion:** OCT could be a useful clinical and valuable research tool for evaluation and monitoring the progression of cognitive disorders by measurement of the RNFL as purely axonal structure (indicative for axonal loss) and the macular ganglion cell complex (evidence of neuronal loss).

Keywords: optical coherence tomography, mild cognitive impairment, retinal nerve fiber layer thickness

Introduction

Pathology of central nervous system has ocular manifestations. They are due to retro-grade and/or direct degeneration of the visual pathways, mostly related to a direct injury to the optic nerve, retinal ganglion cell layer and/or its surrounding cells. Retina is ontogenically a brain-derived tissue and it has been suggested that it may provide an easily accessible and non-invasive way of examining the pathology of the brain: “the eye as a window of the brain” (1, 2). These ocular manifestations can be examined and monitored *in vivo* by Optical Coherence Tomography (OCT). It can detect CNS degenerative pathology at possibly subclinical stages and has a potential of quantifying neuroaxonal loss, and the increasing acceptance of the anterior visual pathway as a model to investigate MCI and other diseases of dementia spectrum (3–8). It was hypothesized that OCT imaging of the retina in patients with MCI may provide a high-resolution, objective, non invasive, and easily quantifiable *in vivo* biomarker of severity of disease.

Aim

The purpose of this study was to compare retinal nerve fiber layer (RNFL) thickness and ganglion cell complex (GCC) between MCI patients and aged matched healthy individuals using 3D Topcon 2000 FA+ OCT.

Methods

This was a pilot observational study conducted on a total of forty eyes: twenty eyes of ten consecutive patients with early-stage cognitive disorder and 20 eyes of ten healthy controls of same age (58 ± 6 y.o.). MCI was diagnosed according to the consensus criteria from the International Working Group on MCI. Cognitively normal controls attended the same clinic but were not impaired in any of the tested domains. Optical coherence tomography was performed using 3D Topcon OCT 2000 FA plus; RNFL thickness and ganglion cell complex (GCC) was detected with the inbuild software. Mean values for the thickness of the peripapillary RNFL and ganglion cell complex (GCC) were

calculated and compared between study groups. All subjects were examined using standard protocols: 3D Disc 6 mm x 6 mm, 3D Macula 6 mm x 6 mm and 3D Macula (V) 7 x 7 mm with 3D Topcon 2000 FA + (Topcon, Tokyo, Japan). At OCT scans center macular thickness was determined automatically and was analyzed by OCT software. Age stratified comparisons of all macular and RNFL measurements between groups were carried out using the independent sample t-test or ANOVA. For significance $p < 0.05$ was considered to be statistically significant. All analyses were performed using SPSS software, version 19.0.

Results

Peripapillary and macular OCT imaging was performed on 20 eyes of patients with mild cognitive impairment. No images were excluded from the final analysis due to poor image quality. Patient age ranged from 51 to 65 years (median 58 ± 6 years). The percentage of subjects who were female was 50%. Disease duration for all subjects with MCI was at least 5 years. The Macular Total Volume in MCI patients was decreased compared to healthy controls, with statistically significant differences. Mean Macular Total Volume for MS patients was $7.84 \pm 0.13 \text{ mm}^3$. For healthy controls mean Macular Total Volume was $8.34 \pm 0.22 \text{ mm}^3$. Peripapillary RNFL thinning was demonstrated: mean thickness was $107.2 \pm 3.8 \mu\text{m}$ ($P < 0.001$) for individuals with MCI when compared with eyes of healthy patients ($115.3 \pm 2.93 \mu\text{m}$). The ganglion cell complex (GCC) in MCI patients were all decreased compared to healthy controls, with statistically significant differences. Mean thickness for MCI patients was $59 \pm 0.2 \mu\text{m}$ for GCL+ and $98 \pm 0.12 \mu\text{m}$ for GCL++. For healthy controls mean thickness was $76 \pm 0.20 \mu\text{m}$ for GCL+ and $119 \pm 0.12 \mu\text{m}$ for GCL++ (Table 1).

Table 1. Comparison of pRNFL and GCL between the two groups

	Study Group ($\pm$ SD)	Control Group ($\pm$ SD)	p
pRNFL	$107.2 \pm 3.8 \mu\text{m}$	$115.3 \pm 2.93 \mu\text{m}$	$p < 0.001$
Macula: GCL+ GCL++	$59 \pm 0.20 \mu\text{m}$ $98 \pm 0.12 \mu\text{m}$	$76 \pm 0.20 \mu\text{m}$ $119 \pm 0.12 \mu\text{m}$	$p < 0.001$

Discussion

Early detection and prophylaxis is important, as well as in other diseases (10, 11). Several studies have explored the relationship between OCT parameters in patients with MCI and AD (9, 12–17). The neurology field has advanced to produce a validated cerebrospinal fluid (CSF) marker for AD (total tau:amyloid-beta), and positron emission tomography (PET) imaging with a radiotracer for amyloid-beta can also help to distinguish AD patients from controls without dementia. However, these biomarkers are both invasive, lumbar punctures have associated risks and contraindications (e.g., anticoagulation), and PET imaging is costly (18–20). Our OCT findings revealed a neuroanatomic difference in the RNFL and GCC thickness among the MCI group and healthy control group. This suggests that a change in these parameters could be a meaningful index for diagnosing early AD, and that OCT could be a valuable research instrument for evaluation and monitoring the progression of cognitive disorders by measurement of the retinal nerve fiber layer (RNFL) as purely axonal structure (indicative for axonal loss) and the macular ganglion cell complex (evidence of neuronal loss). However, there has been conflicting evidence on the effectiveness of ophthalmic scanning in mild cognitive impairment (MCI), the precursor of dementia. A significant correlation between OCT measurements in inner retinal layers with cognitive screening assessments has been reported,

although Ito et al saw no changes on OCT in MCI individuals, recommending further research (20).

Conclusion

To summarize, a decrease in RNFL and GCIPL thickness is associated with a decline in cognitive performance in MCI patients. Although these OCT parameters do not fully explain the variations in cognitive performance in MCI, they may serve as early biomarkers of cognitive decline and neurodegeneration in patients with this disease.

Ethical approval

“All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent was obtained from all individual participants included in the study.”

References

- Huang D, Swanson E, Lin CP, et al. (1991) Optical Coherence Tomography. *Science*; 254: 1178-1181.
- Chen TC, Cense B, Miller JW, et al. (2006) Histologic correlation of in vivo Optical Coherence Tomography- images of the human retina. *Am J Ophthalmol.* ; 141: 1165-1168.
- Xie J, Yi Q, Wu Y, Zheng Y, Liu Y, Macerollo A, Fu H, Xu Y, Zhang J, Behera A, Fan C, Frangi AF, Liu J, Lu Q, Qi H, Zhao Y. Deep segmentation of OCTA for evaluation and association of changes of retinal microvasculature with Alzheimer's disease and mild cognitive impairment. *Br J Ophthalmol.* 2023 Jan 3;bjophthalmol-2022-321399. doi: 10.1136/bjo-2022-321399. Epub ahead of print. PMID: 36596660.
- Hinton DR, Sadun AA, Blanks JC, Miller CA. Optic-nerve degeneration in Alzheimer's disease. *N Engl J Med.* 1986;315:485-487.
- Coppola G, Di Renzo A, Ziccardi L, et al. Optical coherence tomography in Alzheimer's disease: a meta-analysis. *PLoS One.* 2015;10:e0134750.
- Garcia-Martin E, Bambo MP, Marques ML, et al. Ganglion cell layer measurements correlate with disease severity in patients with Alzheimer's disease. *Acta Ophthalmol.* 2016;94:e454-459.
- Moreno-Ramos T, Benito-Leon J, Villarejo A, Bermejo-Pareja F. Retinal nerve fiber layer thinning in dementia associated with Parkinson's disease, dementia with Lewy bodies, and Alzheimer's disease. *J Alzheimers Dis.* 2013;34:659-664
- Liu B, Rasool S, Yang Z, et al. Amyloid-peptide vaccinations reduce {beta}-amyloid plaques but exacerbate vascular deposition and inflammation in the retina of Alzheimer's transgenic mice. *Am J Pathol.* 2009;175:2099-2110.
- Thomson KL, Yeo JM, Waddell B, et al.. A systematic review and meta-analysis of retinal nerve fiber layer change in dementia, using optical coherence tomography. *Alzheimers Dement* 2015;1:136-43. 10.1016/j.dadm.2015.03.001
- K. Dimitrova. MULTIDISCIPLINARY APPROACH IN OPHTHALMOPEDIATRIC PRACTICE—ACTUAL STATE AND OPPORTUNITIES. *Trakia Journal of Sciences* 20 (2), 139
- M. Sabri. Prophylactic Examinations For Cervical Cancer—Mandatory Precaution That Can Save A Life. *Varna Medical Forum* 10 (2), 443-448.
- Oktem EO, Derle E, Kibaroglu S, Oktem C, Akkoyun I, Can U. The relationship between the degree of cognitive impairment and retinal nerve fiber layer thickness. *Neurol Sci* 2015;36:1141-6.
- Gao L, Liu Y, Li X, Bai Q, Liu P. Abnormal retinal nerve fiber layer thickness and macula lutea in patients with mild cognitive impairment and Alzheimer's disease. *Arch Gerontol Geriatr* 2015;60:162-7.
- Kesler A, Vakhapova V, Korczyn AD, Naftaliyev E, Neudorfer M. Retinal thickness in patients with mild cognitive impairment and Alzheimer's disease. *Clin Neurol Neurosurg* 2011;113:523-6.
- Liu D, Zhang L, Li Z, Zhang X, Wu Y, Yang H, et al. Thinner changes of the retinal nerve fiber layer in patients with mild cognitive impairment and Alzheimer's disease. *BMC Neurol* 2015;15:14.
- BENJAMIN J. KIM, MD • DAVID J. IRWIN, MD The Potential Role of Optical Coherence Tomography for Dementia Patients. *Retinal imaging shows promise as a biomarker for dementia. Retinal Physician*, Volume: 15, Issue: Jan/Feb 2018, page(s): 49-51, 53
- Ciudin A, Simó R. New methods for the diagnosis and monitoring of cognitive function in patients with type 2 diabetes. *Front Endocrinol (Lausanne).* 2022 Dec 1;13:1024794. doi: 10.3389/fendo.2022.1024794. PMID: 36531450; PMCID: PMC9751391.
- Dreyer-Alster S, Gal A, Achiron A. Optical Coherence Tomography Is Associated With Cognitive Impairment in Multiple Sclerosis. *J Neuroophthalmol.* 2022 Mar 1;42(1):e14-e21. doi: 10.1097/WNO.0000000000001326. Epub 2021 Jul 21. PMID: 34294657; PMCID: PMC8834165.
- Chua J, Li C, Ho LKH, Wong D, Tan B, Yao X, Gan A, Schwarzhans F, Garhöfer G, Sng CCA, Hilal

S, Venketasubramanian N, Cheung CY, Fischer G, Vass C, Wong TY, Chen CL, Schmetterer L. A multi-regression framework to improve diagnostic ability of optical coherence tomography retinal biomarkers to discriminate mild cognitive impairment and Alzheimer's disease. *Alzheimers Res Ther.* 2022 Mar 10;14(1):41. doi: 10.1186/s13195-022-00982-0. PMID: 35272711; PMCID: PMC8908577

20. Chan VTT, Sun Z, Tang S, Chen LJ, Wong A, Tham CC, et al. Spectral-domain OCT measurements in Alzheimer's disease: a systematic review and

meta-analysis. *Ophthalmology.* 2019;126(4):497–510. 10.1016/j.ophtha.2018.08.009. [PMC free article] [PubMed]

Corresponding author

Vesela Ivancheva
Medical University of Varna
Affiliate Veliko Tarnovo
“1 “Mihai Kefalov” Str., Veliko Tarnovo
e-mail: vesela.lyubenova@gmail.com

Differential Diagnosis of Endocrine Hypertension – a Case Report

Elena Chobankova¹, Dilyana Stoyanova³

Scientific supervisor: Boyan Nonchev^{1,2}

1. Clinic of Endocrinology, UMHAT “KASPELA”, Plovdiv, Bulgaria

2. Department of Endocrinology, Medical University of Plovdiv

3. “Staykov Medical Centre”, Burgas, Bulgaria

Introduction: Arterial hypertension affects about 30% of the general population. The majority of cases are due to primary hypertension. In about 15% of all affected patients, high blood pressure is due to secondary causes such as renal or endocrine pathology. **Case presentation:** A 60-year old male was referred to the Endocrinology department due to resistant hypertension during the last 2 months, despite being on an extensive list of antihypertensive medications. A routine CT scan, performed as part of a follow-up for chronic lymphocytic leukemia, displayed bilateral adrenal incidentalomas. Prior to the hospitalization, blood testing detected remarkably low potassium levels, impaired fasting glycaemia and elevated normetanephrines. The oral glucose tolerance test revealed impaired glucose tolerance and insulin resistance. The aldosterone/renin ratio was extremely high. 24-hour urine cortisol was elevated. In order to distinguish between pseudo-Cushing's syndrome and true hypercortisolism, an overnight suppression test with 1 mg dexamethasone test was performed. Adequate inhibition of cortisol levels was achieved. A retest of normetanephrines in urine showed a negative result. Administration of an aldosterone antagonist was initiated with a good response from the patient. **Conclusion:** Hyperfunction of the suprarenal glands usually presents with hypertension, which can occasionally be a challenge for the differential diagnosis. Disruption of the adrenal hormonal secretion demands a meticulous consideration of possible patient-specific and pre-analytical confounding factors. The clinical presentation, the characteristics of the hypertensive syndrome and serum electrolyte levels are valuable guides in the etiological evaluation.

Keywords: endocrine hypertension, primary hyperaldosteronism

Introduction

Arterial hypertension affects about 30% of the general population. The majority of cases are due to primary hypertension. In about 15% of all affected patients, high blood pressure is due to secondary causes such as renal or endocrine pathology.

Actual presentation of the case and discussion

A 60-year old male was referred to the Endocrinology department due to resistant hypertension. High blood pressure (BP) was diagnosed 10 years ago, with impaired control during the last few months, despite the intake of multiple anti-hypertensive medications. His therapy included: Olmesartan/Amlodipine 20/5 mg x 1 tab q.d., Indapamide 25 mg x 1 tab q.d., Spironolactone 50 mg x 1 tab q.d., Moxonidine 0.4 mg 2 x 1 tab q.d., Doxazosine 2 mg 3 x 1 tab q.d., Nebivolol 5 mg x 1/2 tab q.d. He reported

persistently high BP with frequent hypertensive crises – BP levels up to 220/110 mmHg, leading to several hospitalizations in cardiology departments. K⁺ levels were low on repeated tests – up to 2.7 mmol/l/3.5–5.5/. The patient was diagnosed in 2012 with B-cell chronic lymphocyte leukemia (B-CLL). Since 2017 he has been treated with Ibrutinib. During routine CT scans with i.v. contrast of the abdomen, as a follow-up for to the hematologic disease, a rounded lesion in the left suprarenal gland, sized 26/21 mm and native density 6 HU was described. In some, but not all of the most recent CT scans, another smaller lesion in the right suprarenal gland was observed, with a size of 9/9 cm and native density 11 HU. Ambulatory, fractionated plasma metanephrines (PMET) were tested. The test revealed normal levels of metanephrines – 45 ng/l /<102/ and elevated normetanephrines – 325 ng/l /<189/. Impaired fasting glucose

was noted a year earlier. He reported progressive weight-gain. The patient was a heavy smoker with no family history of endocrine diseases or hypertension. He was admitted in a satisfactory condition. The general status revealed hypersthenic habitus (BMI – 34.38 kg/m²), pale mucous membranes, high BP – 200/100 mmHg. The rest of the status was unremarkable. K⁺ levels were in the lower limit of normal (3.8 mmol/l), despite the administration of 2 tab q.d. Kalinor. Other electrolytes were within reference ranges. Normal kidney and liver function was observed. The patient was biochemically euthyroid. Reference ranges of morning (393,92 nmol/l /140–500/) and midnight cortisol (101,09 nmol/l /< 138/) and preserved rhythm were detected, as well as normal ACTH – 23,5 pg/ml /8,53–57,8/. Blood test revealed suppressed renin – 1,144 μ[IU]/mL /4,2–45,6/, high aldosterone – 406,7 pg/ml /70–300/ and aldosterone/renin ratio (ARR) – 355.5 /< 3.7/. The tests were performed after a preparation with Verapamil and Doxazosin. Elevated urinary free cortisol (UFC) was also detected – 491,19/D-2100 μmol/24 h /130–360/. Oral glucose tolerance test revealed impaired glucose tolerance (IGT) – blood glucose at 120 min – 9.92 mmol/l /< 7.8/, lack of insulin resistance – HOMA index – 1.89. The ultrasound examination of the neck area was unremarkable. Based on the clinical presentation and the laboratory results we narrowed down the differential diagnosis to 3 possible diseases: Cushing's syndrome, pheochromocytoma and hyperaldosteronism. The CT scan image was not specific for neither of those options. IGT could also be observed in all of them. The obesity and high UFC suggested a possible Cushing's syndrome. However, the preserved cortisol rhythm implied pseudo-Cushing's syndrome due to the excess weight. The elevated PMET

indicated pheochromocytoma. Nevertheless, the typical triad (palpitations, perspiration, headache) was not observed during a hypertensive crisis. A careful interrogation of the patient revealed non-observance of the conditions for PMET testing. The necessary 20-min rest before sample collection and tobacco abstinence were not considered. Ignoring these preparations can lead to false-positive results. Hyperaldosteronism was very likely, due to the notable hypokalemia and markedly elevated ARR. The express 1 mg dexamethasone overnight suppression test confirmed pseudo-Cushing's syndrome – cortisol 8 h – 28.2 nmol/l /< 50/. Normal fractionated metanephrines in urine ruled out pheochromocytoma (metanephrines – 181 μg/24 h /43,4–260/, normetanephrines – 312 μg/24 h /128–484/). We asked for a revision of the last CT scans with i.v. contrast of the abdomen. Formations in both adrenals were confirmed with a dominant hypodense nodule in the left gland and a subcentimeter nodule in the contralateral gland. Unfortunately, performance of a lateralization technique was not available. Confirming hyperaldosteronism, we faced the challenge of specifying the exact subtype. We diagnosed the patient with Conn's adenoma due to the marked spontaneous hypokalemia and the extremely high levels of aldosterone > 30 ng/dl, which are a hallmark of autonomic nodules. We suggested that the dominant hypodense nodule on the left was the functioning nodule, and the subcentimeter nodule in the contralateral gland was an incidentaloma (commonly found > 35 years of age). We added a maximum dose of Spironolactone 25 mg 2x2 tab q.d. to the therapy with Verapamil SR 120 mg 2 x 2 tab q.d. and Doxazosin 4 mg 3 x 1 tab q.d. The pros and cons of surgical treatment were discussed with the patient. Due to his preferences he was referred for a

laparoscopic removal of the left adrenal gland. The histological result confirmed a suprarenal adenoma. Postoperatively, K^+ levels normalized promptly, BP control was optimized and the anti-hypertensive therapy was reduced.

Conclusion

The differential diagnosis of endocrine hypertension is primarily directed towards adrenal hypersecretion. Disruption of the adrenal hormonal secretion demands a meticulous overview of possible patient-specific and pre-analytical factors. The clinical

presentation, the characteristics of the hypertensive syndrome and serum electrolyte levels are valuable guides in the search of the etiology.

References

1. Funder J. W. et al. 2016 J Clin Endocrinol Metab. DOI 10.1210/jc.2015-4061
2. Pommer G. et al. 2022. J Clin Endocrinol Metab. DOI: 10.1210/clinem/dgac390
3. Boyd J. et al. 2019 Eur J Endocrinol. 2019. DOI: 10.1530/EJE-19-0176

Corresponding author

Elena Chobankova
Endocrinology Department
UMHAT “Kaspela”, Plovdiv, Bulgaria
Email: elena.chobankova@abv.bg

V. Dental Medicine Session

Er:YAG Laser Debonding of Porcelain Veneers – a Pilot SEM Study

Mariela Tsanova-Stamatova¹, Georgi Tomov²

1. Department of Operative Dentistry and Endodontics, Faculty of Dental Medicine, Medical University of Plovdiv

2. Research Institute at Medical University of Plovdiv, Center for Laser Dentistry

Introduction: The use of ceramic veneers is an integral part of the modern esthetic dentistry. However, some clinical situations require their removal and the procedure is unpredictable both for the integrity of the veneer and the tooth structures. The removal of ceramic veneers with Er:YAG laser is a clinical procedure often reported in the literature (case reports) but only a scanty number of publications address its safety. **Aim:** The purpose of this study is to evaluate the ability of Er:YAG laser to debond successfully porcelain veneers with different design and thickness without damaging the tooth structures and the veneer. **Methods:** Three different design of zirconia ceramic veneers of thickness that varied from 0.1 mm and 1 mm were included in this study. Freshly extracted teeth were used and the samples were decontaminated, stored, prepared and bonded to obtain veneers adhesion. One week after an Er:YAG laser with a non-contact sapphire tip with air-water spray was used for veneer debonding at 20 Hz, 250 mJ (5 W). The overall debonding time of each veneer was recorded and the integrity, the presence of cracks and defects both on the tooth surface and the veneers were analysed using a SEM. **Results:** The average time for the laser-assisted debonding was 52,33 sec. The results demonstrated that veneer debonding seems not related to the preparation design and the veneer thickness. SEM observation confirms that residual tooth structure and the veneers are not altered when using these parameters. **Conclusions:** The Er:YAG laser is able to debond veneers while preserving the tooth structures. The laser-assisted veneer debonding allows the re-use of the detached veneer in some clinical cases. The method is rapid.

Keywords: Debonding, Er:YAG laser, veneer, scanning electron microscopy

Introduction

The use of ceramic veneers is an integral part of the modern esthetic dentistry. The interest to these restorations is attributable to their conservative preparation, esthetic quality, fracture resistance, soft tissue tolerance, low debonding rate and negligible incidence of caries^[1].

However, some clinical situations require their removal. Failure may occur due to several reasons, such as the longevity of adhesive materials or the procedure applied technique. Marginal infiltration, cement decoloration, laminate crack or patient's aesthetic dissatisfaction of shade or shape of all ceramic restoration. Apart from these, microleakage may also occur, which may lead to caries, irreversible pulpitis, staining, and

gingival reactions. There are also failures during the cementation process such as improper positioning. In all of these cases, the procedure of debonding is unpredictable for the integrity of the veneer. The safety removal with respect to preservation of the veneer integrity is still under consideration.

The laser-assisted debonding procedure is considered to be novel and promising method in dentistry. One of these lasers, erbium-doped, yttrium-aluminum-garnet (Er:YAG), has been known and successfully used for debonding ceramic brackets. On the other hand the removal of ceramic veneers with Er:YAG laser is a clinical procedure reported in the literature but only a scanty number of publications address its safety and effectiveness^[2,3].

Aim

The purpose of this study was to evaluate by using scanning electron microscopy the ability of Er:YAG laser to debond successfully porcelain veneers with different designs and thickness without damaging the veneer itself.

Materials and methods

Three different designs of zirconia ceramic veneers with diversified thickness were included in this study. Freshly extracted teeth were used. The samples were decontaminated and stored in a 0.1% thymol solution for 2 days. They were then rinsed on coolant water and veneers were prepared as follows.

Enamel preparation: Vestibular enamel surfaces were prepared under air/water spray with a turbine (Kavo Supertorque 660 B, Germany) with diamond burs (Mani Dia Burs, TR-26F Japan) and polished with TR-26EF burs. Three different designs of zirconia veneers (feathered-edge window, butt joint, palatal chamfer) of thickness that varied from 0.3 mm and 1 mm were included in this study (Fig. 1).

Impression: Once the macroscopic evaluation of the preparation was recognized as acceptable, all of them were fixed in C-silicone impression material (Swisstec, Coltene) and scanned in a laboratory scanner (Autoscan DS-EX, Shining 3D). Then zirconia ceramic veneers (UTML Katana™ Zirconia discs, Kuraray Noritake Dental Inc.) were realized according to the manufacturer's instructions.

Pretreatment procedure: Zirconia veneers were laboratory pretreated with sandblasting with 50 µm AL₂O₃ at a pressure of 2

bars. After that they were ultrasonically cleaned in distilled water.

Sealing: For the sealing procedure, we used Panavia V5 Professional kit (Kuraray Noritake Dental inc.). K-etchant Syringe was applied for selective etching of the prepared teeth. Afterwards the tooth surface was pretreated with Panavia V5 Tooth Primer for 20 sec and air-dried. Clearfil Ceramic Primer Plus was applied on all zirconia veneers and air-dried. Then dual-cure cement Panavia™ V5 was applied and light cured.

Debonding procedure: The veneers were all debonded and removed without the use of diamond tip burs. The laser parameters were: 20 Hz, 250 mJ (5 W) with water/air spray. The application was initiated with 1 min 30 sec duration, with sinusoidal movements along the laminate surface and in a perpendicular position along the tooth axis, 5 mm distant from the tooth. In case the ceramic laminate did not detach, one more minute of laser application was performed and another debonding attempt with the spatula was made (Fig. 2). The integrity of the veneers were analysed using a SEM.

Results

All veneers were completely and easily removed from the tooth using the Er:YAG laser. The total removal time was 157 s. The average time for the laser-assisted debonding was 52,33 sec. The results demonstrated that veneer debonding seems not related to the preparation design and the veneer thickness. On all specimens, the typical structure of the veneer cement covered the surfaces. SEM observation confirms that residual tooth structure and the veneers are not altered when using these parameters (Fig. 3).



Figure 1. Preparation of three different designs of ceramic veneers (feathered-edge window, butt joint, palatal chamfer) and evaluation of their thickness.



Figure 2. Debonding procedure with Er:YAG laser.

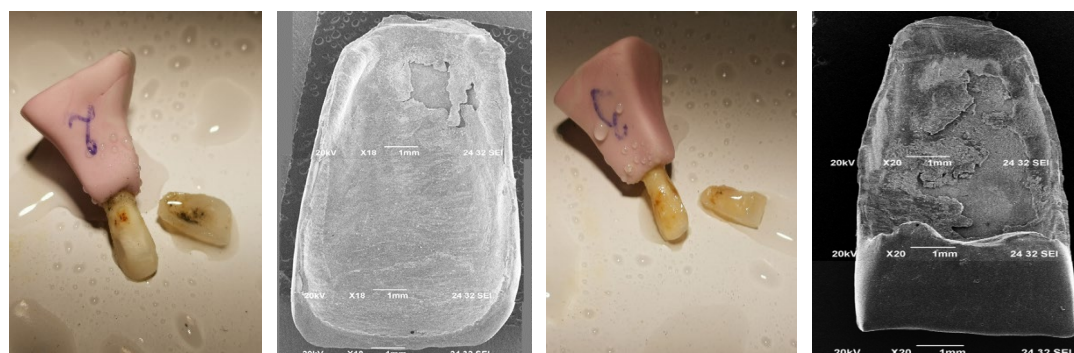


Figure 3. Intact veneers after debonding and consequent SEM evaluation.

Discussion

Clinically, removing a cemented ceramic veneer can consume around 30 to 60 minutes. More importantly, the conventional veneer removal technique with rotary instrument would destroy the restoration and thus make it totally unappropriated if we want to reuse the veneer. Zirconia ceramic is often cracked upon touching by a rotary instrument or US tip. In addition minimal veneer all ceramic preparations are one of the most

preferred procedures in everyday dental practice when there are indications for it. Most common problems related with them are misplacement and their fragility. In particular, for typical enamel-limited and minimal preparations for laminate veneers, laser application can be applied safely for debonding fragile restorations, even as thin as 0.5 mm, and this may be very useful for removing misplaced restorations immediately and then recementing them immediately^[4].

Moreover, using a laser instead of a high-speed handpiece for debonding restorations can avoid enamel substrate removal; thus, the use of a laser is valuable for the long-term success of laminate restorations^[5].

Therefore, there is a clear need for a more conservation approach of all ceramic restorations removal. Previous studies verified that lasers that soften the adhesive resin are effective for the removal of ceramic brackets^[6]. However, there are currently limited studies that confirm the efficacy of laser debonding for laminate veneers^[2,3]. The results from the present study also confirmed that Er:YAG laser can be used as a non-invasive tool to retrieve a ceramic veneer.

Debonding thicker ceramics has been reported to require higher laser energies than those of thin veneers due to the higher optimal laser energy needed to reach and react with the cement–ceramic interface^[7]. The relationship between the restoration thickness and the mechanism of polymer degradation was shown by Culhaoglu et al in 2021^[8]. As the restoration thickness decreases, the laser application becomes more effective. While no damage was observed at the resin cement tooth connection in the thick specimens, ablation areas and melted apatite debris were observed on tooth surfaces in the 0.5-mm thick specimens. Moreover, molten apatite debris may depend on whether the water cooling did not reach the interface between the tooth and resin cement because clean ablated surfaces without smear layers and intact enamel tubules have been reported in the presence of the water spray. In our study strict protocol of veneer preparation was applied and all of the veneers were with thickness between 0,3 mm and 1 mm. All of them were removed for abbreviated time and with no damage to the ceramic surface.

It is important to note the laser settings such as energy (mJ), frequency (Hz), which

result in power (W) differentiate in this study compared to some other authors. Rechmann et al^[9] applied a higher energy setting of 500 mJ for ~4–5 min compared to the energy setting in the present research of 250 mJ for ~2–3 min. Kursoglu (2013)^[10], in his case report paper, applied also laser with higher frequency of 29 Hz and higher energy (320 mJ) again on ceramic laminates. On the other hand Iseri^[11], in his laboratory study used less energy of 100 mJ. However, a higher frequency was used – 50 Hz, and again success was achieved with laminate debonding. Based on the literature data and within the limitations of this pilot study, results therefore indicate that lower energy setting may present a safer and more sufficient method for veneer removal.

Conclusion

The Er:YAG laser is able to debond veneers in a rapid manner while preserving the integrity of the ceramic restoration. The laser-assisted veneer debonding allows the reuse of the detached restoration if needed. Further clinical studies have to confirm the success rate of this method.

References

1. Guruprasada, Rivankar N, Dhiman RK, Viswambaran M. Evaluation of the effect of surface preparation using phosphoric acid and luting cement on the flexural strength of porcelain laminate veneering material. *Med J Armed Forces India* 2015;71:299-305.
2. Zhang Y, Rocca JP, Fornaini C, Zhen Y, Zhao Z, Merigo E. Erbium-doped, yttrium-aluminum-garnet laser debonding of porcelain laminate veneers: An ex vivo study. *Contemp Clin Dent* 2018;9:570-3.
3. Calabro, D.E., Puglisi, R.D., & Mesquita, A.M. (2019). Removal of 11 Laminate Veneers with Er-Yag Laser. *Research & Reviews: Journal of Dental Sciences*, 7.
4. Ghazanfari R, Azimi N, Nokhbatolfoghahaei H, Alikhasi M. Laser aided ceramic restoration removal: A comprehensive review *J Lasers Med Sci*. 2019;10:86
5. Öztürk E, Bolay Ş, Hickel R, Illie N. Shear bond strength of porcelain laminate veneers to enamel, dentine and enamel–dentine complex bonded with

different adhesive luting systems J Dent. 2013;41:97–105

6. Kim, W.T.; Bayome, M.; Park, J.B.; Park, J.H.; Baek, S.H.; Kook, Y.A. Effect of frequent laser irradiation on orthodontic pain. Angle Orthod. 2013;83:611–616.

7. Rechmann P, Buu NC, Rechmann BM, Finzen FC. Laser all-ceramic crown removal and pulpal temperature-A laboratory proof-of-principle study Lasers Med Sci. 2015;30:2087–93

8. Çulhaoğlu AK; Kilicarslan MA; Gökdeniz B; Gür G. The Efficiency of Laser Application for Debonding Laminate Restorations Manufactured of Current CAD-CAM Materials with Different Thicknesses. Nigerian Journal of Clinical Practice 2021; 24(5):705-711.

9. Rechmann P, Buu NCH, Rechmann BMT, Finzen FC. Laser all-ceramic crown removal-a laboratory

proof-of-principle study-Phase 2 crown debonding time. Lasers Surg Med. 2014;46: 636–643.

10. Kursoglu P and Gursay H. Removal of fractured laminate veneers with Er:YAG laser: report of two cases. Photomed Laser Surg. 2013;31:41-43.

11. Iseri U, et al. Effect of Er:YAG laser on debonding strength of laminate veneers. Eur J Dent. 2014;8:58-62.

Corresponding author

Dr. Mariela Tsanova-Stamatova PhD
Medical University of Plovdiv, Bulgaria
Faculty of Dental Medicine
Department of Operative Dentistry and Endodontics
boul. “Hristo Botev” №3
mobile: +359/889813946
e-mail:mariela.pts@abv.bg.

Management of Epulis during Pregnancy by the Dental Practitioner – Literature Review

Vasil Dimitrov¹, Denislav Kitov¹, Rositsa Tsareva¹, Krum Vladov^{2, 3}, Kamen Yamakov^{2, 3}

1. Faculty of Dental Medicine, Medical University of Plovdiv

2. Department of Obstetrics and Gynecology, Faculty of Medicine, Medical University of Plovdiv

3. Clinic of Obstetrics and Gynecology, University Hospital “St. George” EAD Plovdiv

Introduction: Epulis gravidarum is an inflammatory lesion, which develops in the oral mucosa of pregnant women (usually in late first trimester) in response to chronic irritants of low degree such as bacterial biofilm, dental calculus and traumatic agents. These lesions can also present as asymptomatic, pedunculated, red-to-purple raspberry-like nodules, which can be ulcerated due to secondary trauma. Epulis gravidarum has proclivity for intraoral locations, predominately affecting the gingiva followed by the lower lip, tongue, buccal mucosa, upper lip and palate. **Aim:** The aim of this study is to evaluate the literature on rate, clinical signs and possible treatment options of Epulis Gravidarum. **Resources and methods:** A literature search of PubMed data base was conducted. Used keywords were: epulis gravidarum, oral pregnancy tumor, granuloma gravidarum, pregnancy epulis. **Results and discussion:** Publications from 1948 to the present have been found, reflecting the interest of researchers over the years and the relevance of the problem to the present day. **Conclusion:** The literature review on the subject provides information accumulated over more than 75 years on the management of epulis during pregnancy by the dentist in collaboration with the obstetrician.

Keywords: *epulis gravidarum, dental management*

Introduction

Epulis gravidarum is an inflammatory lesion, which develops in the oral mucosa of pregnant women in response to chronic irritants of low degree such as bacterial biofilm, dental calculus and traumatic agents.^[1, 4] The development of this particular kind of gingivitis, typical in pregnancy, suggests the existence of a relationship between the gingival lesion and the hormonal condition observed in pregnancy. Increased levels of progesterone during pregnancy produce dilation and tortuosity of the gingival microvasculature, circulatory stasis and increased susceptibility to mechanical irritation, all of which favor leakage of fluid into the perivascular tissues.^[2] Localized gingival enlargements are often seen in pregnant patients (granuloma gravidarum) and are categorized as ‘conditioned enlargements’ as the systemic condition of the patient exaggerates or distorts the normal gingival response to dental plaque. Although commonly used, the term ‘pyogenic granuloma’ is a misnomer since

the condition is not associated with pus and does not represent a granuloma histologically^[2]. An increased number of periodontal pathogens, especially *Porphyromonas gingivalis*, *Prevotella intermedia* and *Porphyromonas intermedius* in the subgingival biofilm, in pregnancy may directly affect gingival fibroblasts and keratinocytes thus leading to the exuberant tissue response against plaque, ‘increases vascular permeability, favors the infiltration of fluids into the perivascular tissues and enhances the inflammatory response, having an important role in the development of the lesion. *Porphyromonas intermedius* is also able to use progesterone instead of vitamin K as an essential growth factor^[3]. It is a benign, pink, semi-firm, discrete, mushroom-like, exophytic tissue overgrowth that develops within days to weeks and it is attached on a pedunculated or sessile base.^{[1][2]} It is generally associated with bleeding either spontaneously or during tooth brushing and mastication^{[2][4]}

Aim

The aim of this study is to evaluate the literature and present a review about the clinical signs, treatment options and rate of epulis gravidarum.

Materials and methods

A literature search was conducted via PubMed database. Keywords used were “epulis gravidarum”, “oral pregnancy tumor”, “granuloma gravidarum”, “pregnancy epulis”. Only articles in English and Bulgarian were included. Systematic review articles were excluded from the search. The initial search identified 231 results, of which 205 were excluded due to containing non-relevant information, with the remaining 26 included in this review. The search strategy includes only articles that contain treatment options and clinical signs of epulis gravidarum.

Results and discussion

Epulis gravidarum (EG) is observed most commonly in the second and third trimester of pregnancy and its prevalence is estimated to be around 0.2% to 9.6% in pregnant woman.^[4] The location that is most prevalent for EG is the gingiva, as about 73.17% of the lesions are located there. Other locations are – tongue (14.63%), lips (7.32%), buccal mucosa (4.88%). EG is also reported to manifest in subcutaneous and superficial tissues. According to different studies, the prevalence of EG is in the third decade of life with average age of 28 years.^[6] EG is most frequently appearing in areas where gingivitis has already been observed. It is also associated with dental plaque and calculus, as the last have been observed on teeth adjacent to EG.^{[4][1]} Pregnancy is associated with unique hormonal changes in the female body. Estrogen and progesterone are the hormones that are mainly excreted during pregnancy.

Estrogen is related to nutrient transfer and the vascularization of the placenta. Estrogen levels reach their peak in the third trimester. This increased levels of estrogen and progesterone in combination with dental biofilm and irritants (calculus) are causing changes in the oral mucosa – which results in EG.^[4] Estrogen is also observed to be stimulating Nerve Growth Factor (NGF) in macrophages, Fibroblast Growth Factor and Transforming Growth Factor beta1, which leads to production of gingival granulations. Levels of progesterone in saliva during pregnancy are significantly higher, compared to saliva of non-pregnant women, reaching levels of 1543 pg/ml in their peak in third semester^[3, 4]. As a comparison, levels of progesterone in non-pregnant women is around 40 pg/ml^[3]. One of the functions of progesterone is immunosuppressing. Thus, progesterone inhibits the migration of inflammatory cells and fibroblasts especially in the gingival sulcus. This inhibition in combination with the “active” progesterone in the blood, changes the gingival immune system and the cells of the periodontium which alters the reaction of the organism to irritants. This is reported to be one of the main causes of EG in pregnant women.

Dental sulcus is home to many microorganisms. One of them is *Prevotella intermedia*, which is related to periodontal diseases. In third trimester of pregnancy, levels of *P. intermedia* are reported to be significantly higher in comparison to non-pregnant^[8,13]. Progesterone is shown to enhance the growth of oral *P. intermedia*. In fact, *Prevotella* species can use the progesterone as food instead of vit. K. EG have also been observed in woman taking oral contraceptives with higher progesterone dosage. Another microorganism related to periodontal diseases is *Fusobacterium nucleatum*, which can invade all kind of cells in the human

body^[13]. The recent report of a term stillbirth caused by oral *Fusobacterium nucleatum* provided the first human evidence that the bacteria originated from the mother's subgingival plaque and translocated to the placenta and fetus, causing acute inflammation leading to the fetal demise^{[7][5]}.

EG is a smooth or lobulated exophytic lesion manifesting as small, red, erythematous papules on a pedunculated or sessile base, which is usually hemorrhagic and compressible and may develop as dump-bell-shaped masses. It is reported that the two types of EG are clinically different. LCH EG occurs more frequently (66%) as a sessile lesion, whereas non-LCH EG mostly occurs as pedunculated (77%)^[12]. EG may vary in size – from a few millimeters to several centimeters. Although, it rarely exceeds 2.5–3.0 cm and it usually reaches its full size within weeks or months. EG usually has slow, asymptomatic, and painless progression. The surface of the lesion is typically ulcerated and friable, often covered by a yellow, fibrinous membrane. The color of the lesion varies between pink and red nuances, according to the age of the lesion. Clinical development of the lesion is slow, asymptomatic, and painless, but it may also grow rapidly. Young EGs are highly vascular in appearance, because they are composed predominantly of hyperplastic granulation tissue in which capillaries are prominent. Minor trauma to the lesion may cause considerable bleeding, due to its pronounced vascularity, whereas older lesions tend to become more collagenized and pink. Usually, EG is clinically indistinguishable from pyogenic granuloma^[14]. There is a report by Goodman-Topper and Bimstein which informs that in rare cases EG may cause significant bone loss. EG, which manifest in subcutaneous and superficial tissues is rapidly growing lesion. It is typically a painless sessile or

pedunculated mass of varied diameter with size varying from 5–10 mm. It starts as a small pin head sized lesion, which grows rapidly. It is prone to spontaneous hemorrhage or hemorrhage after minor trauma^[15].

Histologically inflamed gingiva in pregnancy has no specific feature distinguishing it from “normally” inflamed gingiva (Maier & Orban 1949). Similarly, pregnancy granuloma is histologically identical to pyogenic granuloma known to be inflammatory by origin (Pindborg 1983). Histologically EG present loose granulation tissue rich in capillary vessels and proliferation of endothelial cells, typically accompanied by a mixture of inflammatory cells^[16]. Microscopic examination of EG shows a highly vascular proliferation that resembles granulation tissue^[17]. Numerous small and large channels are formed which are filled with red blood cells^[17], and lined by flat or plump endothelial cells that are probably mitotically active^[18]. The blood vessels often show a clustered or medullary pattern separated by less vascular fibrotic septum. At lower magnification, particularly at the lateral edges, a lobular arrangement is noticed wherein groups of capillaries proliferate but abruptly stop. Each lobule is surrounded by a thin collagen layer. This arrangement is disrupted at the base, where irregularly shaped larger vascular channels reside and presumably communicate with the proliferation. It is here that some anastomosing channels can occasionally be identified, but they are focal rather than an integral part of the lesion. At higher magnification, another discriminating feature of benign vascular lesion is found: the small capillary endothelial-lined spaces, which are surrounded by a double layer of perithelial or pericytic cells^[18,19]. Polymorphs, as well as chronic inflammatory cells, are consistently present throughout the edematous stroma, with micro-abscess

formation^[20]. The fibroblasts are typically plump and mitotic activity could be noted in the stromal cells. Older lesions demonstrate fibrocytes, which are fewer and more mature cells. Some scarring may be noted in some of these lesions, showing that occasionally there may be maturation of the connective tissue repair process^[21]. The surface is usually ulcerated and replaced by a thick fibrin purulent membrane^[17]. A mixed inflammatory cell infiltrate of neutrophils is mostly prevalent near the ulcerated surface; chronic inflammatory cells are found deeper in the sample^[17,21]. The natural history of the lesions follows three distinct phases – cellular, capillary and involutionary. In the cellular phase, the lobules are compact and cellular with little lumen formation. In the capillary phase, the lobules become frankly vascular with abundant intra-luminal red blood cells. One or more central vessels develop a large lumen with a thick muscular layer resembling a vein. In the involutionary phase, there is a tendency for intra- and perilobular fibrosis with increased venular differentiation^[19]. There are two histological types of EG. The first type is characterized by proliferating blood vessels that are organized in lobular aggregates. Superficially the lesion frequently undergoes no specific change, including edema, capillary dilation or inflammatory granulation tissue reaction. This histological type of EG is called lobular capillary hemangioma (LCH type)^[22], whereas the second type (non-LCH type) consists of highly vascular proliferation that resembles granulation tissue^[17,23]. The lobular area of the LCH EG contains a larger number of blood vessels with small luminal diameter than does the central area of non-LCH EG. In the central area of non-LCH EG, a significantly greater number of vessels with perivascular mesenchymal cells non-reactive for α -smooth muscle actin and muscle-specific

actin is present than in the lobular area of LCH EG. These differences suggest that two histologic types of EG represent distinct formations^[12]. Toida et al.^[24] found that the presence of blood vessels with different luminal diameter in the lobular area of LCH EG and in the central area of non-LCH EG may be because different pathogenic factors influence their development. Epivatianos et al.^[12] observed that foci of fibrous maturation were present in 15% of non-LCH EG but were totally absent in LCH EG. Although it is possible that EG can undergo fibrous maturation, they showed that this happened only in non-LCH EG which suggest that the two types of EG have different pathways of evolution.

According to the National Consensus, pregnant women should be advised to continue with routine dental visits every 6 months and to schedule a dental appointment as soon as possible if oral health problems or concerns arise^[4,11]. In fact, this is never the reality. Only 22 to 34 percent of women in the United States consult a dentist during pregnancy^[10]. Even when an oral problem occurs, only one half of pregnant women attend to it. Recent data suggest that about 50% of women do not have a dental visit during pregnancy, even when they perceive a dental need^[1,4,9]. This data is unsatisfactory because every pregnant woman should be screened for oral risks, counseled on proper oral hygiene, and referred for dental treatment when necessary and should be assessed for dental hygiene habits, access to fluoridated water, oral problems (e.g., caries, gingivitis), and access to dental care^[9]. Comprehensive prenatal health care should include an assessment of oral health^[1,4]. Oral examination should include the teeth, gums, tongue, palate, and mucosa. Patients should be counseled to perform routine brushing and flossing, to avoid excessive amounts of

sugary snacks and drinks, and to consult a dentist^[1]. The pregnant patient should be educated on the importance of oral health care for herself and on expert recommendations for bolstering home oral hygiene care during pregnancy^[11].

Management of EG is usually observational. In the patient's initial treatment visit, root planning and ultrasonic scaling are done, because removal of local irritants may lead the process to regression. The patient is also instructed of good oral hygiene and habits. Chlorhexidine gluconate mouthwash is advised for improving the oral hygiene. The need for surgical intervention during pregnancy should be carefully examined, considering the high rate of GG recurrence, as well as the possibility of regression after childbirth. The surgical manipulation is risky because the epulis bleeds heavily and may result in serious morbidity or fetal mortality. Another thing about the surgical incision is that, if done during pregnancy, the lesion is likely to recur, and the patient should be informed of that possibility^[1,26]. If, despite all of this, the lesion is bleeding, causing irritation, interfering with mastication or is causing esthetic problems, it can be surgically removed. The surgical manipulation can be carried out with a scalpel and a mucoperiosteal flap. However, other treatment methods can also be used – laser therapy, electro-cautery, chemical cautery^[26]. Good results are obtained with cryocautery^[16]. Some cases of the EG resolve spontaneously after delivery, mainly due to the regularization of hormone levels. If the lesions persist after childbirth, surgical excision can be carried out. Different non-surgical therapies have been developed. One of them is the angiogenesis therapy - with angiostatin and endostatin^[25]. Angiostatin is capable of inhibiting the migration of monocytes and neutrophils to the inflammatory sites. It can also inhibit

pathological angiogenesis without affecting the development of physiological vascularization. All of this leads to decrease in inflammatory cytokines^[9], and the granuloma cells enter a default pathway of apoptosis. Recombinant human endostatin has entered phase I clinical study and has been proven safe and well tolerated^[25].

Conclusion

Epulis during pregnancy is a benign neoplasm with a low incidence manifesting more frequently in the second trimester. In some cases, it can reach large sizes and provoke associated complications or lead to recurrent gingivorrhagia. A review of the literature shows that the dentist can monitor the lesion, which in most cases regresses after delivery, or undertake conservative or surgical treatment. For pregnant women with epulis, a multidisciplinary approach and collaboration with the obstetrician who monitors the course of pregnancy are very important.

Bibliography

1. Cardoso JA, Spanemberg JC, Cherubini K, Figueiredo MA, Salum FG. Oral granuloma gravidarum: a retrospective study of 41 cases in Southern Brazil. *J Appl Oral Sci.* 2013;21(3):215-8. doi: 10.1590/1679-775720130001. PMID: 23857656; PMCID: PMC3881906.
2. Krishnan B, Arunprasad G, Madhan B. Giant granuloma gravidarium of the oral cavity. *BMJ Case Rep.* 2014 Apr 8;2014:bcr2014204057. doi: 10.1136/bcr-2014-204057. PMID: 24713716; PMCID: PMC3987557.
3. Ojanotko-Harri AO, Harri M-P, Hurtia HM and Sewon LA: Altered tissue metabolism of progesterone in pregnancy gingivitis and granuloma. *J Clin Periodontol* 1991; 18: 262-266
4. Steinberg BJ, Hilton IV, Iida H, Samelson R. Oral health and dental care during pregnancy. *Dent Clin North Am.* 2013 Apr;57(2):195-210. doi: 10.1016/j.cden.2013.01.002. Erratum in: *Dent Clin North Am.* 2013 Jul;57(3):ix. Iida, Hiroko [corrected to Iida, Hiroko]. PMID: 23570802.
5. Sarwal P, Lapumnuaypol K. Pyogenic Granuloma. 2022 Oct 23. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. PMID: 32310537.
6. Dassatti L, Manicone PF, Iavarone F, Stefanelli N, Nicoletti F, Lazzareschi I, Luciano RPM,

- Castagnola M, D'Addona A. Proteomic evaluation of GCF in the development of pregnancy related periodontal disease: a pilot clinical study. *Eur Rev Med Pharmacol Sci*. 2019 Jun;23(12):5030-5039. doi: 10.26355/eurrev_201906_18166. PMID: 31298357.
7. Chaikin BS. Incidence of gingivitis in pregnancy. *Quintessence Int Dent Dig*. 1977 Oct;8(10):81-9. PMID: 351665.
8. Schlagenhauf U, Jakob L, Eigenthaler M, Segerer S, Jockel-Schneider Y, Rehn M. Regular consumption of *Lactobacillus reuteri*-containing lozenges reduces pregnancy gingivitis: an RCT. *J Clin Periodontol*. 2016 Nov;43(11):948-954. doi: 10.1111/jcpe.12606. Epub 2016 Aug 12. PMID: 27461133; PMCID: PMC6299356.
9. Kaur M, Geisinger ML, Geurs NC, Griffin R, Vassilopoulos PJ, Vermeulen L, Haigh S, Reddy MS. Effect of intensive oral hygiene regimen during pregnancy on periodontal health, cytokine levels, and pregnancy outcomes: a pilot study. *J Periodontol*. 2014 Dec;85(12):1684-92. doi: 10.1902/jop.2014.140248. PMID: 25079400; PMCID: PMC4372244
10. Vasiliauskiene I, Milciuviene S, Bendoraitiene E, Narbutaite J, Slabsinskiene E, Andruskeviciene V. Dynamics of pregnant women's oral health status during preventive programme. *Stomatologija*. 2007;9(4):129-36. PMID: 18303278.
11. Geisinger ML, Geurs NC, Bain JL, Kaur M, Vassilopoulos PJ, Cliver SP, Hauth JC, Reddy MS. Oral health education and therapy reduces gingivitis during pregnancy. *J Clin Periodontol*. 2014 Feb;41(2):141-8. doi: 10.1111/jcpe.12188. Epub 2013 Nov 19. PMID: 24164645
12. Epivatianos A, Antoniadis D, Zaraboukas T, Zairi E, Pouloupoulos A, Kiziridou A, Iordanidis S (2005) Pyogenic granuloma of the oral cavity: comparative study of its clinicopathological and immunohistochemical features. *Pathol Int* 55, 391-397
13. Siddiqui R, Makhoul Z, Alharbi AM, Alfahemi H, Khan NA. The Gut Microbiome and Female Health. *Biology (Basel)*. 2022 Nov 21;11(11):1683. doi: 10.3390/biology11111683. PMID: 36421397; PMCID: PMC9687867.
14. Sills ES, Zegarelli DJ, Hoschander MM, Strider WE. Clinical diagnosis and management of hormonally responsive oral pregnancy tumor (pyogenic granuloma). *J Reprod Med*. 1996;41(7):467-470.
15. Park HY, Houh D, Houh W. Subcutaneous superficial granuloma pyogenicum. *Int J Dermatol* 1996;35:205-6.
16. Kamra SB, Panigrahi P, Vavre S, Janki R, Ghorpade A, Mercy P, Case Report - Granuloma gravidarum, *J Obstet Gynecol India* Vol. 59, No. 4 : July/August 2009 pg 349-351
17. Neville BW, Damm DD, Allen CM, Bouquot JE (2002) *Oral & maxillofacial pathology*. 2nd ed, WB Saunders, Philadelphia, 437-495
18. Pilch BZ (2001) *Head and neck surgical pathology*. Lippincott Williams & Wilkins, Philadelphia, 389-390
19. Sternberg SS, Antonioli DA, Carter D, Mills SE, Oberman H (1999) *Diagnostic surgical pathology*. 3rd ed, Lippincott Williams & Wilkins, Philadelphia, 69, 174
20. Greenberg MS, Glick M (2003) *Burket's oral medicine: diagnosis and treatment*. 10th ed, BC Decker, Hamilton, 141-14
21. Regezi JA, Sciubba JJ, Jordan RCK (2003) *Oral pathology: clinical pathologic considerations*. 4th ed, WB Saunders, Philadelphia, 115-116
22. Mills SE, Cooper PH, Fechner RE (1980) Lobular capillary hemangioma: the underlying lesion of pyogenic granuloma. A study of 73 cases from the oral and nasal mucous membranes. *Am J Surg Pathol* 4, 470-479
23. Bouquot JE, Nikai H (2001) Lesions of the oral cavity. In *Diagnostic surgical pathology of the head and neck*, Gnepp DR ed, WB Saunders, Philadelphia, 141-233
24. Toida M, Hasegawa T, Watanabe F, Kato K, Makita H, Fujitsuka H, Kato Y, Miyamoto K, Shibata T, Shimokawa K (2003) Lobular capillary hemangioma of the oral mucosa: clinicopathological study of 43 cases with a special reference to immunohistochemical characterization of the vascular elements. *Pathol Int* 53, 1-7
25. Yuan K, Lin MT. The roles of vascular endothelial growth factor and angiopoietin-2 in the regression of pregnancy pyogenic granuloma. *Oral Dis*. 2004 May;10(3):179-85. doi: 10.1046/j.1601-0825.2003.00997.x. PMID: 15089929.
26. Purwar P, Dixit J, Sheel V, Goel MM. 'Granuloma gravidarum': persistence in puerperal period an unusual presentation. *BMJ Case Rep*. 2015 Jan 9;2015:bcr2014206878. doi: 10.1136/bcr-2014-206878. PMID: 25576506; PMCID: PMC4289774.

Corresponding author

Vasil Dimitrov
vasildimitrov1999@abv.bg
GSM: 0897380573

Assessment of Different Techniques for Adhesive Cementation of All-Ceramic Systems

Ebru Ramadanova¹, Elena Vasileva²

1. Faculty of Dental Medicine, Medical University of Plovdiv

2. Department of Prosthetic Dental Medicine, Faculty of Dental Medicine, Medical University of Plovdiv

Introduction: Modern esthetic dentistry is based on all-ceramic restorations. Knowledge of the different techniques for cementation can allow dentists to choose the proper ones. Acknowledging the properties of ceramic materials and their cementation protocol is essential for long-term clinical success of fixed restorations. **Purpose:** The purpose of this study is to introduce different techniques for adhesive cementation of all-ceramic restorations. **Material and methods:** The study was done by researching and comparing different sources in publicly available scientific online databases (such as PubMed, Scopus, Science Direct). **Results and discussion:** Silica-based ceramics (feldspathic porcelain, leucite-reinforced ceramic, lithium disilicate and lithium silicate ceramics) should be bonded with adhesive or esthetic resin cements using appropriate bonding agents and primers. Zirconium oxide based ceramics are a rapidly growing type of esthetic restoration. Due to their high strength, zirconia-based ceramic restorations can be cemented with traditional cements or bonded with adhesive resin cements. Self-adhesive resin cements offer less techniques sensitivity than traditional cements, making them excellent choices for the cementation of appropriate zirconia restorations. **Conclusion:** The choice of the right cement and cementation protocol can raise or decrease the aesthetic effect of the all-ceramic restoration. It has to be considered to the type of ceramic material and its manufacturer's recommendations.

Keywords: all-ceramic restorations, adhesion, cementation, ceramics

Introduction

Modern esthetic dentistry is based on all-ceramic restorations. Knowledge of the different techniques for cementation can allow dentists to choose the proper ones. Acknowledging the properties of ceramic materials and their cementation protocol is essential for long-term clinical success of fixed restorations. Ceramic materials are divided by their microstructure and elemental composition into two main classes: mainly silica-based with fillers – typically including feldspathic, leucite, or lithium disilicate – and the oxide-based alumina and zirconia. The infinite variability of the ceramic components can confuse dentists, and without proper guidelines for their use, this can lead to wrong clinical decisions and failure. Achieving the bond between any resin cement and a ceramic material requires surface conditioning. For regular glass-ceramic restoration is recommended 4–5% Hydrofluoric acid etching and the usage of a silane coupling agent in order to obtain a chemical network between silica from the ceramic

surface and resin matrix. The lack of vitreous phase (below 1%) of densely sintered alumina and yttria-tetragonal zirconia polycrystal (Y-TZP) ceramics makes them resistant to acid etching. As a consequence, alternative conditioning methods have been proposed: sandblasting and use of a MDP (10-methacryloyloxydecyl dihydrogenphosphate) primer/cement or silica coating of zirconia surface and use of regular adhesive cement. Nevertheless, different primers and adhesives coupled with specific bonding procedures drastically reduce the microleakage and its consequences. Currently, glass ionomer cement (GIC) and resin-based cements are the primary choices for bonding ceramic restorations to the remaining tooth structure. GIC and resin-modified GIC (RMGIC) are often used to cement acid-resistant ceramics, mostly because these cements are very easy to use. Yet, the most popular and effective cements for all types of ceramic restorations are the resin-based composites, especially the systems containing the 10-methacryloyloxydecyl dihydro-

genphosphate (MDP) monomers. These cement systems contain special adhesive molecules that can potentially promote chemical bonding with all dental ceramics. However, the longevity of this bond remains unclear (1, 2, 3, 4, 5, 6, 7, 8).

Aim

The aim of this study is to introduce different techniques for adhesive cementation of all-ceramic restorations.

Material and methods

The study was carried out in the databases of PubMed, Scopus and Science Direct using the terms “all-ceramic restorations”, “adhesion” and “cementation”. Preferred were studies in the period from 2003 to 2021. 21 articles were selected that summarize the factors associated with the occurrence of this condition.

Results and discussion

Silica-based ceramics (feldspathic porcelain, leucite-reinforced ceramic, lithium disilicate and lithium silicate ceramics) should be bonded with adhesive or esthetic resin cements using appropriate bonding agents and primers. Zirconium oxide based ceramics are a rapidly growing type of esthetic restoration. Due to their high strength, zirconia-based ceramic restorations can be cemented with traditional cements or bonded with adhesive resin cements. Self-adhesive resin cements offer less techniques sensitivity than traditional cements, making them excellent choices for the cementation of appropriate

zirconia restorations (9, 10). The zirconia based ceramic is a glass-free (vitreous phase below 1%) polycrystalline microstructure, with high fracture strength and fracture toughness but in the same time an acid-resistant or non-etchable material. This fact determines poor adhesion for resin cements. For this reason, there have been some efforts of manufacturers and researchers to modify the surface properties of zirconia by using various methods. The most used two methods are: The first recommends a ceramic primer or resin cement, which contains a bio-functional monomer, 10-methacryloyloxydecyl dihydrogen-phosphate (MDP). This monomer has the same basic chemical structure as any primer or bonding. Its property to combine directly with metal oxides as ZrO_2 , Al_2O_3 , etc. In the same time it provides the highest bond strength to dentin among the functional monomers from adhesive systems. The cementation protocol for natural teeth supposes dentin conditioning, zirconia surface conditioning with a special MDP primer and MDP or non-MDP composite cement. The second method called “silicatisation” is usually applied in dental laboratories and is an airborne particle abrasion using 50–110 μm Al_2O_3 coated with silica (Table 1). Due to the very high speed of these particles, about 1000 km/h, these are embedded on the ceramic surface with the result of a chemically silica-modified surface. Furthermore this surface is acting as a usual glass ceramic surface, and resin cement bonds to it with silane coupling agent (1, 11, 12).

Table 1. Pretreatment and cementation of different type of all-ceramic restorations

	Porcelain	Oxide-based ceramics, Zirconia	Glass ceramics
Mechanical pretreatment	-	Grit blasting with alumina powder	Grit blasting with adhesive discs
Chemical pretreatment	▪ HF 5% or 9% ▪ Silanisation	▪ 10-MDP ▪ Silicatisation	▪ HF 5% or 9% ▪ Silanisation
Type of adhesive cement system	▪ Light or dual-cured (preferably amine-free) resin composite cement ▪ Composite cement	▪ Dual-cured resin composite cement containing 10-MDP ▪ Resin-modified glass ionomer cement	▪ Dual-cured resin composite cement ▪ Composite cement

Lithium disilicate can be conventionally or adhesively cemented to tooth structure. The choice of cementation, conventional or adhesive, should be in correspondence with the abutment dimension. Conventional cementation is associated with technique simplicity, whereas adhesive cementation is associated with a technique-sensitive multistep procedure that is problematic in cases of subgingival margins. The clinical success of adhesive ceramic restorations is heavily dependent on luting resin cement and cementation procedures, including ceramic surface treatment. Surface treatment of lithium disilicate is attained by combined hydrofluoric (HF) acid and subsequent silane application. During the cementation procedure, chemical bonds and micromechanical interlocking are formed at the resin-ceramic interface (13, 14). Micromechanical retention is provided by HF acid etching of the ceramic surface, while chemical coupling is provided by the application of a silane coupling agent. The HF removes the glass matrix and the second crystal phase (lithium orthophosphate), creating a rough surface with irregularities within the lithium disilicate for bonding. Furthermore, the silane coupling agent, promotes the chemical adhesion between the silica in the glass phase of the ceramics and the organic phase (the methacrylate groups) of the resin cement through siloxane bonds. A variety of silane primers have been marketed, but the majority contain dilute (2–5 wt%) alcoholic solutions of 3-methacryloxypropyltrimethoxysilane (MPTMS), either as two-bottle systems for hydrolytic activation prior to application or as a single-bottle prehydrolyzed form.

According to the manufacturer's directions of use, etching the lithium disilicate surface is recommended with 5% HF acid for 20 s. (6, 9, 10). However, in many studies, a higher concentration (9.5–10%) of HF

acid has been used. This higher concentration corresponds to the recommended surface treatment of feldspathic ceramic, which is usually 9–10% HF (15, 16). Studies examining the effect of overetching (higher acid concentration and/or longer exposure time) on the bond strength (SBS) of lithium disilicate to resin cement have led to contradictory results evaluated the effects of different HF acid concentrations on the cyclic load-to-failure of CAD-CAM lithium disilicate crowns cemented by resin cement to dentin and found no negative effect of the different HF concentrations. Many studies the influence of HF acid concentration and etching time on the micro-shear bond strength (μ SBS) of lithium-silicate glass ceramics to resin cements and demonstrated that while material and HF concentration influenced the μ SBS, the etching time was not an influential factor. Their conclusion was that the most effective etching treatment for lithium disilicate was the use of 4.9% HF for 20 s. Their conclusion was that the highest roughness value was obtained with 10% HF for 20–40 s, but that the highest bond strength value was produced with 5% HF for 40 s (10, 11, 17, 18, 19, 20, 21).

Conclusion

The dentist could choose between different cements available on the dental market. Each one of them has advantages, disadvantages and a precise working protocol. The choice of the right cement and cementation protocol can raise or decrease the aesthetic effect of the all-ceramic restoration. It has to be considered to the type of ceramic material and its manufacturer's recommendations.

References

1. Petre A., Ruxandra S. Adhesive cementation protocol of zirconia restorations. *Romanian Journal of Oral Rehabilitation*, 2011 July; 3(3)

2. Passos SP, May LG, Barca DC, Ozcan M, Bottino MA, Valandro LF. Adhesive quality of self-adhesive and conventional adhesive resin cement to Y-TZP ceramic before and after aging conditions.
3. Kenneth J. Anusavice, Phillips Science of Dental Materials, 11th edition.
4. Casucci A., Osorio E., Osorio R., Monticelli F., Toledano M., et al. Influence of different surface treatments on surface zirconia frameworks. *J Dent.* 2009 Nov
5. Kitayama S, Nikaido T, Maruoka R, Zhu L, Ikeda M, Watanabe A, Foxton RM, Miura H, Tagami J. Effect of an internal coating technique on tensile bond strengths of resin cements to zirconia ceramics. *Dent Mater J.* 2009 July
6. Cuzic C., Marius Pricop M.O., Jivanescu A., et al. Assessment of Different Techniques for Adhesive Cementation of All-Ceramic Systems. *Medicina (Kaunas)* 2022 July 27
7. Blatz M.B., Vonderheide M., Conejo J. The effect of resin bonding on long-term success of high-strength ceramics. *J. Dent. Res.* 2018
8. Sriamporn T., Thamrongananskul N., Busboy C., Poolyhong S., Uo M., Tagami J. Dental zirconia can be etched by hydrofluoric acid. *Dent. Mater. J.* 2014
9. Blatz M.B., Sadan A., Kern M. Resin-ceramic bonding: A review of the literature. *J. Prosthet. Dent.* 2003
10. Guers P., Wille S., Strunskus T., Polonskyi O., Kern M. Durability of resin bonding to zirconia ceramic after contamination and the use of various cleaning methods. *Dent. Mater.* 2019
11. Afrasiabi A., Mostajir E., Golbari N. The effect of Z-primer on the shear bond strength of zirconia ceramic to dentin: In vitro. *J. Clin. Exp. Dent.* 2018
12. Saleh N.E., Guven M.C., Yildirim G., Erol F. Effect of different surface treatments and ceramic primers on shear bond strength of self-adhesive resin cement to zirconia ceramic. *Niger J. Clin Pract.* 2019
13. Levartovsky Sh., Bohbot H., Brosh T. et al. Effect of Different Surface Treatments of Lithium Disilicate on the Adhesive Properties of Resin Cements. 2021 June15
14. Kwon S.J., Lawson N.C., McLaren E.E., Nejat A.H., Burgess J.O. Comparison of the mechanical properties of translucent zirconia and lithium disilicate. *J. Prosthet. Dent.* 2018
15. Wille S., Lehmann F., Kern M. Durability of resin bonding to Lithium disilicate and zirconia ceramic using a self-etching primer. *J. Adhes. Dent.* 2017
16. J.Y., Stoner B.R., Smith R., et al. Adhesion/cementation to zirconia and other non-silicate ceramics: Where are we now? 2010 Nov20.
17. Maroulakos G., Thompson G.A., Kontogiorgos E.D. Effect of cement type on the clinical performance and complications of zirconia and lithium disilicate tooth-supported crowns: A systematic review. Report of the Committee on Research in Fixed Prosthodontics of the American Academy of Fixed Prosthodontics. *J. Prosthet. Dent.* 2019
18. Kumbuloglu O., Lassila L.V.J., User A., Toksavul S., Vallittu P.K. Shear bond strength of composite resin cements to lithium disilicate ceramics. *J. Oral Rehabil.* 2005
19. Nagai T., Kawamoto Y., Kakeashi Y., Matsumura H. Adhesive bonding of a lithium disilicate ceramic material with resin-based luting agents. *J. Oral Rehabil.* 2005;
20. Klosa K., Wolfart S., Lehmann F., Wenz H.J., Kern M. The effect of storage conditions, contamination modes, and cleaning procedures on the resin bond strength to lithium disilicate ceramic. *J. Adhes. Dent.* 2009
21. Yoshida K. Influence of cleaning methods on the bond strength of resin cement to saliva-contaminated lithium disilicate ceramic. *Clin. Oral Investig.* 2020

Corresponding author

Ebru Ramadanova
ebru981@abv.bg
GSM: 0879030605

Comparison of the Opacity of Conventional and Resin Modified Glass Ionomer Cements

Dushjanth Ganeshapooban¹, Saroash Shahid², Vesela Stefanova³, Veselina Todorova³

1. Faculty of Dental Medicine, Medical University of Plovdiv

2. School of Bioengineering and Materials Science Queen Mary University of London

3. Department of Operative Dentistry and Endodontics, Faculty of Dental Medicine, Medical University of Plovdiv

Introduction: Glass-Ionomer cements have many advantages as restorative materials: adhesion to tooth structure, fluoride releasing capacity, biocompatibility. However, in terms of aesthetics, especially opacity, they are not as adequate as desired. **Aim:** The aim of this study was to compare the opacity of two encapsulated commercial glass ionomer cements – a conventional glass ionomer (Riva Self Cure) and a resin-modified glass ionomer cement (Riva Light Cure). **Materials and methods:** 3 encapsulated Riva self-cure and 3 Riva light-cure capsules of shade A2 were used to prepare 6 samples. The specimens were stored in 10ml of deionised water for an hour in an incubator. Then they were moved into a clear petri disk and placed on black and white backgrounds. Digital photographs were taken to obtain a greyscale measurement. The disks were then stored again for 24 hours in water at 37°C and photographed again. Digital images were analysed with Image J software. **Results:** Conventional glass ionomer cement showed higher opacity (mean value 0,755) than resin-modified (mean value 0,707) after 1 hour. The opacity was again higher for the conventional glass ionomer cement (mean value 0,725) than the resin-modified glass ionomer cement (mean value 0,673) after 24 hours. Opacity for both specimens decreased after 24 hours. **Conclusion:** Resin-modified glass ionomer cements have lower opacity than conventional glass ionomers. There is further scope for improving aesthetic properties of glass ionomer cements.

Keywords: *glass-ionomer cement, opacity*

Introduction

Glass-ionomer cements also referred to as glass polyalkenoate cements are widely used as restorative and adhesive dental materials mainly because of the ability to bond to enamel and dentine and to release fluoride (1, 2). Glass ionomers have been separated into two groups – conventional glass-ionomer cements (C GICs) and resin-modified glass-ionomer cements (RMGICs) (3). The development of RMGIC emerged in an attempt to overcome some of the limitations of C GICs. The addition of resin chemistry to glass ionomers has improved properties such as a prolonged working time with a rapid set when light cured, strength resistance to acid attack and wear resistance (1). An essential clinical consideration of GICs is the ability to release fluoride ions from the bulk of restoration due to the leaching of fluoride ions from the matrix to the surrounding environment. During the setting reaction, the glass releases the ions (freely ready to migrate) which are present within the matrix (4, 5).

Aim

The aim of this study was to compare the opacity of two encapsulated commercial glass ionomer cements – a conventional glass ionomer (Riva Self Cure) and a resin-modified glass ionomer cement (Riva Light Cure).

Materials

A conventional glass ionomer cement and a resin-modified glass ionomer cement were evaluated for this study. The conventional glass ionomer was Riva Self Cure shade A2 and A3 (SDI, Bayswater, Australia). The self-cure batches (shade A2 and A3) consisted of 0.45 g powder 0.14 g liquid (0.13 mL) per capsule. The resin-modified glass ionomer used was Riva Light cure high viscosity shade A3 (SDI, Bayswater, Australia). The light-cure high viscosity A3 batch consisted of 0.43 g of powder, 0.14 g liquid (0.13 mL) per capsule.

Method

3 encapsulated Riva self-cure and 3 Riva light-cure capsules of shade A2 were used to prepare specimens to compare the aesthetics. Each sample was placed in separate test tubes consisting of 10 ml deionised water. The specimens were then placed into an incubator for one hour. After an hour the samples were taken out of the incubator and dried using filter paper. All 6 samples were placed in the middle of a clear petri dish consisting of 5 ml of deionised water. The petri dish was placed on an A4 sheet of paper; an illuminated white background (reflectance 70%) and illuminated black background. The A4 sheet of paper was placed on top of a light with the petri dish positioned in the middle of the paper. An iPhone was used to take digital images of the samples in order to give a greyscale measurement. The samples were placed in test tubes containing 10 ml of deionised water and kept at 37°C for 24 hours. Digital images were taken of the specimens after 24 hours.

The paste was filled into a mould (ISO standard 4 x 6 cm) (Figure 2). The Riva Self-cure was extruded into the mould and compressed using acetate sheet on either side. 7–8 capsules were extruded into the mould and compressed using acetate sheet as self-cured specimens would consist of possible defects, therefore making more than the desired amount was necessary. The mould consisting of prepared samples were placed at 37°C in a drying and heating chamber (Series FD, BINDER GmbH, Tuttlingen, Germany) for 15 min before being removed using a syringe. The finished samples were placed in 10 ml of deionised water test tubes and kept in an incubator for the desired amount of time. The procedure for Riva light-cure samples was the same as Riva self-cure until the paste was filled into the mould. The next step consisted of blue light being applied on

either of the sample (covering the sample completely) for 20 s. A syringe was then used to remove the samples from the mould and placed in 10 ml of deionised water.



Figure 1. ESPE Rotomix machine

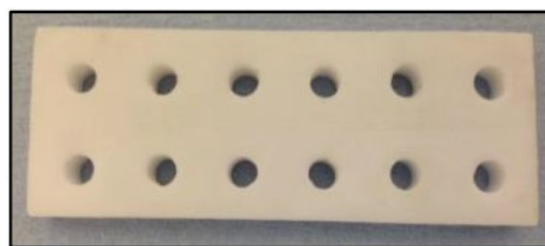


Figure 2. Mould plate Results

Results

From Figure 3 it can be seen that 1 hour after preparation the RMGIC samples show less opacity within the illuminated white and black background. When comparing the zoomed in versions in Figure 3 this is evident. In addition, the 3 RMGIC specimens show very little contrast, which was an expected observation. Similarly, the 3 C GIC specimens portray minimal distinctions in opacity.

A similar trend can be observed from the images present in Figure 4 – after 24 hours. The C GIC samples expose a greater level of opacity when compared to the RMGIC samples. However, it is difficult to compare the images taken an hour after preparation and after 24 hours, as the Figures above do not show a conclusive difference.

ImageJ was the software used to analyse the digital images. The first tool was used to draw square boxes in order to calculate the reflectance given by the illuminated white background and the reflectance given by the illuminated black background.

The procedure undergone for calculating opacity values for each specimen is shown in Figures 5, 6, 7 and 8. A constant value of 570 was used for each sample in order to

maintain consistency throughout all of the data collected. It can be seen that the mean value for RMGIC Sample 1 under the white illuminated background was 147.53; with the mean value for the sample under black illuminated background being 108.05. The same technique was completed for the 3 RMGIC and C GIC samples for both illuminated white and black backgrounds.

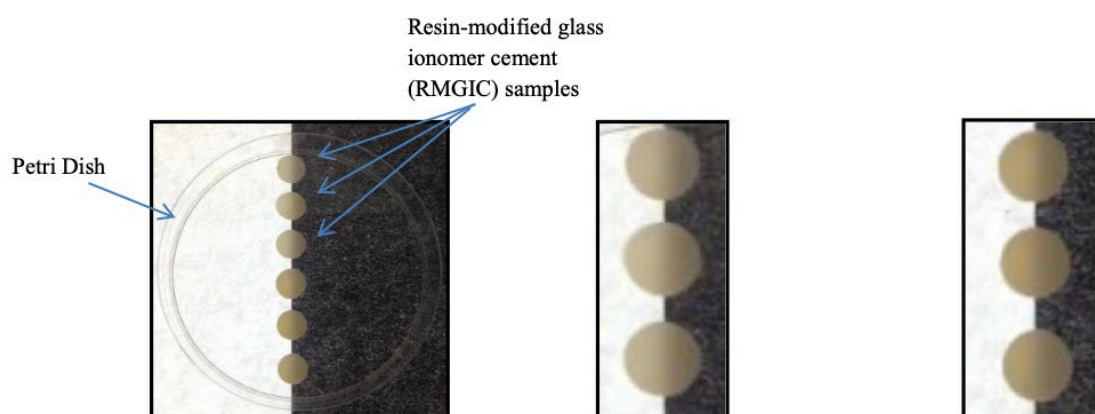


Figure 3. A petri dish consisting of 3 RMGIC samples and 3 C GIC samples, taken an hour after preparation.

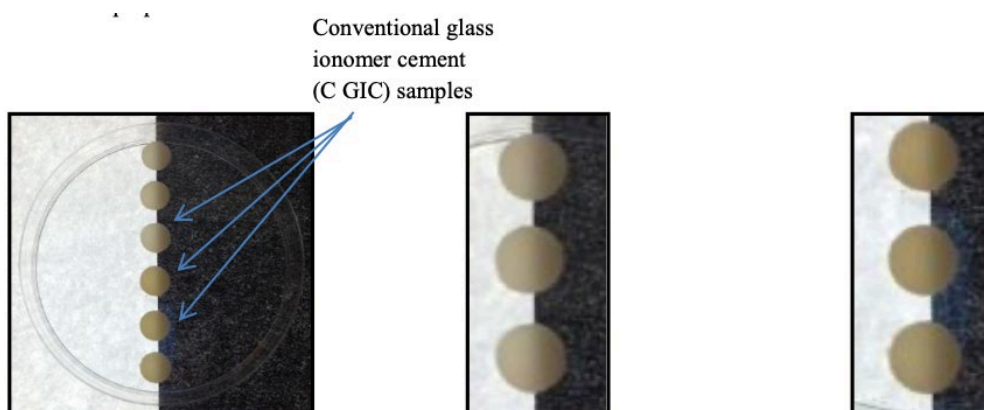


Figure 4. A petri dish consisting of 3 RMGIC and 3 C GIC samples 24 hours after preparation.

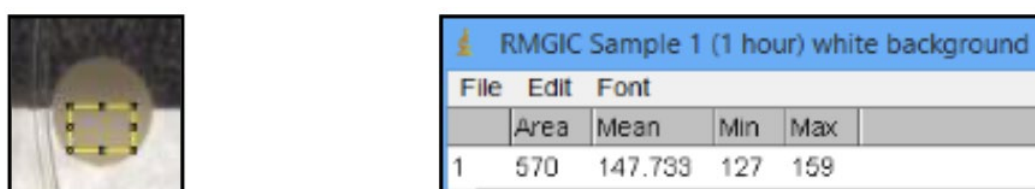


Figure 5. An image representing a square tool box covering the white illuminated background.

Figure 6. An image showing the area and mean values for sample no. 1 (RMGIC) under the white illuminated background.



RMGIC Sample 1 (1 hour) black background					
File Edit Font					
	Area	Mean	Min	Max	
1	570	108.051	58	134	

Figure 7. An image representing a square tool box covering the black illuminated background for sample no.1 (RMGIC). **Figure 8.** An image showing the area and mean values for sample no.1 (RMGIC) under the black illuminated background.

Opacity was calculated using the following equation:

$$C_{0.70} = R_B / R_{0.70}$$

The following calculation represents the step taken for calculating the opacity for RMGIC Sample 1 using the mean values for both illuminated backgrounds:

$$R_B = 108.051$$

$$R_{0.70} = 147.733$$

$$C_{0.70} = ?$$

$$C_{0.70} = 108.051 / 147.733 = 0.731393798$$

From Figure 9 it is evident that the average opacity for RMGIC specimens after 1 hour is 0.707 (3 significant figures, 3.s.f).

However, after 24 hours the average opacity for the specimens had decreased to 0.673 (3.s.f). This shows that the RMGIC specimens had a decline of 0.034 over the time scale of 24 hours. Similarly, C GIC samples represented a comparable trend in opacity values. The mean value for C GIC samples after 1 hour was 0.755 (3.s.f) and 0.723 (3.s.f) after 24 hours – a difference of 0.032. This indicated that the difference in mean values between 1 hour and 24 hours after sample preparation was the same for both types. Furthermore, C GIC had more opacity after 24 hours in comparison to RMGIC. Although the opacity for both specimens decreased after 24 hours.

RMGIC	Sample	White background reflectance	Black background reflectance	Opacity
After 1 hour	1	147.733	108.051	0.731393798
	2	150.561	102.905	0.683477129
	3	144.016	101.758	0.706574270
	Mean = 0.707148399			
After 24 hours	1	148.074	100.309	0.677424801
	2	148.146	103.075	0.695766339
	3	152.704	98.454	0.644737531
	Mean = 0.67264289			

Figure 9. The data collected for RMGIC samples after 1 hour and 24 hours.

C GIC	Sample	White background reflectance	Black background reflectance	Opacity
After 1 hour	1	131.491	97.325	0.740164726
	2	127.258	97.046	0.762592529
	3	126.539	96.275	0.760832629
	Mean = 0.754529961			
After 24 hours	1	134.632	95.370	0.708375423
	2	132.881	97.693	0.735191638
	3	135.335	98.960	0.731222522
	Mean = 0.724929861			

Figure 10. The data collected for C GIC samples after 1 and 24 hours.

Discussion

From the aesthetics results gathered from the experiments conducted it is evident that RMGIC specimens consist of better translucency than C GIC specimens an hour and 24 hours after preparation. The maturation of the matrix within conventional GICs (within the Riva Self Cure samples) affects the aesthetics and results in the shade match improving a couple of days after preparation. Moreover, the type of material may play a role in the amount of time taken for the shade match to be achieved (6). However, a long-term benefit of C GICs over RMGICs is the greater capability to resist colour changes over the restoration life period. This shows that observing the opacity of C GIC specimens after 2–3 days would have possibly given a lower opacity.

Conclusion

Resin-modified glass ionomer cements have a lower opacity than conventional glass ionomers. There is further scope for improving aesthetic properties of glass ionomer cements.

References

1. Nicholson, J. Chemistry of glass-ionomer cements: a review. *Biomaterials*. 1998; 19(6): 485-494.
2. Noort R. Introduction to dental materials. Edinburgh: Mosby/Elsevier. 2007
3. Bagheri R, Burrow M, Tyas M. Surface characteristics of aesthetic restorative materials? an SEM study. *J Oral Rehabil*, 2007; 34(1): 68-76.
4. Tyas M. Clinical evaluation of glass-ionomer cement restorations. *J. Appl. Oral Sci.* 2006; 14(spe).
5. Smith, D. Development of glass-ionomer cement systems. *Biomaterials*. 1998; 19(6): 467-478.
6. Bonsor, Stephen J, and Gavin J Pearson. (2013). *A Clinical Guide To Applied Dental Materials*. Amsterdam: Elsevier/Churchill Livingstone.

Corresponding author

Dushjanth Ganeshapoban

Email: dush_ganesh@hotmail.com

Phone: +447984647364

Management of a Patient with Multiple Carious Lesions – a Case Report

Minahill Hussain Sadiq, Stela Nicheva

Department of Operative Dentistry and Endodontics, Faculty of Dental Medicine,
Medical University of Plovdiv

In this case report a 24-year-old male patient with multiple carious lesions was treated with multidisciplinary evidence based approach. The management of this patient was conducted according to evidence based clinical protocol including long term glass ionomer cement (GIC) temporary restorations. The patient's short term dental management was successful due to the implementation of preventive strategies coupled with accurate diagnosis and treatment planning. Enhancing the actual awareness of the patient of his oral health status is of paramount importance for the success of any complex dental treatment in high caries risk patients.

Keywords: *multiple carious lesions*

Introduction

Dental caries is an important public health problem worldwide. Today it is recognized as an infectious disease that affects children and adults throughout life (1). A poor oral health has psychosocial impacts that diminish a quality of life and restrict activities in school, at work, and home (2). Multiple caries predominantly affects teenagers and young adults, with some etiological factors being reduced salivary flow (possibly due to certain diseases or drugs), drug abuse and high sugar/carbohydrate diets, coupled with poor oral hygiene (3).

Case report

Background

The presented case is of a 24-year-old male patient with multiple carious lesions. His complains were of severe provoked pain on sweet, cold and sour food that ceases upon stimulus removal, along with poor aesthetics due to numerous visibly decayed teeth (Fig. 1). The radiographic examination (Fig. 2) showed previous root canal treatment, failing restorations with poor quality, secondary caries and deep decay. The patient had previously undergone several extractions in the past due to non-restorable teeth,

hinting at the neglect of oral hygiene. The Initial periodontal evaluation (Fig. 3) showed bleeding on probing and plaque index 100%, highlighting the dire need of intense education and motivation.

Although the thorough anamnesis could not confirm any drug abuse or infectious disease great protective care was taken when treating the patient in the clinical room.



Figure 1. Initial clinical picture showing numerous acute & chronic carious lesions, heavy plaque & calculus and inflamed gingiva.



Figure 2. OPG and segment X-ray of teeth 24, 26, 27, 12-22, 33, 34, 37

Patient Last Name: Ivanov First Name: Georg Date Of Birth: 16/01/1999

☒ Initial Exam ☐ Reevaluation Clinician: Mirehli Hussain Sadiq

	16	17	18	19	20	21	22	23	24	25	26	27	28
Waxing	0	0	0	0	0	0	0	0	0	0	0	0	0
Inspection	0	0	0	0	0	0	0	0	0	0	0	0	0
Function	0	0	0	0	0	0	0	0	0	0	0	0	0
Bleeding on Probing	0	0	0	0	0	0	0	0	0	0	0	0	0
Plaque	0	0	0	0	0	0	0	0	0	0	0	0	0
Gingival Margin	0	0	0	0	0	0	0	0	0	0	0	0	0
Probing Depth	0	0	0	0	0	0	0	0	0	0	0	0	0

Buccal

Palatal

Ungual

Buccal

Figure 3. Initial periodontal examination

Material and methods

The management of this patient was conducted using the following protocol:

1. Assessment of the caries risk of the patient as extremely high (4);
2. Motivation and oral hygiene instructions;
3. Elimination of supra- and sub-gingival plaque and calculus;
4. Removal of all the carious lesions in a single visit and obturate with GIC to decrease plaque retentive areas and cariogenic bacteria, allowing time for remineralization (Fig. 2, 3);
5. Extractions of non-restorable teeth;
6. Prescription 5000 ppm fluoride toothpaste 2x daily;
7. Topical Fluoride application after 1 week of GIC placement to optimise the Fluoride release;
8. Chlorhexidine gluconate (0,12%) rinse 1x daily for 1 week every month until the next check up;

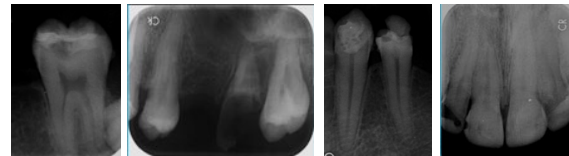


Figure 4. Thorough removal of all carious lesions



Figure 5. Temporisation with GIC for 2 weeks and topical Fluoride application after 1 week to optimise the FI- release.

9. Re-evaluation and re-motivation;

10. Final composite restorations and metal-acrylic bridge placement (24–27) after significant reduction in the bleeding on probing index and plaque index.

It was apparent that a heavy focus on oral hygiene was needed, so the first step of the treatment plan was to motivate the patient using visualisation of the presence of the large build-up of debris and plaque with a disclosing agent, along with providing an in-depth consultation and explanation (using a typodont) of the most effective brushing technique for his case. After taking the Periodontal probing depth and debris and calculus indices, ultrasonic scaling to remove the calculus, followed by polishing to remove any leftover debris was conducted. The second stage was to excavating all caries lesions (on teeth 13–23, 16, 17, 33, 34, 37, 47) with a carbide bur and slow handpiece. Temporisation with a chemically setting GIC for 2 weeks was chosen as it releases more

Fluoride than the Resin Modified one (5). To further optimise the Fluoride release, a topical Fluoride varnish was applied after 1 week of placing the GIC to effectively “re-charge” the GIC (6). The patient was advised to brush 2 times daily with prescription 5000 ppm NaF toothpaste and use a 0.2% Chlorhexidine rinse twice daily for 4 weeks. A xylitol chewing gum was also recommended to increase the saliva stimulation. After 2 weeks, the patient’s updated oral hygiene was assessed, to ensure he was suitable for the final obturations and then composite was used to replace the GIC. Unfortunately, two of the patient’s teeth (26 & 48) were decayed beyond repair, therefore had to be extracted during this time too. After a month of healing, a metal acrylic bridge was cemented in the region of 24–27 to complete all the restorations. This patient will be recalled every 3 months to ensure their improved oral hygiene is being maintained.

Results

The short term dental management of the patient was successful due to the implementation of preventive strategies coupled with accurate diagnosis and treatment planning (Fig. 6–9). The long term success depends of the compliance of the patient.

Discussion and conclusion

Dental caries is a multifactorial disease that necessitates complex interactions between fermentable carbohydrates, acid-producing microorganisms, and host factors. It is noteworthy that the prevalence of the disease has reduced due to better awareness, improved oral hygiene, and access to regular dental care. Even though there is reduced mean caries prevalence, approximately 15–20% of the population belong to the high-risk category with about 60% contributing toward caries development (7).

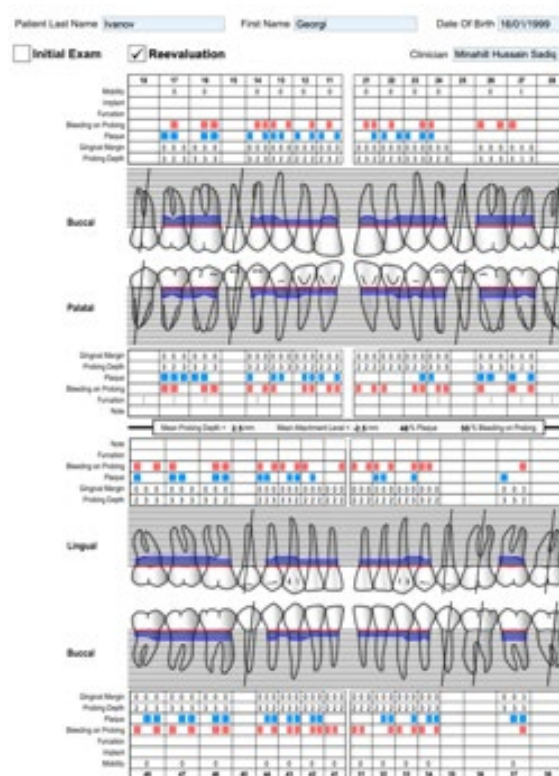


Figure 6. The periodontal re-evaluation after 1 month clearly shows a decrease in the Bleeding on Probing and Plaque index, indicating the patient compliance and suggesting good prognosis after treatment if patient maintains to improve his oral hygiene



Figure 7. After front teeth restorations (2 months after the initial visit) with direct composite.



Figure 8. After cementation of a posterior metal-acrylic bridge, following several extractions and direct composite restorations



Figure 9. After numerous direct composite restorations of the lower jaw

The treatment plan in this case was tailored around motivating and educating the patient using various means to ensure the success of the treatment, due to the poor initial oral hygiene status. The initial aim of the treatment was to remove all of the carious lesions in a single visit and obturate the defects with glass ionomer cement. In that way we could stop the progression of the active carious lesions and reduce the cariogenic bacterial load in the oral cavity by eliminating the plaque retentive zones. This was coupled together with professional and personal oral hygiene instructions in order to regulate the oral biofilm from cariogenic to non-cariogenic state. Glass ionomer cements (GIC) possess certain unique properties that made them useful as temporary restorative materials in this case including adhesion to the tooth structure which potentially reduces the microleakage, remineralization potential due to fluoride release, which will improve the bonding quality of the final restorations, anticariogenic properties again due to fluoride release, thermal compatibility with the tooth enamel, and biocompatibility (8, 9).

The final restorations were done after significant improvement of the bleeding on probing and plaque indices upon

reevaluation to ensure a long term stable result. In conclusion the enhancement of the actual awareness of the patient of his oral health status is of paramount importance for the success of any complex dental treatment in high caries risk patients.

References

1. Marcenes W., Kassebaum N. J., Bernabé E., et al. Global burden of oral conditions in 1990–2010. *Journal of Dental Research*. 2013;92(7):592–597.
2. Beyene DH, Shashamo BB, Digesa LE, Tariku EZ. Oral Hygiene Practices and Associated Factors among Patients Visiting Private Dental Clinics at Hawassa City, Southern Ethiopia, 2018. *Int J Dent*. 2021 Mar 26;2021:8868308.
3. Sabharwal A, Stellrecht E, Scannapieco FA. Associations between dental caries and systemic diseases: a scoping review. *BMC Oral Health*. 2021 Sep 25;21(1):472.
4. Rechmann P, Kinsel R, Featherstone JDB. Integrating Caries Management by Risk Assessment (CAMBRA) and Prevention Strategies Into the Contemporary Dental Practice. *Compend Contin Educ Dent*. 2018 Apr;39(4):226-233;
5. Upadhyay S, Rao A, Shenoy R. Comparison of the amount of fluoride release from nanofilled resin modified glass ionomer, conventional and resin modified glass ionomer cements. *J Dent (Tehran)*. 2013 Mar;10(2):134-40.
6. Panigrahi A, Sudeep S, Sharma S, Mohanty S. Comparative Evaluation of Fluoride Recharge Ability of Conventional and Hydroxyapatite Modified Glass Ionomer Cement with Daily Low Fluoride Exposure- An Invitro Study. *J Clin Diagn Res*. 2016 Feb;10(2):ZC53-5.
7. Almeahmadi AH. Evidence-based Decisioning on the Management of a High Caries Risk Patient-A Case Report. *J Multidiscip Healthc*. 2021 Jul 20;14:1893-1900.
8. Khere CH, Hiremath H, Sandesh N, Misar P, Gorie N. Evaluation of antibacterial activity of three different glass ionomer cements on streptococcus mutans: an in-vitro antimicrobial study. *Med Pharm Rep*. 2019 Jul;92(3):288-293.
9. Fúcio SB, Carvalho FG, Sobrinho LC, Sinhoreti MA, Puppini-Rontani RM. The influence of 30-day-old Streptococcus mutans biofilm on the surface of esthetic restorative materials--an in vitro study. *J Dent*. 2008;36:833–839

Oral Manifestations in a Patient with Bulimia – a Case Report

Margarita Hadzhieva¹, Ivanina Totova², Georgi Tomov¹

1. Department of Periodontology and Oral Mucosa Diseases, Faculty of Dental Medicine, Medical University of Plovdiv

2. Faculty of Dental Medicine, Medical University of Plovdiv

Eating disorders (ED) belong to the psychiatric nosology and is characterized with serious mental, behavioral and somatic problems. The most common eating disorders in the Bulgarian population are anorexia and bulimia. Bulimia is characterized by loss of control over food intake and inappropriate behavior in order to lose weight. In this paper we report a 45-year-old male with bulimia, accompanied by significant oral manifestations. Clinical evaluation includes extra and intraoral inspection and assessment of dental status using BEWE and DMFT index, periodontal status, and paraclinical examination of saliva parameters. The examination revealed some significant characteristics attributed to ED, namely enlargement of the parotid glands, advanced erosion, oral mucosal lesions. Dental practitioners are usually one of the first healthcare professionals to whom a previously undiagnosed ED may present, so adequate knowledge about oral manifestations of bulimia is necessary. Creating a dental protocol for management of these patients should be a priority, as well as providing preventive and treatment modalities corresponding with the individual needs of the patient.

Keywords: *eating disorders, bulimia, oral findings*

Introduction

Eating disorders (ED) are psychosomatic diseases associated with a permanent disorder of eating behavior with the aim of weight loss. They have severe physical and psychosocial consequences¹. American Psychiatric association divides them into three groups: (1) Anorexia nervosa, (2) Bulimia nervosa and (3) Other unclassified ED². The etiology of eating disorders is multifactorial and includes a combination of biological, psychological, socio-cultural factors^{3,4}. Bulimia nervosa is characterized by recurrent, uncontrollable episodes of large amounts of food intake within a short period of time, followed by inappropriate compensatory behavior – self-induced vomiting, use of laxatives and diuretics, intense exercises⁵. The rate of diffusion of anorexia is keeping fairly constant – 0.3%, as opposed to bulimia – 1%, that is continuously increasing⁶. Bulimic behavior which includes vomiting ranges from 4.8% – 12.5%⁷. Oral manifestations in eating disorders may vary in severity, depending on their duration, purging

frequency, diet and oral hygiene habits^{8,9}. The most common are – dental erosion, caries, xerostomia, parotid gland hypertrophy, oral lesions of the mucosa and periodontal diseases^{6,9,10}.

Case presentation

A 48-year-old man is referred to the Department of Periodontology and Oral Mucosa Diseases, with complaints of hypersensitivity of the teeth from thermal and chemical irritants, and acute pain in tooth 37. From the anamnesis, no history of alcohol and cigarettes use was found. The BMI index was 37.9 corresponding to class 2 obesity. At the next examination after 2 months, the BMI index changed to 33.1 – class 1 obesity. During the first visits he concealed the presence of harmful habits and behaviors. After more extensive questioning during the following visits, he shared about weight loss attempt and diets. The extraoral examination revealed bilaterally enlarged parotid glands, as well as Russell's sign on the dorsal surface of the fingers of the left hand.

During the intraoral examination, was registered perimolysis of the hard dental tissues, which we evaluated by the BEWE index – 12, and also decrease in the vertical bite dimension. The erosions are localized on the palatal surfaces on the upper front teeth and the occlusal surfaces of the lower molars. The DMFT index was also calculated – 13. During the periodontal examination by registering FMBS – 34%, FMPS – 28%, CI – 2.8, and CAL we made a diagnosis – generalized plaque-induced gingivitis. When we examined the oral mucosa we found morsicatio buccarum, as well as a hemorrhagic lesion on the palatal mucosa. Quantitative and qualitative measurements of saliva were done including pH and buffer capacity of unstimulated and stimulated saliva. The results showed small amount of unstimulated Saliva – 2.5 ml with increased viscosity, moderate acidity – 6 and low buffer capacity – 6. After gland stimulation the amount of saliva was normalized – 6.5 ml, as well as the pH – 7.4 and the buffer capacity – 10. The first step in the treatment plan was to establish good communication with the patient, building trust and gathering detailed information about the ED. To affect the hypersensitivity, we covered the affected teeth with composite restorations. The tooth 37 was devitalized as a result of acute pulpitis – a complication of the advanced erosion. We have prepared a preventive plan of treatment in order to protect the rest of the dental tissues, emphasizing on instructions to the patient not to brush his teeth immediately after the vomiting episode, but to rinse with fluoride or alkaline solution. We recommended the use of protective splints as well as topical fluoride. Correction of the diet with restriction of carbohydrates and acidic foods, as well as guidelines for maintaining adequate oral hygiene were provided.



Bilateral Parotid swelling in bulimia



Erosion on the palatal surfaces of maxillary teeth



Russell's sign on the dorsal surface of the fingers of the left hand

Discussion and conclusion

Patients with bulimia, unlike those with anorexia, have fluctuations in weight for short periods of time⁴. Typical for them is the Russell's sign – lesions or calluses on the dorsal surface of the fingers, as a result of the multiple traumas caused by the incisal edges when induce vomiting^{6,11}. Hypertrophy of the parotid glands in patients with bulimia has frequency at 10–50% and the facial appearance is considered typical⁶. The very characteristic for the chronic vomiting is the erosion of the hard dental tissues due to acidic substances in the gastric juice, with typical localization on the palatal surfaces of

upper frontal teeth. Xerostomia and reduced buffering capacity, acidic pH and increased carbohydrate intake reduce the protection of hard dental tissues against erosion and creates conditions for increased caries risk^{9,10,12}. Hemorrhagic lesions on the palatal mucosa are usually result of the mechanical trauma from the fingers and the chemical irritation from the gastric juice^{9,13}. Oral manifestations of ED can be detected at different stages of their course. The early ones, manifested at the beginning of the ED can be recognized by the dentist^{6,12}. The literature revealed that the knowledge of the symptoms of eating disorders are insufficient among dentists and the need to include more information about ED in the dental curriculum is conditional^{10,14}. There is a need for development of algorithm for a complex dental management of these patients, as well as a program for their training^{14,15}. The dentist should take part in the multidisciplinary treatment team together with various other specialists^{4,6}.

The study was financed by IP № P-2833/PPDP 2022.

References

1. Hasan, Shamimul, et al. "Oral cavity and eating disorders: An insight to holistic health." *Journal of Family Medicine and Primary Care* 9.8 (2020): 3890.
2. Askounes, Sara E., and K. A. William. "Eating disorders within the dental practice: A literature review." *Global J Med Res J DentOtolaryngol* 16 (2016): 1-4.
3. Manevski, Jovana, et al. "Dental aspects of purging bulimia." *Vojnosanitetski pregled* 77.3 (2020).
4. Balshi, Thomas J., et al. "Oral rehabilitation and psychosocial evaluation of a patient with bulimia nervosa following Teeth in a Day® immediate loading protocol." *Compendium* 36.4 (2015).
5. Garbin, Cléa Adas Saliba, et al. "Oral manifestations in patients with anorexia and bulimia nervosa: a systematic review." *Journal of Public Health* 28 (2020): 765-771.
6. Lumbau, Aurea, and Giovanni Spano. "Oral Implications of Eating Disorders." *Eating Disorders: A Paradigm of the Biopsychosocial Model of Illness* (2017): 71.
7. Bretz, Walter A. "Oral profiles of bulimic women: Diagnosis and management. What is the evidence?." *Journal of Evidence Based Dental Practice* 2.4 (2002): 267-272.
8. Vieira Esteves, Camilla, et al. "Oral profile of eating disorders patients: case series." *Special Care in Dentistry* 39.6 (2019): 572-577.
9. De Moor, R. J. G. "Eating disorder-induced dental complications: a case report." *Journal of oral rehabilitation* 31.7 (2004): 725-732.
10. Rosten, A., and T. Newton. "The impact of bulimia nervosa on oral health: A review of the literature." *British dental journal* 223.7 (2017): 533-539.
11. Burkhart, Nancy, et al. "Communicating effectively with patients suspected of having bulimia nervosa." *The Journal of the American Dental Association* 136.8 (2005): 1130-1137.
12. Uhlen, Marte-Mari, et al. "Self-induced vomiting and dental erosion—a clinical study." *BMC oral health* 14.1 (2014): 1-7.
13. Маргарита Хаджиева, Георги Томов, Мария Мутафчиева. „Некротизираща сиалометаплазия при пациент с булимия: клиничен случай“, СУБ, 2020, 24: 181-185
14. Dynesen, Anja W., et al. "Eating disorders: experiences of and attitudes toward oral health and oral health behavior." *European journal of oral sciences* 126.6 (2018): 500-506
15. Conviser, Jenny H., Sheehan David Fisher, and Kristin B. Mitchell. "Oral care behavior after purging in a sample of women with bulimia nervosa." *The Journal of the American Dental Association* 145.4 (2014): 352-354.

Corresponding author

Margarita Hadzhieva
Faculty of Dental Medicine
Medical University of Plovdiv
Hristo Botev 3, Plovdiv 4000, Bulgaria
Email: Margarita.Hadzhieva@mu-plovdiv.bg

Prevalence of Black Stain and Associated Risk Factors in Children

Kristina Tabutova, Maria Shindova

Department of Pediatric Dentistry, Faculty of Dental Medicine, Medical University of Plovdiv

Black stain is a common discoloration in children. It is characterized by distinctive dark dots to continuous lines, which are localized at the tooth surface parallel to the gingiva, firmly attached to the tooth. The prevalence is reported to be 2.4–21% with equal sex distribution in literature and it depends on the study design, patients' age as well as country of origin. The main therapeutic approach is frequent professional oral hygiene upon the reappearance of the black stain, which can cause damage to the enamel and can lead to dental hypersensitivity. However, to treat the black stain properly, it is important to explore the etiological factors. The aim of this review article is to analyze the scientific literature regarding black stain prevalence. A search was performed to summarize the evidence behind the numerous risk factors including some bacterial species, factors related to oral hygiene, diet, type of tap water, intake of iron supplements and properties and composition of the saliva. The findings of the literature review give grounds to undertake studies investigating the aetiology of the black stain. Future explorations will be interesting as its negative effect on dental aesthetic perception causes concerns in parents and can have significant effects on the personality and self-confidence of the child.

Keywords: black stain, prevalence, risk factors, children

Introduction

In pediatric clinical practice, the topic about the presence of Black stain on teeth in children and teenagers has been widely discussed. Most of the studies concerning this issue were conducted in the pediatric population. Its negative effect on dental aesthetic perception causes concerns in parents and can have significant effects on the personality and self-confidence of the child.

Definition and origin

Black stain (BS) is characterized by distinctive dark dots to continuous lines, which are localized at the tooth surface parallel to the gingiva, firmly attached to the tooth /1/. Oral hygiene at home is not sufficient for removing this black stain and the main therapeutic approach is frequent professional oral hygiene to solve this aesthetic problem. Although a simple scaling and using a brush with pumice can remove the stain, frequently Black stain can reappear /2/. Repeated "routine scaling and polishing" can cause *undesirable alterations* of enamel and can lead to dental hypersensitivity.

There have been discussions about the

origin of BS and its caries protective properties for a long time. In 1890, Miller noted the presence of BS in members of the same family and suggested a hereditary constitutional factor. At the beginning of the 20th century, Pickerill described these stains as a thin dark brown line around the cervical area of the teeth, appearing in the form of a deposited film of calculus. He concluded that the occurrence of BS can correlate to the immunity to caries lesions /3/.

The aim of this review article is to analyze the scientific literature regarding BS prevalence. A search was performed to summarize the evidence behind the numerous risk factors including some bacterial species, factors related to oral hygiene, diet, type of tap water, intake of iron supplements and properties and composition of the saliva.

Prevalence of black stain in children

In scientific literature, the prevalence of BS depends on the study design, the subject's age and gender, dentition, and country of origin.

The prevalence of BS is reported to be 2–22% with equal sex distribution in literature.

Zhang et al. confirmed the affection of both primary as well as permanent dentition and equal prevalence in both genders /4/.

Żyła et al. found that the number of stained teeth increases with age ($p < 0.007$) /3/. The majority of the studies are about BS among children and there is no data about the prevalence of this issue in adults /3/. In addition, the most investigated age groups in studies reporting the prevalence of Black tooth stain are children aged 6–12 years old or adolescents.

Thus, the epidemiological data about the deposition of BS on primary dentition is limited. In one report, more stained teeth were observed in permanent dentition compared to primary dentition /5/. Other authors reported affection for primary teeth several times more than permanent ones /3,5/.

Concerning the region, the prevalence in Europe ranges from 1.6% (UK), 4.45% (Poland), 6.3% (Italy), to 7.54% (Valencia, Spain). In South America, it ranges from 6.5% (Peru) to 14.8% (Brazil). On the Asian continent, it varies from 16% (Philippines) to 18% (India). There is no research about this issue in Bulgaria yet. These different values in the studies can be explained by the differences in dietary habits, oral hygiene and the related oral microbiome /4/.

Etiology of Black stain and associated risk factors in children

The main therapeutic approach is frequent professional dental cleaning when the BS reappears, which can cause damage to the enamel thus leading to hypersensitivity. However, to treat the black stain correctly, it is important to determine the etiological factors. The etiology of BS has been debated throughout the 20th century. So many studies identified significant risk factors.

The numerous risk factors include factors related to oral hygiene, diet, type of tap water, intake of iron supplements and properties and composition of the saliva, some bacterial species, and others /Table 1/.

Oral hygiene

The first group can be related to oral hygiene – toothbrushing with fluoride toothpaste and rinsing with fluoride mouthwashes may influence the appearance of BS in children. While low levels of fluoride help strengthen and protect tooth enamel, too much fluoride swallowed during the early childhood years may cause brown or black spots or streaks on the gingival margin in the cervical half of the crown. This discoloration is usually combined with a different level of mineralization.

Table 1. Risk factors in children associated with BS

Classification of risk factors in children associated with BS		
1st group: Oral hygiene	2nd group: Dietary factors	3rd group: Saliva factors
• Brushing teeth with fluoride toothpaste • Rinsing with mouthwashes containing fluoride	• Snacking between meals • Frequent consumption of foods rich in iron (vegetables) • Intake of iron supplements • Type of drinking water	• pH • Buffer capacity • Salivary flow • Composition of the saliva

Diet

One of the groups that can influence the appearance of BS includes dietary factors such as snacking between meals or the frequency of consumption of foods rich in iron (vegetables). Type of drinking water, intake of iron supplements. Even regular consumption of foods rich in iron during pregnancy could favor the development of chromogenic bacteria. One study conducted in Nepal found a positive correlation between iron levels in water and stain distribution (Pushpanjali et al. 2004). Another study in Spain found a statistically significant correlation between BS and the frequent consumption of foods rich in iron (Garcia Martin et al. 2013). A small survey made in a private practice suggests that the diet of patients with BS is rich in cheese, which contains lactoferrin, a protein known for its high affinity for iron (Mesonjesi 2012). As one of the most important metal elements in the human body, it is likely to find iron in both BS and dental plaque /6/.

Saliva

Another group is in favor of the properties and components of the saliva and such as buffer capacity, pH and many others. Saliva has an important role in oral health and protection against caries lesions. Salivary parameters such as pH, buffering capacity, and calcium and phosphate ion concentrations are important caries protective factors /7–8/. There are studies that investigate the saliva composition in subjects with BS. Surdacka obtained stimulated saliva using paraffin wax. Significantly higher levels of calcium, inorganic phosphates, copper, sodium, and total protein were found in patients with BS compared to controls. Glucose levels were significantly lower in the Black stain group. No differences were found in iron, zinc, and magnesium concentrations. The authors also

showed that pH was higher in children with stains. The salivary flow rate did not differ between groups /7–8/. In comparison with the study above Aysun et al. also explored salivary parameters in children with BS. However, the results were different. Calcium levels and saliva buffering capacity were significantly higher in BS group. There were no significant differences in phosphorus levels and salivary pH. The salivary flow rate was lower in children with BS compared to children without Black stain /8/.

Bacterial species

Other group risk factors reported some bacterial species that could play a role in the formation of BS. Chromogenic bacteria were proposed as an etiological factor in the production of black pigment. Periodontal pathogens such as *Porphyromonas gingivalis*, *Prevotella intermedia* and *Prevotella nigrescens* are reported to produce black pigment in the oral cavity /9/. Some studies suggest that *Prevotella melaninogenica* is closely related to BS /10/. The traditional method of exploring oral microorganisms was bacterial culture but its disadvantage is that almost 50% of oral bacteria are nonculturable. In addition, this conventional method is time-consuming. In comparison, molecular techniques have the advantage of detecting nonculturable and difficult to cultivate bacteria. Only expected bacterial species can be detected using polymerase chain reaction (PCR). In one study, they used next-generation sequencing of the bacterial 16S rRNA gene to detect oral microorganisms in children with or without BS /1/.

Saba et al. detected the presence of four distinct subspecies in dental plaque samples of 100 BS-affected children between 6 and 12 years old and 100 stain-free control subjects by PCR and electrophoresis. The prevalence of *Actinomyces* and *Aggregatibacter*

actinomycetemcomitans is higher in the group with BS compared to the plaque of the control group. Porphyromonas gingivalis and Prevotella melaninogenica were absent in both groups (with BS and control group) /11/.

In addition, real-time PCR was used to reveal the microbial compositions of samples of BS from 46 children aged from 3 to 10 years and non-discolored dental plaque from 47 counterparts without BS. BS group contained higher numbers of Actinomyces naeslundii and lower numbers of Fusobacterium nucleatum and Lactobacillus spp. No significant differences were observed for Aggregatibacter actinomycetemcomitans and Prevotella intermedia. The above studies indicate a unique microbiota in patients who have Black stain. On the other hand, only, a few selected species were evaluated which leads to the conclusion that the relationship between microorganisms and BS remains uncertain /9/.

The microflora of this deposit is dominated by Actinomyces having lower cariogenic potential and a lower number of Lactobacillus spp than non-discolored dental plaque (Heinrich-Weltzien et al. 2009; Heinrich-Weltzien et al. 2014; Chen et al. 2013).

Role of iron

A study published by Zhang shows a higher level of iron in BS when compared to the standard plaque by using the method of coupled plasma mass spectrometry. Analysis of the black material taken from the teeth indicates that it contains insoluble ferric salt, which is likely to be ferric sulfide and a high content of calcium and phosphate /1/. These studies suggested that the ferric sulfide might be formed by the reaction between hydrogen sulfide produced by bacterial metabolism and iron in the saliva or gingival exudates. In one study, a group of 6-year-old

Spanish children with BS were found to have consumed a significantly higher proportion of iron supplements than children without black stain; an increased rate of iron supplementation was also found among the mothers of these children during pregnancy /1/. This study also reported higher consumption of specific foods rich in iron, such as vegetables, dairy products and eggs, in children with BS. Furthermore, another study revealed a positive correlation between BS and the concentration of iron in water sources /12/. These studies suggested that the formation of Black stain may be associated with ferric sulfide.

Conclusion

This issue can cause significant concerns for parents and can lead to social and psychological issues in children

The etiology of BS as a form of extrinsic stain is not completely understood. However, it is essential to understand the nature of BS and know how to realize the correct treatment. The findings of the literature review give grounds to undertake studies investigating the etiology of the BS. Future explorations will be interesting as its negative effect on dental aesthetic perception causes concerns in parents and can have significant effects on the personality and self-confidence of the child. Professional oral hygiene is necessary, but the formation of BS is repetitive. However, frequent professional hygiene can cause damage to the dental enamel. Nowadays, new technologies as well as innovative scientific findings suggest noninvasive treatment methods.

Reference

1. Li Y, Zhang Q, Zhang F, Liu R, Liu H, Chen F. Analysis of the Microbiota of Black Stain in the Primary Dentition. Al-Ahmad A, editor. PLOS ONE [Internet]. 2015 Sep 4;10(9):e0137030. Available from: www.ncbi.nlm.nih.gov/pmc/articles/PMC4560370/
2. Garcia Martin JM, Gonzalez Garcia M, Seoane

- Lleston J, Llorente Pendas S, Diaz Martin JJ, Garcia-Pola MJ. Prevalence of black stain and associated risk factors in preschool Spanish children. *Pediatrics International*. 2013 Jun;55(3):355–9.
3. Żyła T, Kawala B, Antoszevska-Smith J, Kawala M. Black Stain and Dental Caries: A Review of the Literature. *BioMed Research International*. 2015;2015:1–6.
4. Zhang Y, Yu R, Zhan JY, Cao GZ, Feng XP, Chen X. Epidemiological and Microbiome Characterization of Black Tooth Stain in Preschool Children. *Frontiers in Pediatrics* [Internet]. 2022 Jan 26;10. Available from: [www.ncbi.nlm.nih.gov/pmc/articles/PMC8826690/](https://pubmed.ncbi.nlm.nih.gov/pmc/articles/PMC8826690/)
5. Surdacka A. [Black stain and caries in children and adolescents in schools]. *Czasopismo Stomatologiczne* [Internet]. 1987 [cited 2023 Apr 29];40(11-12):763–9. Available from: <https://pubmed.ncbi.nlm.nih.gov/3507302/>
6. Zhang F, Li Y, Xun Z, Zhang Q, Liu H, Chen F. A preliminary study on the relationship between iron and black extrinsic tooth stain in children. *Letters in Applied Microbiology* [Internet]. 2017 Jun 1;64(6):424–9. Available from: <https://pubmed.ncbi.nlm.nih.gov/28266718/>
7. Surdacka A. [Amount and pH of the saliva in children and adolescents with black tartar]. *Czasopismo Stomatologiczne* [Internet]. 1989 Jun 1 [cited 2023 Apr 29];42(6):381–6. Available from: <https://pubmed.ncbi.nlm.nih.gov/2488211/>
8. Surdacka A. [Chemical composition of the saliva in children and adolescents with black tartar]. *Czasopismo Stomatologiczne* [Internet]. 1989 [cited 2023 Apr 29];42(10-12):525–33. Available from: <https://pubmed.ncbi.nlm.nih.gov/2487836/#:~:text=In%20the%20saliva%20of%20children>
9. Soukos NS, Som S, Abernethy AD, Ruggiero K, Dunham J, Lee C, et al. Phototargeting Oral Black-Pigmented Bacteria. *Antimicrobial Agents and Chemotherapy*. 2005 Apr;49(4):1391–6.
10. Slots J. The microflora of black stain on human primary teeth. *Scandinavian Journal of Dental Research* [Internet]. 1974;82(7):484–90. Available from: <https://pubmed.ncbi.nlm.nih.gov/4612701/>
11. Hwang JY, Lee HS, Choi J, Nam OH, Kim MS, Choi SC. The Oral Microbiome in Children with Black Stained Tooth. *Applied Sciences*. 2020 Nov 13;10(22):8054.
12. Pushpanjali K, Khanal SS, Niraula SR. The relationship of dental extrinsic stains with the concentration of trace elements in water sources in a district of Nepal. *Oral Health & Preventive Dentistry* [Internet]. 2004 [cited 2023 Apr 29];2(1):33–7. Available from: <https://pubmed.ncbi.nlm.nih.gov/15641763/>

Corresponding author

Kristina Tabutova, Assistant Professor
Department of Pediatric Dentistry
Faculty of Dental Medicine
Medical University – Plovdiv
3 Hristo Botev Bvd, 4000 Plovdiv, Bulgaria
Mobile: + 359 896 700 635
email: ktabutova@abv.bg

VI. Surgical Session

Effect of Operative Approaches on Functional Outcomes in Distal Humerus Fractures

Stanislav Karamitev, Vladimir Stavrev

Department of Orthopedics and Traumatology, Faculty of Medicine, Medical University of Plovdiv

Purpose: To present the approaches we used in the surgical treatment of intra-articular fractures of the distal humerus, as well as to analyze the functional results after their application. **Material and methods:** For a period of 12 years (2010–2022), 98 patients with bicondylar interarticular fracture of the distal humerus were surgically treated. In 78 of them, the classic dorsal trans-olecranon approach was used, and in the remaining 20 patients, we used an approach with osteoplastic splitting of the triceps brachii muscle. **Results:** All fractures and osteotomies ended with consolidation. In 7 of the cases of “V”-shaped osteotomies of the olecranon and Weber fixation, we observed migration of the K-needles and maceration of the skin, which necessitated their removal. In 10 of the operated patients, hypoesthesia was established in the autonomous zone of the ulnar nerve, which disappeared within two months. In one patient, we observed a serious complication with osteosynthesis debricolage, which necessitated a new intervention, by means of autoosteoplasty and plate fixation. **Conclusions:** The triceps brachial division approach is an alternative to the classic trans-olecranon approach in simple intra-articular and all extra-articular fractures of the distal humerus. Its main advantage is the absence of prominent implants, which often requires a new intervention to remove them. In contrast, the trans-olecranon approach provides good visualization and enables precise repositioning of the intra-articular and metaphyseal component in this type of fracture.

Keywords: *distal humerus, olecranon osteotomy, triceps brachii muscle*

Introduction

Displaced fractures at the lower end of the humerus require open reduction and stable internal fixation. A number of surgical approaches to the distal humerus have been described in the literature. (1, 2) The trans-olecranon approach, i.e. through osteotomy of the olecranon, provides a good view of the articular surface and metaphysis of the humerus, allows anatomical reposition of the fracture, but at the same time is associated with complications such as non-union, delayed union, migration of the osteosynthesis and neurological device. (3, 4) These access deficiencies lead to outflow from it and are created in which the extensor mechanism is prepared from the proximal ulna and distal humerus, i.e. by splitting the triceps brachii muscle. (5, 6) This approach avoids these inconveniences, but at the expense of poor articular visualization, due to the fact that the olecranon remains preserved and does not

allow the visualization of the trochlea humeri.

Purpose

To present the approaches we used in the surgical treatment of intra-articular fractures of the distal humerus, as well as to analyze the functional results after their application.

Material and methods

For a period of 12 years (2010–2022) in the Orthopedics and Traumatology Clinic of UMBAL “St. Georgi” – Plovdiv, 98 patients with bicondylar interarticular fracture of the distal humerus were surgically treated. In 78 of them, the classic dorsal trans-olecranon approach (Shavron osteotomy) was used, and in the remaining 20 patients, we used an approach with osteoplastic splitting of the triceps brachii muscle (split approach). The follow-up period is one year. The AO classification was used to determine the type of

fracture in the region of the distal humerus. All fractures were reported as C type. Chevron osteotomy was applied to C1 type – 10 patients; C2 type – 26 patients; C3 type – 42 patients. With the split approach, the following distribution of fractures was observed: C1 type – 12 patients, C2 type – 6 patients and C3 type – 2 patients. We did not treat open fractures.

In patients from the first group, access to the articular surface of the distal humerus is dorsal through a Chevron (V-shaped) osteotomy of the olecranon. The patient is in a lateral position with the arm flexed at the elbow joint. We open the joint to determine the central part of the trochlear arch. We center the osteotomy there. The osteotomy itself is V-shaped with the tip pointing distally. Then, with a chisel, we perform the osteotomy reaching the subchondral bone and finish by levering movement in a proximal direction until the fracture of the olecranon through the subchondral bone. The uneven bony ends formed in the subchondral bone facilitate repositioning and fixation of the osteotomy. The osteotomized olecranon is resected proximally to free the triceps medially and laterally from the intermuscular septum until the metaphyseal part of the fracture is exposed. After repositioning and imposed osteosynthesis of the fracture, we place and fix the performed osteotomy by means of tension osteosynthesis according to the Weber method.

In the second group of patients, we applied a posterior approach with splitting of the triceps brachii m. proposed by Campbell. The muscle layers bluntly from the tip of the olecranon in a proximal direction. Distal division of the muscle is carried out osteoplastically, taking care not to disturb the integrity of the periosteal insertion. Medial disinsertion is performed after identification of the ulnar nerve. After repositioning and

subsequent fixation of the fracture, we restore the integrity of the extensor apparatus by means of sufficiently thick and strong non-absorbable sutures through bone tunnels.

In both accesses, we impose aspiration drainage for one day, with the hand placed in the cuff until the sutures are removed on the 14th postoperative day. We allow movement already on the next postoperative day, as loading of the limb occurs after X-ray data on consolidation of the fracture.

Results

The patients were followed for a period of one year. All fractures and osteotomies ended with consolidation. (fig. 1–6)



Figure 1, 2. 39♀ C-type Fx AP/lateral view



Figure 3, 4. CT 3D reconstruction

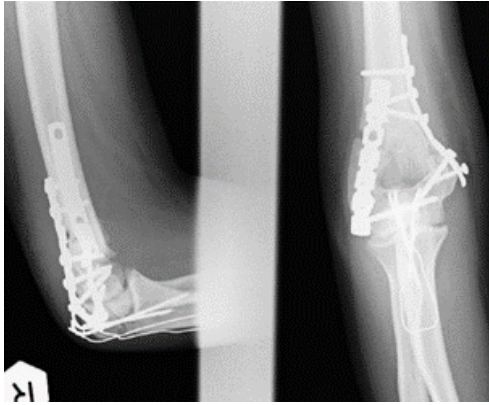


Figure 5. 2 month post surgery

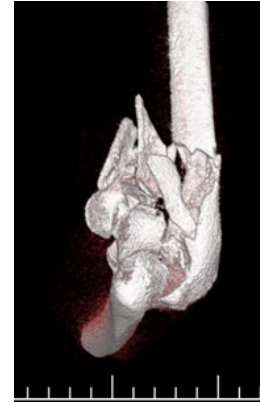


Figure 7. 68♀ C-type Fx



Figure 6. 8 month later



Figure 8. ORIF with plates

In 7 of the cases of “V”-shaped osteotomies of the olecranon and Weber fixation, we observed migration of the K-needles and skin maceration, which necessitated their removal. In 10 of the operated patients, hypoesthesia was found in the autonomous zone of the ulnar nerve, which disappeared in two months, after drug therapy. In one patient, we observed a serious complication with osteosynthesis debricolage, which necessitated a new intervention, by means of autoosteoplasty and plate fixation. (fig. 7–10)

The functional evaluation of the results was performed based on the abbreviated version of the DASH score (the so-called quick DASH). There was no difference in the functional results of the two approaches, in terms of flexion contracture and range of motion.



Figure 9. 50 day post surgery (debricolage)



Figure 10. Reoperation (ORIF+autoosteoplasty)

Discussion

The ideal operative access should provide sufficient exposure for anatomic repositioning and fixation, with minimal soft tissue or bony damage. The choice depends on the type of fracture, joint involvement, accompanying soft tissue lesions, rehabilitation protocol, and surgeon preference. Compared with all posterior approaches, the olecranon osteotomy offers the best visualization of the distal humerus. (4) According to Wilkinson, 57% of the articular face is reached in this way, while only 35% of it is accessible when dividing the m. triceps brachii. Specific complications are also observed – irritation of the K-wire and the need for their removal in 27% to 80% of cases. (7) There is also a chance of nonunion of the osteotomy, which in Stanley's study was as high as 15%. Proximal continuation of the opening is difficult, as well as possible arthroplasty.

Posterior access with division of the triceps brachii m. allows this more easily, but at the expense of reduced visualization of the articular facet and postoperative extension weakness.

The similar functional results with the two approaches show us that the outcome of severe trauma depends rather on the severity of the fracture and the quality of the achieved reposition and stabilization.

Conclusion

The results of our study show that the triceps brachii split approach is a valid alternative to the classic olecranon osteotomy for simple intra-articular and all extra-articular

fractures of the distal humerus. Its main advantage is the absence of prominent implants, which often necessitates surgical intervention. The transolecranon approach provides good exposure and allows for precise repositioning of the intra-articular and metaphyseal component in distal humerus fractures.

Reference

1. Cheung EV, Steinmann SP. Surgical approaches to the elbow. *J Am Acad Orthop Surg.* 2009; 17(5):325–33
2. Ring D, Gulotta L, Chin K, Jupiter JB. Olecranon osteotomy for exposure of fractures and nonunions of the distal humerus. *J Orthop Trauma.* 2004;18(7): 446–9.
3. Elmadag M, Erdil M, Bilsel K. The olecranon osteotomy provides better outcome than the triceps-lifting approach for the treatment of distal humerus fractures. *Eur J Orthop Surg Traumatol.* 2014;24(1):43–50.
4. Zhang C, Zhong B. Comparing approaches to expose type C fractures of the distal humerus for ORIF in elderly patients: six years clinical experience with both the triceps-sparing approach and olecranon osteotomy. *Arch Orthop Trauma Surg.* 2014;134(6):803–1145.
5. Schildhauer TA, Nork SE et al. Extensor mechanism-sparing paratricipital posterior approach to the distal humerus. *J Orthop Trauma.* 2003;17(5):374–8
6. Ziran BH, Smith WR, Balk ML et al. A true triceps-splitting approach for treatment of distal humerus fractures: a preliminary report. *J Trauma.* 2005;58(1):70–5.
7. Wilkinson JM, Stanley D. Posterior surgical approaches to the elbow: a comparative anatomic study. *J Shoulder Elbow Surg.* 2001;10:380-382.

Corresponding author

Stanislav Karamitev, MD, PhD
Clinic of Orthopaedics and Traumatology
“Saint George” University Hospital,
66 Peshtersko shousse bld. 4002 Plovdiv, Bulgaria
E-mail: dokstanly@yahoo.com
GSM +359888811879

Tumor of the Heart – a Clinical Case

Ali Vehbi¹, Spas Kitov^{1,2}, Maria-Florans Kitova³, Galabin Markov³

1. St. George University Hospital, Cardiac Surgery Clinic, Plovdiv

2. Clinic of Cardiology, Medical Faculty, Medical University, Plovdiv

3. Faculty of Medicine, Medical University of Plovdiv

A 58-year old man came in for a check-up which included echocardiography, magnetic resonance. Both showed a formation in the posterior mitral valve cusp /fibroelastom with mediastinal lymphadenomegalia/ in absence in the mitral valve disfunction. Pre-operation coronary angiography showed absence of the significant coronary stenosis. The whole operation included resection of the posterior mitral valve cusp, septal myectomy and a mitral valve prothesis Sorin Carbomedics number 33. The result from the histology exam which is confirmed by two unrelated pathoanatoms – hyalinized fibres tissue with excessive degenerative calcinosis without papillar structures. After a 3-month clean period the patient was admitted to the hospital with acute left ventricle insufficiency. The result from the transoesophagoes echocardiography was severe paraprothesis mitral insufficiency. He underwent a mitral reprothesis with Sorin Carbomedics Standart number 33. After that he was admitted for the third time with temperature 38.2 and acute left ventricle insufficiency. He underwent a third operation- suture of the mechanic valve prothesis and occlusion of the auricular of the left atrium. After another clean period that continued 1 month he was admitted to the cardio-surgery department with hepato-renal syndrome, polyorgan insufficiency and it ended with exitus letalis. In conclusion, the possible causes behind the prothesis dehiscence are: 1. Weak connective tissue. Located area of paraprothesis infective endocarditis. 3. Excesive local enlargement of the neoplastic tissue in the prothesis suture area.

Keywords: tumor, mitral valve, prothesis, cardio-surgery

Introduction

Papillary fibroelastoma is a rare benign tumor that most commonly affects the heart valves. Most papillary fibroelastomas are asymptomatic; they are rarely diagnosed because of cardiac symptoms or after an embolic event. In this case, the 58-year-old man was asymptomatic. The evaluation for a potential source of embolism revealed a tumor formation on the posterior mitral valve sheath. MRI tissue characterization findings of the mass supported the diagnosis of mitral valve fibroelastoma.

Clinical case

A 58-year-old man with a formation captured by the posterior mitral leaflet of the mitral valve, diagnosed during a preventive examination by echocardiography, confirmed by MRI, /observed fibroelastoma with mediastinal lymphadenomegalia/, in the absence of mitral valve dysfunction. (Figure 1, 2, 3)

Conducted pre-operative selective coronary angiography with data on insignificant stenoses on the coronary arteries, not

requiring treatment at the moment. Operative intervention performed on 26.05.2022 in volume – resection of posterior mitral sheet, septal myectomy and mitral valve prothesis with mechanical valve Sorin Carbomedics No. 33. Subsequent light period of 3 months until 12/09/22. presenting with complaints of easy fatigue, progressive shortness of breath with minimal physical exertion from the performed transthoracic and transesophageal echocardiography with evidence of severe paraprothesis mitral regurgitation, taken blood cultures, urine culture, nasal and throat secretions – sterile results, with elevated inflammatory markers. On 13.09.22 performed reoperative intervention – mitral valve re-prothesis with S. Carbomedics Standard №33 prothesis. On 07/11/22 he comes in with complaints of easy fatigue, severe shortness of breath and chest discomfort, fever up to 38.2 degrees Celsius. On 11/24/22. operative re-intervention was performed in volume suture of a mechanical valve prothesis and occlusion of the ear of the left atrium. An antibiotic course of

treatment was carried out according to the protocol for prosthetic infective endocarditis and, during the stay, on the occasion of another paraprosthetic mitral regurgitation, a re-operative intervention was performed on 02/12/22. in volume mitral valve re-prosthesis with S. Carbomedics Standard #33 prosthesis and suture of secondary interatrial defect. Subsequent bright period of 1 month

without complaints until 04/01/23. presenting once again with persistent complaints of easy fatigue, shortness of breath and chest discomfort with minimal physical exertion. Without febrile-intoxication manifestations, in the course of hospitalization with the development of hepato-renal syndrome, multiple organ failure and on 08/01/23 ended with exitus lethalis.

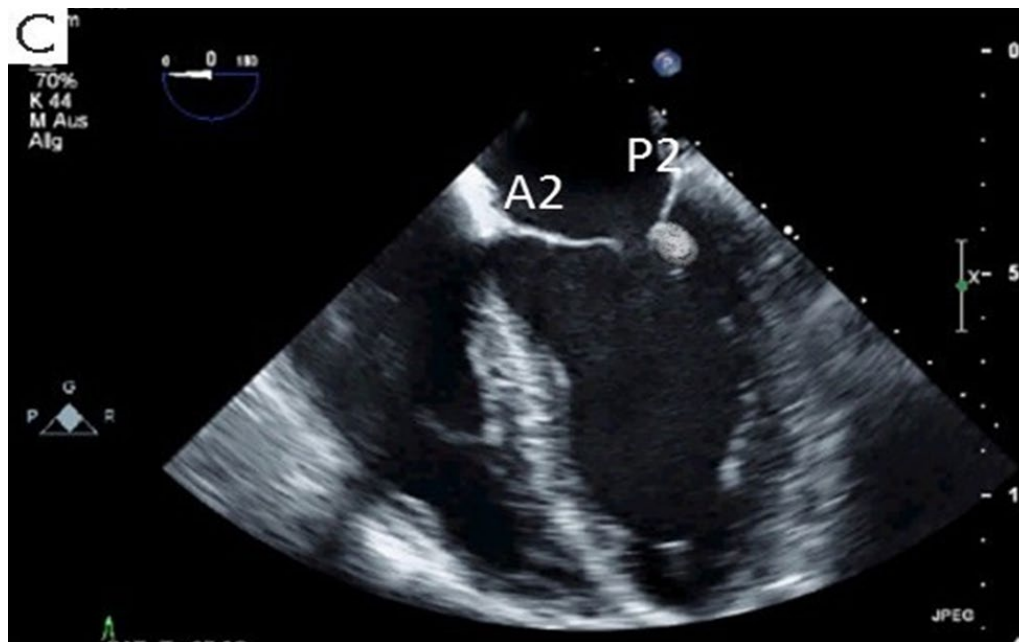


Figure 1. Transthoracic echocardiography showing the posterior mitral leaflet formation

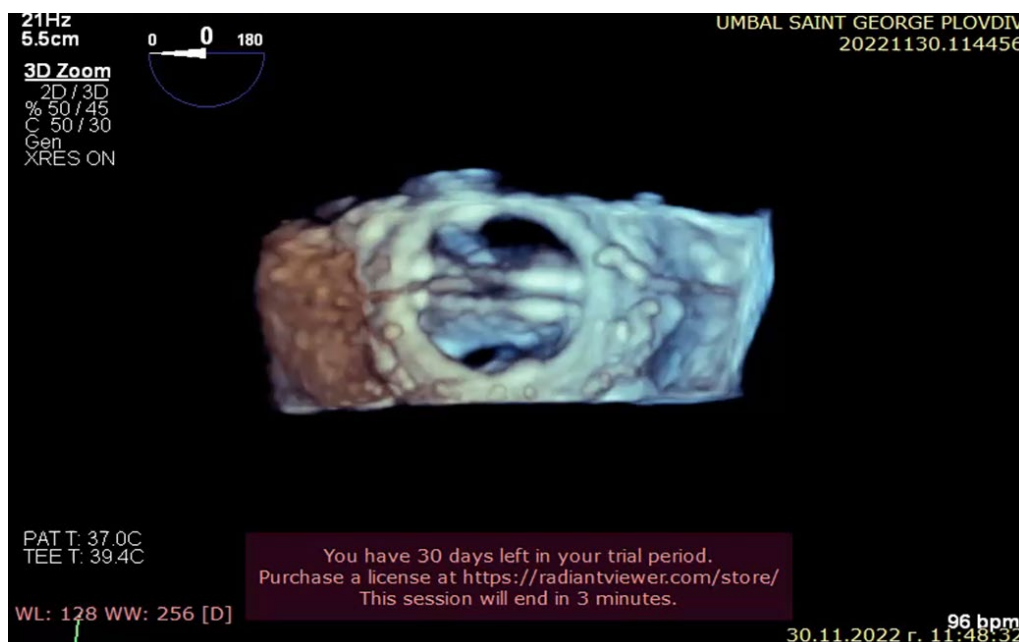


Figure 2. 3-D echocardiography of the mitral valve

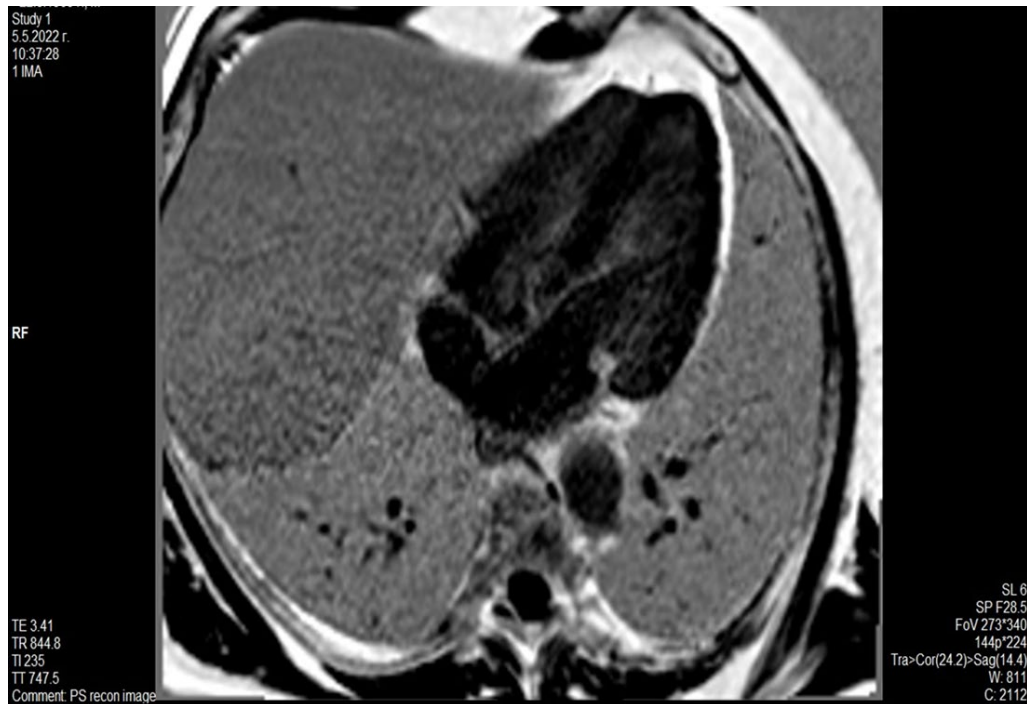


Figure 3. Magnetic resonance imaging of the heart showing the posterior mitral leaflet formation

Discussion

Possible causes of prosthetic dehiscences.

1. Connective tissue weakness
2. Localized focus of paraprosthetic infective endocarditis
3. Excessive growth of neoplastic tissue in the area of the prosthetic suture.

Conclusion

Histiocytosis, also called Langerhans cell histiocytosis or histiocytosis X, is a group of

rare diseases involving specific cells that normally have an important role as part of the immune system. Although the cause of Langerhans cell histiocytosis is unknown, the condition can often have a neoplastic course. Histiocytosis is the name given to a group of syndromes characterized by an abnormal increase in the number of certain immune cells called histiocytes. These include monocytes, macrophages and dendritic cells.

Management Options for Cervical Intraepithelial Neoplasia

Gita Yamakova-Vladova^{1,2}, Krum Vladov^{1,2}, Peter Uchikov³, Radoslav Terjumanov^{1,2}, Kamen Yamakov^{1,2}

1. Department of Obstetrics and Gynecology, Faculty of Medicine, Medical University of Plovdiv

2. Clinic of Obstetrics and Gynecology, University Hospital "St. George" EAD Plovdiv

3. Department of Special Surgery, Faculty of Medicine, Medical University of Plovdiv

Introduction: Each year, more than half a million women are diagnosed with cervical cancer and the disease results in over 300 000 deaths worldwide. High-risk subtypes of the human papilloma virus (HPV) are the cause of the disease in most cases. The disease is largely preventable. Cervical cancer incidence and mortality have more than halved over the past 30 years since the introduction of formal screening programmes. Treatment depends on disease extent at diagnosis and locally available resources. **Aim:** Management of cervical intraepithelial neoplasia (CIN) needs to protect women at risk from developing cervical cancer and to avoid over-treatment as well as obstetrical complications in women undergoing invasive treatment. **Resources and methods:** A literature search of PubMed data base was conducted. Used keywords were: management options for cervical intraepithelial neoplasia; cervical cancer; surgical treatment. **Results and discussion:** Excisional treatments are referred to as cone biopsies or cervical conization and include cold knife conization, loop electrosurgical excision procedure (LEEP; also called large loop excision of the transformation zone [LLETZ]), and laser conization. Ablative treatments include cryotherapy, CO₂ laser ablation, and thermal ablation (eg, diathermy, cold coagulation). **Conclusion:** Chronic persistent infection of the cervix with oncogenic types of HPV leads, as well as other risk factors such as smoking, to induce precancerous lesions of the cervix. Under the influence of risk factors, cervical intraepithelial neoplasia progresses to invasive cervical carcinoma. The literature review summarizes minimally invasive surgical methods for the treatment of low- and high-grade dysplasia of the cervix. Early diagnosis by colposcopy with targeted biopsy and subsequent ablative or excisional treatment would reduce the incidence of invasive cervical cancer.

Key words: *intraepithelial neoplasia, excisional and ablative treatments*

Introduction

According to GLOBOCAN 2020 data, cervical cancer has been diagnosed in 600,000 worldwide and more than 340,000 women have lost their lives as a result of disease progression [1]. In Europe, cervical cancer is the second most common cancer among women aged 15 to 44. In Bulgaria, the newly diagnosed cases for 2018 are 850, mainly women in the third and fourth stage. Our country ranks third in Europe in terms of morbidity and fourth in terms of mortality from cervical cancer, which is caused by the human papillomavirus (HPV) [2]. There is an effective primary prevention – a vaccine against HPV, but the vaccine coverage in Bulgaria is only 3%, although the vaccine is free for girls aged 10–13 [2].

At the same time, the WHO has launched an initiative for 90% of girls worldwide to receive the HPV vaccine by 2030[3]. At the

same time, in Bulgaria every day one woman dies from cervical cancer (HPV), and 44 women are diagnosed with HPV [2]. The study by Nagao Y. et al. confirms that diagnostic conization is used for early detection of cervical cancer [4].

Aim

Management of cervical intraepithelial neoplasia (CIN) needs to protect women at risk from developing cervical cancer and to avoid over-treatment as well as obstetrical complications in women undergoing invasive treatment.

Resources and methods

A literature search of PubMed data base was conducted. Used keywords were: management options for cervical intraepithelial neoplasia; cervical cancer; surgical treatment.

Results and discussion

Chronic persistent infection of the cervix with high-risk oncogenic HPV types leads to cervical intraepithelial lesions (CIN I, CIN II and CIN III) – low-grade (LSIL) and high-grade (HSIL) [5]. The progression of the oncological disease is for a period of 4–5 years, necessary for progression to a high-grade lesion and more than ten years for malignant transformation to invasive carcinoma [6]. Cervical conization is required in women with colposcopically proven HSIL or in cases where CIN2 persists for two years or progresses to CIN3, according to the 2019 American Society for Colposcopy and Cervical Pathology (ASCCP) recommendations [7].

Excisional treatments are referred to as cone biopsies or cervical conization and include cold knife conization, loop electrosurgical excision procedure (LEEP; also called large loop excision of the transformation zone [LLETZ]), and laser conization. Ablative treatments include cryotherapy, CO₂ laser ablation, and thermal ablation eg, diathermy, cold coagulation [8, 9, 10, 11, 12].

Cold knife conization (CKC) is a traditional procedure for CIN. A cone-shaped piece of abnormal tissue is removed from the cervix using a scalpel [8]. It is typically performed under general or local anesthesia in the hospital. Accurate evaluation of the surgical margins is the major advantage of this procedure. According to the volume and height of the cone, CKC increases the risk of premature birth [8, 9].

Widely performed to treat high-grade CIN in recent years is LEEP – Loop Electrosurgical Excision Procedure. According to Abdulaziz AMA. et al. article, LEEP is associated with a higher positive margin than some other treatment procedures [10].

A positive margin is associated with an increased risk of recurrent and residual

disease. The author also points out some of the leading advantages of this method as short operating time with fewer complications, the possibility of performing it in an outpatient practice under local anesthesia [10].

Rezniczek GA, Large loop excision of the transformation zone (LLETZ) is the standard of care for CIN II. In a meta-analysis of 26 randomized trials, the incidence of persistent or recurrent CIN after LLETZ was comparable to that after cold knife conization. A leading advantage is that LLETZ is faster and causes less intraoperative bleeding. The shorter hospital stay and the option for outpatient treatment are advantages of the method. The authors' study shows that LLETZ cones have an overall smaller volume and weight and result in less cervical stenosis and fewer unsatisfactory studies [11].

Laser conization (LC) can be performed under general or local analgesia. Through a highly focused laser spot, an ectocervical peripheral incision is made at a depth of 1 cm.

A major disadvantage of laser conization is the limited ability to assess margins due to the thermal damage that is created. Various non-randomized studies indicate a success rate of this type of conization over ninety percent – 93% to 96%. The main advantages of laser conization are precise adjustment of cone size, low blood loss in most cases, and less trauma to the cervix compared to cold knife conization [12].

Cryotherapy refers to ablative methods. This is an ablation method that is most often used for the treatment of ectocervical premalignant cervical lesions. The leading advantage of the method is the high percentage of effectiveness, i.e. no disease after its application. Studies indicate good results in 92% for CIN II and 85% for CIN III. Another major advantage over other methods is the “single visit approach”. Disadvantages

of cryotherapy are related to the availability and transport of large gas tanks, as well as the high cost of the necessary equipment [13].

Conclusion

Chronic persistent infection of the cervix with oncogenic types of HPV leads, as well as other risk factors such as smoking, to induce precancerous lesions of the cervix.

Under the influence of risk factors, cervical intraepithelial neoplasia progresses to invasive cervical carcinoma.

The literature review summarizes minimally invasive surgical methods for the treatment of low- and high-grade dysplasia of the cervix. Early diagnosis by colposcopy with targeted biopsy and subsequent ablative or excisional treatment would reduce the incidence of invasive cervical cancer.

Literature resources

1. Cancer, I A F R O, GLOBOCAN. Cervix uteri, Cancer Incidence and Mortality Statistics Worldwide and by Region. 2020.
2. National program for primary prevention of cervical cancer in the Republic of Bulgaria. 2021–4.
3. WHO updates recommendations on HPV vaccination schedule [Internet]. Available from: <https://www.who.int/news/item/20-12-2022-WHO-updates-recommendations-on-HPV-vaccination-schedule>
4. Nagao Y, Yokoi A, Yoshida K, Sumi M, Yoshihara M, Tamauchi S, et al. Clinical effects of cervical conization with positive margins in cervical cancer. *Sci Rep* [Internet]. 2021;11(1).
5. Ntanasis-Stathopoulos I, Kyriazoglou A, Liontos M, A Dimopoulos M, Gavriatopoulou M. Current

trends in the management and prevention of human papillomavirus (HPV) infection. *J BUON*. 2020;25(3):1281–5.

6. Jain MA, Limaïem F. Cervical Squamous Cell Carcinoma. *StatPearls Treasure Island*. 2023;
7. Perkins RB, Guido RS, Castle PE, Chelmow D, Einstein MH, Garcia F, et al. 2019 ASCCP risk-based management consensus guidelines for abnormal cervical cancer screening tests and cancer precursors. *J Low Genit Tract Dis* [Internet]. 2020;24(2):102–31
8. Zebitay AG, Güngör ES, İlhan G, Çetin O, Dane C, Furtuna C, et al. Cervical conization and the risk of preterm birth: A population-based multicentric trial of Turkish cohort. *J Clin Diagn Res* [Internet]. 2017;11(3):QC21–4.
9. Kyrgiou M, Mitra A, Arbyn M, Stasinou SM, Martin-Hirsch P, Bennett P, Paraskevaidis E, Fertility and early pregnancy outcomes after treatment for cervical intraepithelial neoplasia: systematic review and meta-analysis *BMJ* 2014 349:6192
10. Abdulaziz AMA, You X, Liu L, Sun Y, Zhang J, Sun S, et al. Management of high-grade squamous intraepithelial lesion patients with positive margin after LEEP conization: A retrospective study: A retrospective study. *Medicine (Baltimore)* [Internet]. 2021;100(20):e26030.
11. Rezniczek GA, Neghabian N, Rehman S, Tempfer CB. Video colposcopy versus headlight for large loop excision of the transformation zone (LLETZ): a randomised trial. *Arch Gynecol Obstet*. 2022;305(2):415–423.
12. Martin-Hirsch PP, Paraskevaidis E, Bryant A, Dickinson HO. Surgery for cervical intraepithelial neoplasia. *Cochrane Database Syst Rev*. 2013; 2013(12):CD001318.
13. Duan L, Du H, Belinson JL, et al. Thermocoagulation versus cryotherapy for the treatment of cervical precancers. *J Obstet Gynaecol Res*. 2021;47(1):279–286

Corresponding author

Dr. Gita Yamakova-Vladova
Address: 15A Vasil Aprilov Blvd
Email: gitayamakova@yahoo.com
mobile phone: +359894766467

A Rare Presentation of Orbital Dermoid Cyst in Adulthood: a Case Report

Plamen Penchev¹, Petar-Preslav Petrov², Ivan Ivanov⁵, Bogomil Iliev^{3,4}, Yavor Enchev^{3,4}

1. Faculty of Medicine, Medical University of Plovdiv

2. Department of Anatomy, Histology and Embriology, Faculty of Medicine, Medical University of Plovdiv

3. Department of Neurosurgery, Medical University of Varna

4. Neurosurgery Clinic, University Hospital “Saint Marina”, Varna

5. Faculty of Medicine, Medical University of Varna

Introduction: Dermoid cysts are common, benign, soft tissue cysts of embryonic origin that can occur in a variety of craniofacial spaces. They may contain hair follicles, skin tissue, and glands that produce sweat and sebum. Over time, the glands continue to produce these substances, resulting in cyst growth. **Aims:** The purpose of this case report is to introduce a patient to a dermoid orbital cyst and assess the post-operative clinical outcome. **Materials and methods:** We present a case report of a 38-years old male patient who has entered the Neurosurgery clinic of “Saint Marina” University Hospital with clinical manifestation of rapidly growing tumor formation in the left orbital region. MRT discovered evidence of tumor formation in the left orbital area, suspected of a dermoid cyst. **Results and discussion:** Under general anesthesia, a left lateral orbitotomy was performed. The tumor mass was extirpated completely by microsurgical technique. A cranioplasty with titanium mesh was followed. Postoperatively, the patient had a relief in the neurological status. He was mobilized the day after the operation and released on the fifth day. **Conclusion:** Dermoid cysts are rare embryological benign lesions that make up 0.04–0.6% of all intracranial tumours. Management of these lesions requires complete excision although it is complicated because of the compressive effect of the deep component.

Keywords: dermoid cyst, orbit, orbital tumors, orbitotomy

Introduction

Dermoid cysts are rare benign cysts that are squamous epithelium-lined sacs that result from abnormal migration of ectodermal cells. These lesions are composed of keratinized squamous epithelium, have a fibrous capsule, and also have skin appendices like sweat glands, sebaceous glands and hair follicles. [1, 2] Orbital dermoid cysts represent around 3 to 9% of all primary orbital tumors. [3] Orbital dermoid cysts are mostly seen in childhood. Patients usually present with mass lesion, proptosis and inflammatory reaction. In this report, the authors describe a case in its adult stage.

Case report

We present a case report of a 38-years old male patient who has entered the Neurosurgery clinic of “Saint Marina” University Hospital with clinical manifestation of rapidly growing tumor formation in the left

orbital region. MRT has discovered evidence of tumor formation in the left orbital region, suspicious of a dermoid cyst (Figure 1).

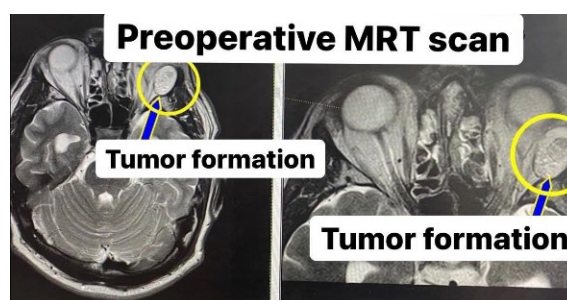


Figure 1. Preoperative scan. MRI indicated a suspected dermoid cyst tumor in the left orbital region.

Under general anesthesia, a left lateral orbitotomy was performed. The tumour mass was completely removed by micro-surgical technique (Figure 2).

A cranioplasty with titanium mesh was followed. After surgery, the patient received relief from the neurological condition. He

was mobilised the day following the intervention and released on the 5th day (Figure 3).



Figure 2. Intraoperative photography. Dermoid cyst

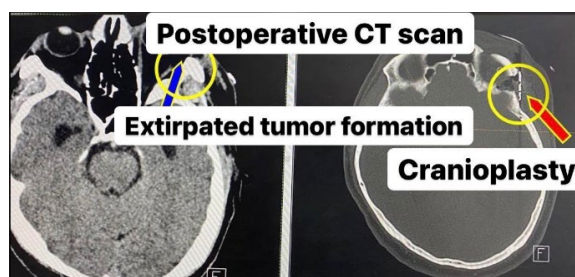


Figure 3. Postoperative scan. Incised dermoid cyst with titanium cranioplasty.

Discussion

Dermoid cysts are abnormally localized lesions resulting in abnormal involution of the superficial ectoderm during closure of sutures between the bones. [2] These lesions occur mainly in children, with few reported cases in adults. [5] Clinical manifestations are variable and depend upon orbit position. Usually the superficial forms manifest as a subcutaneous palpebral swelling of the superolateral location, deforming the eyebrow. [6] Deeper located forms are expressed as slowly progressive, painless, irreducible and nonaxial exophthalmitis. Lesions, contiguous to the optic nerve, can induce a decrease in the visual acuity. [7] The differential diagnosis of orbital cysts include teratoma, choristoma and colobomatous cyst. [8] The management of dermoid cysts

is surgical with complete excision of the cyst lining, taking care in order to avoid rupture. The approach is chosen according to the size and the location of the cyst. A supra-lateral orbitotomy is recommended for cysts in the superior-external quadrant or it can be a wider subfrontal approach for retrobulbar forms. [9]

Conclusion

Comprehensive surgical excision is the only effective method of treating these lesions. Long-term follow-up is required in cases of re-offending.

Acknowledgements

The authors have no support to report.

Funding

The authors have no funding to report.

Competing Interests

The authors have declared that no competing interests exist.

Consent

Written informed consent was obtained from the patient for publication of this case report and accompanying images.

References

1. Saumitra S. et. al. A case report on Intraorbital Exrtraconal Dermoid Cyst. *Science Repository*. 17th June 2020. Accessed: 30.01.2023. DOI: 10.31487/j.SCR.2020.06.15
2. Shepherd G Pryor, Jean E Lewis, Amy L Weaver, Laura J Orvidas (2005) Pediatric dermoid cysts of the head and neck. *Otolaryngol Head Neck Surg* 132: 938-942.
3. Taha S et al. (2009) Intraorbital Dermoid Cyst: Case Report. *Neurochirurgie* 55: 577-580.
4. Jerry A Shields, Carol L Shields (2004) Orbital cysts of childhood--classification, clinical features, and management. *Surv Ophthalmol* 49: 281-299
5. Nguyen Si Pham, Arthur Brooks Dublin, Edward Bradley Strong (2010) Dermoid cyst of the orbit and frontal sinus: a case report. *Skull Base* 20: 275-278
6. Civit T, Joud A, Klein O. [Congenital orbital tumors in adults (dermoidcysts)]. *Neurochirurgie*. 2010; 56(2-3): 183-186, doi:

10.1016/j.neuchi.2010.02.019, indexed in Pubmed: 20303551.

7. Taoufik Abdellaoui et al. (2021) A giant dermoid cyst of the orbit present in adulthood- a case report. *Ophthalmology J*: 2021; 6:53-56. DOI: 10.5603/OJ.2021.0008

8. Ouassime Kerdoud et al. Dumbbell-shaped dermoid cyst of the orbit: Case report, *Advances in Oral and Maxillofacial Surgery*, Volume 3, 2021, 100135, ISSN 2667-1476, DOI: <https://doi.org/10.1016/j.adoms.2021.100135>.

9. G.T. Emerick et al. Chewing-induced visual impairment from a dumbbell dermoid cyst. *Ophthalmic Plast Reconstr Surg*, 13 (1) (Mar. 1997), pp. 57-61, 10.1097/00002341-199703000-00010

Corresponding author

Plamen Nikolaev Penchev
Medical University of Plovdiv
bul. "Vasil Aprilov" 15A
Tel: 089 296 2278
email: plamen_penchev00@abv.bg

"Happy" New Year or Why You Should Stay away from Pyrotechnics: a Case Report of Firework-related Injury of Both Eyes

Sinan Aptikadir^{1,2}, Yumyut Idriz², Nina Stoyanova^{1,2}, Vesela Mitkova-Hristova^{1,2}, Nelly Sivkova^{1,2}

1. Department of Ophthalmology, Faculty of Medicine, Medical University of Plovdiv

2. University Eye Clinic, St. George University Hospital, Plovdiv

Eye injuries are the leading cause of monocular visual impairment and blindness in the world. Worldwide, pyrotechnics are used to celebrate popular events such as holidays, national and cultural festivals. Firework-related injuries affect patients of all ages however, they have a higher prevalence in male adults and children. The incidence of these injuries is particularly high around New Year's celebrations. In this case report, we present a 25-year-old male patient who suffered ocular trauma to both eyes due to a firework explosion hitting his face on New Year's Eve. The patient arrived at the UMHAT St. George Eye Clinic shortly after midnight on January 1. He presented with bilateral penetrating injuries with prolapse of intraocular structures. Both eyes had a visual acuity of 0 /zero/ at the time of admission to the hospital. An imaging study was performed, which included a CT scan of the retrobulbar space and the brain. Among the findings were a fractured floor of the right orbit and its medial wall, and a prolapse of the lower oculomotor muscle into the homolateral maxillary sinus. The eyeballs were bilaterally deformed and asymmetric, metallic foreign bodies were also detected. A multidisciplinary team, including an ophthalmologist and a craniomaxillofacial surgeon, performed surgery on the patient. It is important to use pyrotechnics in a controlled environment, especially during celebrations where alcohol is consumed, which can slow down reactions. It is true that fireworks can be a pleasing sight for the eyes, but they can also cause fatal damage to them.

Keywords: *pyrotechnics, firework-related injury, bilateral ocular trauma*

Introduction

Monocular blindness is most commonly caused by eye injuries (1). About 19 million people worldwide suffer from monocular blindness and reduced visual acuity, caused by trauma (2), (3).

Approximately 3.5 people per 100,000 suffer monocular blindness as a result of trauma worldwide each year (4). Male adults and children are more likely to suffer fireworks-related eye injuries than females (5,6). Fireworks are a common way to celebrate holidays, national holidays, and cultural festivals across the globe. The incidence of these injuries is particularly high around New Year's celebrations (7). This case report illustrates ocular trauma caused by a firework explosion hitting the face of a male patient on New Year's Eve.

Clinical case

We present a clinical case report of a 25-year-old male who was admitted to UMHAT

St. George Eye Clinic shortly after midnight on January 1st with bilateral penetrating eye injuries and facial injuries sustained after getting hit by a firework explosion during a New Year's celebration. The examination of the eyeballs proved very challenging upon his admission due to the significant eyelid edema and hematoma. The visual acuity of both eyes was zero /0/

- lack of light perception. Slit-lamp examination revealed subconjunctival hemorrhage at 360 degrees and total hyphema. The manual examination of the eye pressure in both eyes was suggestive of low intraocular pressure. (**Fig. 1, 2**)

CT scan results showed that the inferior rectus muscle had prolapsed into the right maxillary sinus and the floor and medial wall of the right orbit were fractured. The optic nerves were bilaterally preserved in thickness and anatomical course. Moreover, foreign bodies were detected in the intrabulbar space of both eyes. (**Fig. 3**)



Figure 1. Right eye on admission



Figure 2. Left eye on admission

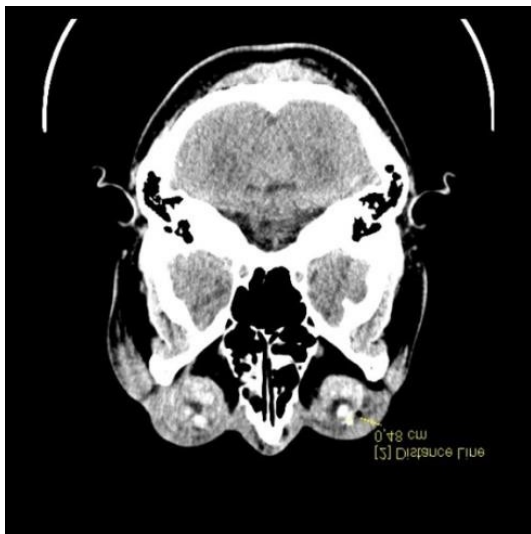


Figure 3. CT scan

B-scan examination revealed total vitreous hemorrhage in both eyes with no clear visualization of the retinal contours.

The operative treatment, performed under general anesthesia, required a multidisciplinary approach – the team included an ophthalmologist and a craniomaxillofacial surgeon. During surgery, the scleral tear in the right eye was observed to be behind the upper and lateral rectus muscle attachment points, protruding behind the equator and causing uveal, retinal, and vitreous prolapse. The scleral wounds were sutured with 7/0 Silk. The scleral wounds of the left eye started at the limbus and continued posteriorly – reaching behind the medial rectus muscle, which was delaminated and torn. Similarly to the right eye, uveal, retinal, and vitreous prolapse were also present. Limbal wounds were sutured with 10/0 Silk. The conjunctival wounds were adapted with 7/0 Vicryl. Vancomycin was applied intravitreally to both eyes. The eyelid wounds were adapted with 6/0 Silk. The lacerations on the tip of the nose and upper lip were sutured by a craniomaxillofacial surgeon (**Fig. 4**).



Figure 4. An intraoperative image of the left eye

The patient's visual acuity was still poor on his follow-up appointment

- twenty days after discharge. The patient did not attend any subsequent follow-up appointments (**Fig. 5**).

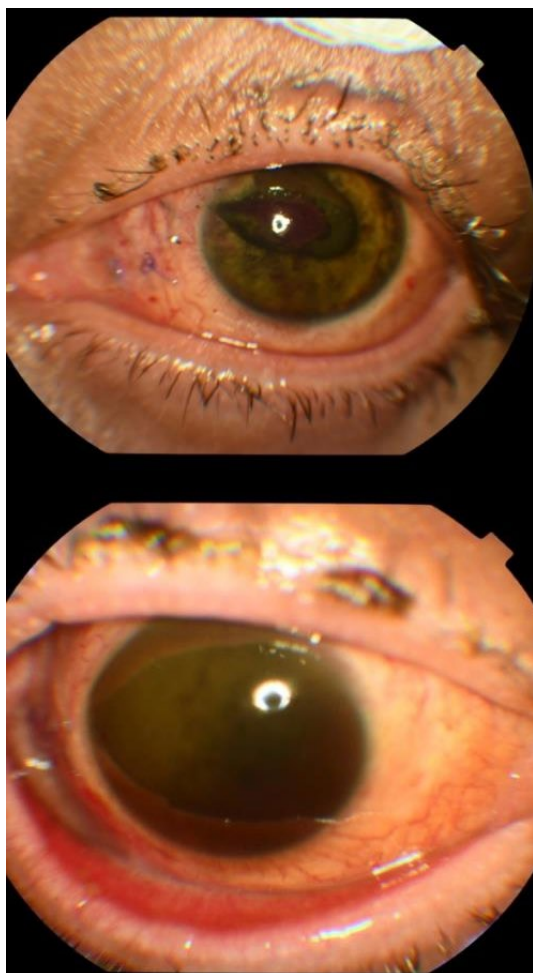


Figure 5. Postoperative images of the left and right eye

Discussion

Fireworks and other types of pyrotechnics are used in Bulgaria and around the world during various celebrations. Their use is especially widespread during New Year's celebrations (7, 8). Ocular trauma, particularly penetrating traumas, are the leading cause of monocular visual impairment and blindness worldwide (1). In various parts of the world, it has been found that during national celebrations and especially around

New Year, cases of eye injuries associated with pyrotechnics increase.

For example, in Switzerland, a study showed that the cases of patients with eye injuries related to fireworks had 2 (two) peaks. The first one is in January (around the New Year holidays) and the second is in August (during the Swiss National Holiday) when pyrotechnics are used the most (7). In the USA, similar results have been published – 85% of eye trauma cases related to fireworks are in the period around New Year's Day and Independence Day (8). Until 1996 the sale and use of pyrotechnics were banned in Ireland, and after the ban was lifted, the incidence of eye injuries associated with fireworks increased dramatically (9). In most cases, these injuries lead to severe vision loss or blindness, resulting in reduced quality of life. The focus should therefore be on prevention, such as banning the sale of pyrotechnics or providing special training for handling explosives. A similar restriction is in place in Sofia, Bulgaria – pyrotechnic products of categories F2 and F3 can only be used on New Year's Eve or after prior permission from the regional administration. It is believed that the incidence of firework-related injuries can be reduced by limiting the use of pyrotechnics and conducting educational campaigns on how to handle them.

Conclusion

Injuries caused by pyrotechnics can range from erosion of the corneal epithelium to perforation with loss of intraocular structures. Injuries with such genesis can be considered socially significant since they predominantly affect young people. Primary prevention must be emphasized to reduce the frequency of this type of trauma, which includes banning the sale and use of pyrotechnics in a controlled environment.

References

1. KALAYCI M, ÇETİNKAYA E. Epidemiology of Open Globe Injuries in Somalia. *Acibadem Universitesi Saglik Bilimleri Dergisi*. 2021 Apr 1;12(2).
2. Négrel AD, Thylefors B. The global impact of eye injuries. <http://dx.doi.org/10.1076/oep531438364> [Internet]. 2009 [cited 2023 Apr 4];5(3):143–69. Available from: <https://www.tandfonline.com/doi/abs/10.1076/oep531438364>
3. Park J, Yang SC, Choi HY. Epidemiology and Clinical Patterns of Ocular Trauma at a Level 1 Trauma Center in Korea. *J Korean Med Sci*. 2021 Jan 4;36(1).
4. Fujikawa A, Mohamed YH, Kinoshita H, Matsumoto M, Uematsu M, Tsuiki E, et al. Visual outcomes and prognostic factors in open-globe injuries. *BMC Ophthalmol*. 2018 Jun 8;18(1).
5. Dessislava Stateva CBBPKS. Epidemiological study of eye injury in Pleven, Bulgaria. [cited 2023 Jan 24]; Available from: <https://citeseerx.ist.psu.edu/document?repid=rep1&type=pdf&doi=9dae516308eebf3574cbb54f322836318cbb20d8>
6. Thylefors B. Epidemiological patterns of ocular trauma. Vol. 20, *Australian and New Zealand Journal of Ophthalmology*. 1992. p. 95–8.
7. Turgut F, Bograd A, Jeltsch B, Weber A, Schwarzer P, Ciotu IM, et al. Occurrence and outcome of firework-related ocular injuries in Switzerland: A descriptive retrospective study. *BMC Ophthalmol*. 2022 Dec 1;22(1).
8. Shiuey EJ, Kolomeyer AM, Kolomeyer NN. Assessment of Firework-Related Ocular Injury in the US. *JAMA Ophthalmol*. 2020 Jun 1;138(6):618–29.
9. Chan WC, Knox FA, McGinnity FG, Sharkey JA. Serious eye and adnexal injuries from fireworks in Northern Ireland before and after lifting of the firework ban- an ophthalmology unit's experience. *Int Ophthalmol* [Internet]. 2004 May 1 [cited 2023 Apr 9];25(3):167–9. Available from: <https://link.springer.com/article/10.1007/s10792-004-1958-z>

Corresponding author

Sinan Aptikadir
Department of Ophthalmology
Medical University of Plovdiv
sinan.aptikadir@mu-plovdiv.bg
Tel. 0876628802

Arteriovenous Malformation Associated with Intracerebral Haematoma and Subarachnoid Hemorrhage: a Case Report

Ivan Ivanov¹, Ivo Kehayov^{2,3}, Petar-Preslav Petrov⁴, Plamen Penchev⁵

1. Faculty of Medicine, Medical University of Varna

2. Department of Neurosurgery, Faculty of Medicine, Medical University of Plovdiv

3. Neurosurgery Clinic, University Hospital "St. George", Plovdiv

4. Department of Anatomy, Histology and Embryology, Faculty of Medicine, Medical University of Plovdiv

5. Faculty of Medicine, Medical University of Plovdiv

Introduction: Brain arteriovenous malformations (AVM) are type of intracranial high-flow vascular lesions which are a nidus of enlarged feeding blood vessels, associated with the brain parenchyma. These lesions occur approximately in 4% of the population but become symptomatic in only 12% of affected individuals. Cerebral arteriovenous malformations are clinically presented with hemorrhages, seizures and headaches. **Aims:** This case report aims to present a patient with an arteriovenous malformation and to evaluate the postoperative clinical outcome. **Materials and methods:** We present a case report of a 26-years-old female patient who has entered the Neurosurgery clinic of "Saint George" University Hospital with clinical manifestation of headache, vomiting and tinnitus. CT scan has discovered intracerebral hematoma and subarachnoid hemorrhage in the right frontal lobe with perifocal edema and mass effect. **Results and discussion:** Under general anesthesia, an operative treatment was performed with a right frontal parasagittal craniotomy. The arteriovenous malformation was removed by microsurgical technique. Subcortical and medial to the malformation an intracerebral hematoma was discovered and was aspirated. Postoperatively surgery-related complications were not observed. The patient was verticalized on the day after intervention and discharged on the 5th day. **Conclusion:** Arteriovenous malformations are dangerous vascular lesions which can be fatal. Proper imaging and timely diagnosis can prevent further complications. Surgical treatment is the preferred method for treating these lesions.

Keywords: AVM, intracerebral hematoma, cerebrovascular lesion, subarachnoid haemorrhage.

Introduction

Brain arteriovenous malformations (bAVMs) are rare lesions, which are presented as directly connected arterial and venous blood vessels, without a capillary network in between. This results in a haemodynamic imbalance, occurring in the nidus and leading to dilation of the draining veins or the arterial sector. [1] Pathological findings on the vessel wall could be interruptions of tunica media or of the internal elastic membrane, fibrosis of the vascular wall, carrying the risk of complications, such as haemorrhagic stroke. [1, 2] These lesions can be congenital (AVMs, localized on the cortex) or acquired (dural AVMs). Subpial AVMs are seen more often than dural AVMs. The diameter of an AVM can vary from a few millimeters (usually by dural AVMs and in some cases of subpial AVM with a nidus, localized on the cerebral cortex) to several

centimeters (often by subpial AVMs). The afferent arterial vessels to the nidus are usually branches of the external carotid, vertebral and basilar artery. [1] The main manifestations of bAVMs are headaches, seizures and life-threatening complications as subarachnoid haemorrhage (SAH). [2]

Case report

We present a case report of a 26-years-old female patient who has entered the Neurosurgery clinic of "Saint George" University Hospital with clinical manifestation of headache, vomiting and tinnitus. CT scan has discovered intracerebral haematoma and subarachnoid hemorrhage in the right frontal lobe with perifocal edema and mass effect (Figure 1).

Under general anesthesia, an operative treatment was performed with a right frontal parasagittal craniotomy. The arteriovenous

malformation was removed by microsurgical technique. Subcortical and medial to the malformation an intracerebral haematoma was discovered and was aspirated (Figure 2).



Figure 1. CT scan, visualizing an intracerebral haemorrhage with edema

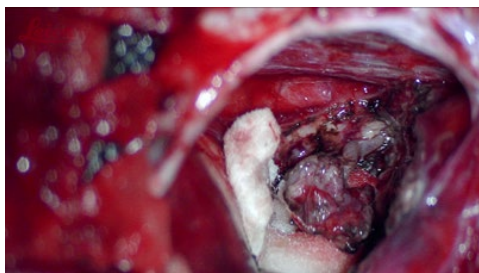


Figure 2. Intraoperative view of the AVM

Postoperatively surgery-related complications were not observed. The patient was verticalized on the day after intervention and discharged on the 5th day (Figure 3).

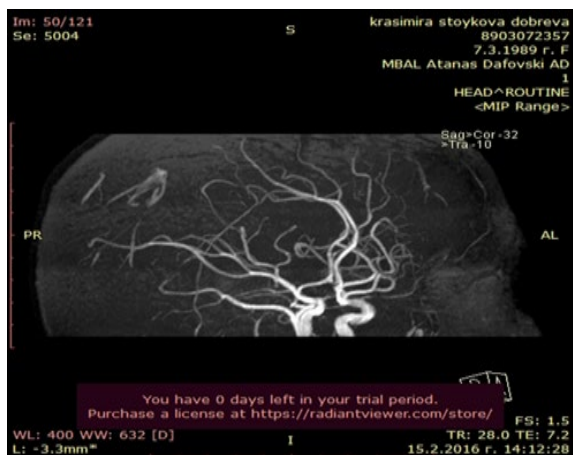


Figure 3. Postoperative MR angiography

Discussion

The prevalence of bAVMs is between 10 and 15/100 000 inhabitants and the incidence is around 1.3 new cases/100 000/year. Dural AVMs are discovered by only around 10–15% of the patients and occur due to venous hypertension, cerebral phlebitis and events, potentially leading to venous thrombosis. [1] Patients with subarachnoid haemorrhage (SAH) experience the so called “thunderclap headache” with severe intensity of the pain. The fact, that SAH is often misdiagnosed as migraine, puts the emphasis on the importance of properly taken status and laboratory testing – SAH is characterized with neck pain and stiffness, severe headache and leukocytosis (not common for migraine). Non-contrast CT is preferred for imaging of SAH, because of the high sensitivity for intracranial bleed. [2, 3] Risk factors for rupture of an AVM are smaller AVM size, young age and deep localization. [5] Pregnancy is also reported as a risk factor for rupture of an AVM, because of the increased cardiac output. [6] Gold standard for the diagnosis of AVMs is the cerebral angiography. Other imaging techniques that can be used are CT and MRI. [2] The differential diagnoses of cerebral AVMs include intracranial aneurysms, stroke, migraine, cluster headaches and carotid/vertebral artery dissection. [4] The surgical treatment of AVMs includes microsurgery, endovascular embolization and radiosurgery. Several trials compare the outcomes from conservative and surgical treatment of AVMs (ARUBA). Some of them show better results from the conservative treatment in preventing stroke and death over certain follow-up periods, and others showed that microsurgery is safe and effective for low-grade AVMs; radiosurgery is appropriate for deeply localized lesions; and endovascular embolization is performed to reduce blood flow to an AVM before microsurgery. [5]

Conclusion

In many cases, patients with undiscovered AVMs are without any clinical manifestation but carry a high risk of life-threatening acute haemorrhage. [6] Technical improvements nowadays give us the opportunity to treat AVMs with significantly lowered risk of follow-up seizures or fatal outcome, compared to the past. [7]

Acknowledgements

The authors have no support to report.

Funding

The authors have no funding to report.

Competing interests

The authors have declared that no competing interests exist.

Consent

Written informed consent was obtained from the patient for publication of this case report and accompanying images.

References

1. X. Barreau, G. Marnat, F. Gariel, V. Dousset, Intracranial arteriovenous malformations, *Diagnostic and Interventional Imaging*, Volume 95, Issue 12, 2014, Pages 1175-1186, ISSN 2211-5684, DOI: <https://doi.org/10.1016/j.diii.2014.10.004>.

2. Smith, John. (2019). Subarachnoid Hemorrhage due to a Ruptured Arteriovenous Malformation. *Clinical Medical Image Library*. DOI: 5. 130. 10.23937/2474-3682/1510130.

3. Al Rasyid, Taufik Mesiano, Mohammad Kurniawan, Rakhmad Hidayat, Rahmad Mulyadi, Setyo Widi Nugroho, Sophie Yolanda, Elvan Wiyarta, Salim Harris, Spontaneous subarachnoid hemorrhage due to arteriovenous malformation mimicking migraine: A case report, *Radiology Case Reports*, Volume 17, Issue 3, 2022, Pages 790-793, ISSN 1930-0433, DOI: <https://doi.org/10.1016/j.radcr.2021.12.015>.

4. Bokhari MR, Bokhari SRA. Arteriovenous Malformation Of The Brain. [Updated 2022 Sep 6]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2023 Jan-. Accessed: 17.04.2023 Available from:

<https://www.ncbi.nlm.nih.gov/books/NBK430744/>

5. Makiko Oomori, Sayaka Ito, Kazushi Higuchi. Fatal ruptured occult arteriovenous malformation in a young adult: An autopsy case report. 01-Jul-2022;13:284

6. English LA, Mulvey DC. Ruptured arteriovenous malformation and subarachnoid hemorrhage during emergent cesarean delivery: a case report. *AANA J*. 2004 Dec;72(6):423-6. PMID: 15633365.

7. Guidetti B, Delitala A. Intracranial arteriovenous malformations. Conservative and surgical treatment. *J Neurosurg*. 1980 Aug;53(2):149-52. doi: 10.3171/jns.1980.53.2.0149. PMID: 6159458.

Corresponding author

Ivan Tsvetanov Ivanov
Medical University of Varna
55 "Marin Drinov" str.
Tel: 088 939 0009
e-mail: iivanov09022001@gmail.com

Flesh-eating Disease – a Case Report

Velizar Hadzhiminev¹, Georgi E. Markov¹, Aleksandar Sarpanov², Krasi Kalacheva³,
Atanas S. Ivanov⁴

1. Department of Propedeutics of Surgical Diseases, Section of General Surgery, Faculty of Medicine, Medical University of Plovdiv; Clinic of General Surgery, UMHAT “St. George”, Plovdiv

2. Clinic of General Surgery, UMHAT “St. George”, Plovdiv

3. Department of Medical Oncology, MHAT “Central Onco Hospital”, Plovdiv

4. Department of Urology and General Medicine, Faculty of Medicine, Medical University of Plovdiv; Clinic of Urology, UMHAT “St. George”, Plovdiv

Necrotizing fasciitis (NF) also known as “flesh-eating disease” is aggressive and life-threatening infection that causes necrosis of muscle fascia and subcutaneous tissues. It is a result of bacterial infection introduced through a break in the skin’s integrity. Key concepts for treatment of NF are early diagnosis, appropriate empiric antibacterial coverage, adequate control of infection sources with aggressive surgical interventions. A 65-years old female patient with no comorbidities and no previous surgeries was admitted to the Department of General surgery with clinical and imaging findings of NF of the right abdominal flank, right ureterolithiasis with hydronephrosis. A locally advanced right flexure colon cancer with fistulisation into the retroperitoneum was found as a source of the infection. The patient underwent several surgeries including necrectomies, right lumbotomy with extraction of the concrement, right hemicolectomy with ileostomy, skin-graft plastic reconstruction. Wound management was performed by using proteolysis enzyme therapy, debridement and daily dressing change.

Key words: necrotizing fasciitis, flesh-eating disease, soft tissue infection, skin necrosis

Introduction

Necrotizing fasciitis (NF) also known as “flesh-eating disease” is aggressive and life-threatening infection that causes necrosis of muscle fascia and subcutaneous tissues. This infection typically spreads rapidly along the fascial plane, which has a poor blood supply, leaving the overlying tissues initially unaffected, potentially delaying diagnosis and surgical intervention. The infectious process usually affects the limbs, perianal area and the trunk. The majority of patients have history of diabetes, alcoholism, liver cirrhosis or previous oncologic disease. NF can occur post-surgery or any invasive procedure. [1, 2]

Necrotizing fasciitis is a result of bacterial infection introduced through a break in the skin’s integrity in approximately 80% of all cases. Gram-positive strains of *Staphylococcus aureus* and *Streptococci* are responsible for the majority single-site source infections. Polymicrobial infections occur as well because of a combination of gram-negative (*Escherichia coli*, *Pseudomonas* spp., and

Klebsiella spp.) and anaerobic involvement (*Clostridium perfringens* “gas gangrene”) and anaerobic streptococcus. [3]

Necrotizing fasciitis affects about 0.4 in every 100,000 people per year in the United States. In some areas of the world, it is as common as one in every 100,000 people. [4]

NF is more commonly presented with excruciating pain and systemic septic signs than non-necrotizing infections. Physical findings may include tenderness to palpation beyond the erythematous border, crepitus, and cellulitis. The presence of bullae, ecchymotic changes to the skin, and dysesthesia or paresthesia should also be treated as a necrotizing infection. Subcutaneous emphysema and crepitus are almost always present. The infection can spread rapidly within hours hence suspicion should be high for necrotizing fasciitis in the presence of intense pain. [5, 6]

Key concepts for treatment of NF are early diagnosis, appropriate empiric (wide-spectrum) antibacterial coverage, adequate control of infection sources such as

aggressive surgical intervention for abscess drainage and debridement of necrotizing soft tissue infections, identification of infection-causing pathogen and applicable adjustment of the antibacterial therapy. [7]

Case report

A 65-years old female patient with no comorbidities and no previous surgeries was admitted to the Department of General surgery of University Hospital “St. George” with complaints of pain, swelling and redness of the skin with necrosis and blistering located in the right abdominal flank. She did not informed about nausea, vomiting, constipation or urination problems. Physical exam revealed fever up to 38.5 degrees, pale skin, dry and coated tongue, tachycardia – 120 beats/min, blood pressure 100/70 mm Hg; distended abdomen. The local status was following: skin necrosis with bullae, hyperemia, oedema and crepitations engaging the right abdominal flank along the projection of the right ureter (Figure 1). Relevant laboratory findings at the time of hospitalization were following: HGB – 86 g/l; RBC – 3.29; WBC – 36.7; PLT – 718; glucose – 5.6 mmol/l; CRP – 417 mg/l; t. protein – 63 g/l; PT – 42.7%. X-ray of the abdomen revealed air-collection in the soft tissues of the right abdominal flank and a concrement (2/1 cm) in the right ureter (Figure 2). Ultrasonography findings were right-sided hydronephrosis and ureterolithiasis and subcutaneous emphysema. CT scan of the abdomen was performed. It revealed soft-tissue infection with subcutaneous emphysema of the flank and anterior abdominal wall, right-sided ureterolithiasis with hydronephrosis, intact right and left paranephral spaces and intrabdominal organs. Chest X-ray of the patient revealed a tumor formation (4/4 cm) in the inferior lobe of the right lung. Our

therapeutic approach and management, presented chronologically were following:

DAY 1: Preoperative preparation – fluid resuscitation, blood and plasma transfusion, empiric antibacterial coverage with Meropenem 3 x 1 g/24 h; Metronidazol 3 x 500 mg; Surgical debridement with wide incisions and necrectomies were performed (Figure 3). Percutaneous nephrostomy was performed by urologist due to right-sided hydronephrosis;

DAY 3: Revision of the wounds and necrectomies; The patient was consulted with urologist. Urinary infection with perinephric abscess were discussed as possible source of soft-tissue infection.

DAY 4: Right-sided lumbotomy and stone-extraction from the right ureter were performed by urologist. A locally advanced right flexure colon carcinoma with fistulisation in the retroperitoneal and right perinephric space was discovered. Midline laparotomy with right hemicolectomy and ileostomy were performed followed by surgical debridement of the wounds.

DAY 7: Revision of the wounds and necrectomies (Figure 4).

For daily management of the wounds we used proteolysis enzyme therapy. *Escherichia coli* were isolated from the wound. The histology from the colon tumor revealed low differentiated adenocarcinoma. The postoperative stage of the patient was pT4 Nx M1, G3. The patient was consulted with oncologist and plastic surgeon.

DAY 26: She was transferred to the Department of Plastic and reconstructive surgery for skin-grafting (Figure 5).

DAY 50: After successful skin-graft surgeries the patient was discharged and transferred to the Department of Medical oncology for further chemotherapy (Figure 6).



Figure 1. Necrotizing fasciitis – skin necrosis with bullae, hyperemia, oedema engaging the right abdominal flank



Figure 2. X-ray of the abdomen – air-collection in the soft tissues of the right abdominal flank and a concrement in the right ureter.



Figure 3. *Day 1* – Surgical debridement with wide incisions and necrectomies



Figure 4. *Day 7* – Results after lumbotomy, right hemicolectomy and daily wound management



Figure 5. *Day 26* – Wound status (fresh granulation tissue) after multiple surgical debridements and proteolysis enzyme therapy



Figure 6. *Day 50* – Postoperative result after skin-graft surgery

Discussion

NF is an infection of the deep soft tissues causing progressive destruction of the muscle fascia and overlying subcutaneous fat. Two major classifications of NF are generally accepted and several new classifications have been proposed to create further subdivisions. The following classifications are based on the underlying bacteria that initiated the cascade of injury:

*Polymicrobial (type I) NF. Type I NF stems from polymicrobial infection identified via microbiological culture. This type of infection is caused by both aerobic and anaerobic bacteria. The complex microbiological profile of offending organisms leads to gaseous infiltration of subcutaneous tissue similar to gas gangrene. Type I NF accounts for most reported cases of NF and is more prevalent in older adults with chronic diseases.

*Monomicrobial (type II) NF is most commonly associated with Gram-positive organisms such as group A *Streptococcus* (GAS) and methicillin-resistant *Staphylococcus aureus* (MRSA). Endotoxins released by type II NF organisms are responsible for some clinical presentations, including toxic shock syndrome. Type II NF is not associated with a specific age group. Some patients do not have comorbidities or an obvious portal of entry that predisposes them to severe infection. [8–11]

No specific lab studies have been proven to be reliable for the diagnosis of NF. Wong and colleagues developed the Laboratory Risk Indicator for Necrotizing Fasciitis (LRINEC) to help clinicians screen for NF using serum levels of the total white blood cell count, hemoglobin, sodium, glucose, creatinine, and C-reactive protein. LRINEC scores of 6 or more were thought to be associated with NF. However, in subsequent

reports LRINEC scores failed to accurately predict NF. [4], [12]

Computed tomography (CT) and MRI are imaging methods of choice. They may show edema extending along the fascial plane, although these findings may be absent in early stages of NF. Surgical exploration is the only way to establish the diagnosis of necrotizing infection.[13] Surgical debridement is the mainstay of treatment of NF and results in significantly improved mortality compared to cases in which surgery is delayed for even a few hours. When NF is suspected, patients should be brought to an operating room as soon as possible for a “search and destroy” mission of aggressive and extensive debridement. Involved tissues should be resected thoroughly until there is no further evidence of infection. Initial surgery is the most important determinant for survival and the wound must be inspected closely after the initial debridement. A “second-look” surgery is typically done 12 to 24 hours after the initial debridement. Patients with NF may require anywhere from five to 40 sessions of surgery, and one study found an average of 33 debridements and grafting procedures. Once the infection is controlled, daily dressing is needed at the bedside under sedation, followed by secondary suturing of the wounds with or without split skin grafts to cover the exposed underlying tissues. [5], [10], [14]

The mortality of NF has been reported in recent studies to be approximately 25% but it is higher in patients with toxic shock syndrome, advanced age (> 65 years old), hypotension, leucopenia, bacteremia and may reach 67%. [9], [15]

We presented an interesting case of severe form of necrotizing fasciitis. The underlying cause was oncologic disease – locally advanced colon cancer with lung metastasis. We kept to the major principles in diagnosis

and treatment of NF: timely clinical, laboratory and imaging diagnosis; aggressive surgical debridement, broad-spectrum antimicrobial coverage, “second-look” surgeries, definitive treatment of the main source of the infection, adequate wound-care management; skin-graft reconstruction. We had an excellent outcome in our patient regarding the surgical pathology.

Conclusion

Necrotizing fasciitis remains disease with high morbidity and mortality, despite improvements in care. Patients with NF must be identified early, treated expeditiously, and supported with the best available critical care. Timely diagnosis, multidisciplinary approach and supportive therapies, including antimicrobials and timely referral to surgery are crucial to improve patient outcomes.

References

1. Heijkoop B, Parker N, Spornat D. Fournier's gangrene: not as lethal as previously thought? A case series. *ANZ J Surg*. 2019 Apr;89(4):350-352.
2. Erichsen Andersson A, Egerod I, Knudsen VE, Fagerdahl AM. Signs, symptoms and diagnosis of necrotizing fasciitis experienced by survivors and family: a qualitative Nordic multi-center study. *BMC Infect Dis*. 2018 Aug 28;18(1):429.
3. Khalid M, Junejo S, Mir F. Invasive Community Acquired Methicillin-Resistant Staphylococcal Aureus (CA-MRSA) Infections in Children. *J Coll Physicians Surg Pak*. 2018 Sep;28(9):S174-S177.
4. Fernando SM, Tran A, Cheng W, Rochweg B, Kyeremanteng K, Seely AJE, Inaba K, Perry JJ. Necrotizing Soft Tissue Infection: Diagnostic Accuracy of Physical Examination, Imaging, and LRINEC Score: A Systematic Review and Meta-Analysis. *Ann Surg*. 2019 Jan;269(1):58-65.
5. Bonne SL, Kadri SS. Evaluation and Management of Necrotizing Soft Tissue Infections. *Infect Dis Clin North Am*. 2017 Sep;31(3):497-511.
6. Semenič D, Kolar P. Fournier's Gangrene Does Not Spare Young Adults. *Wounds*. 2018 Jul;30(7):E73-E76.
7. Baraket O, Triki W, Ayed K, Hmida SB, Lahmidi MA, Baccar A, Bouchoucha S. [Therapeutic factors affecting the healing process in patients with gangrene of the perineum]. *Pan Afr Med J*. 2018;29:70.
8. Stevens DL, Baddour LM. Necrotizing soft tissue infections. UpToDate. 2020. www.uptodate.com.
9. Jabbour G, El-Menyar A, Peralta R, et al. Pattern and predictors of mortality in necrotizing fasciitis patients in a single tertiary hospital. *World J Emerg Surg*. 2016;11:40. [PubMed: 27508002]
10. Stevens DL, Bryant AE. Necrotizing soft-tissue infections. *N Engl J Med*. 2017;377(23):2253–2265. [PubMed: 29211672]
11. Shiroff AM, Herlitz GN, Gracias VH. Necrotizing soft tissue infections. *J Intensive Care Med*. 2014; 29(3):138–144. [PubMed: 23753218]
12. Wong C-H, Khin L-W, Heng K-S, Tan K-C, Low C-O. The LRINEC (Laboratory Risk Indicator for Necrotizing Fasciitis) score: a tool for distinguishing necrotizing fasciitis from other soft tissue infections. *Crit Care Med*. 2004;32(7): 1535–1541. [PubMed: 15241098]
13. Stevens DL, Bisno AL, Chambers HF, et al. Practice guidelines for the diagnosis and management of skin and soft tissue infections: 2014 update by the Infectious Diseases Society of America. *Clin Infect Dis*. 2014;59(2):e10–e52. [PubMed: 24973422]
14. File TM Jr, Tan JS, DiPersio JR. Group A streptococcal necrotizing fasciitis. Diagnosing and treating the “flesh-eating bacteria syndrome”. *Cleve Clin J Med* 65: 241-249, 1998.
15. Elliott DC, Kufera JA, Myers RA. Necrotizing soft tissue infections. Risk factors for mortality and strategies for management. *Ann Surg* 224: 672-683, 1996.

