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Review

A Comparison of Surgical Treatments for Tertiary Hyperparathyroidism. A Systematic Review

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Abstract

Introduction: Tertiary hyperparathyroidism develops in patients who have secondary hyperparathyroidism that persists despite successful kidney transplantation or in patients who are on chronic dialysis.

Aim: This study aims to present a comparison of surgical treatments of tertiary hyperparathyroidism.

Materials and methods: A systematic review of studies published in English that reported on the surgical management of tertiary hyperparathyroidism was conducted using PubMed databases in accordance with the PRISMA guidelines. Two authors independently reviewed the full text of potentially selectable articles and selected appropriate studies. Surgical treatment options were evaluated.

Results: This review contains thirteen relevant studies. The treatments recommended by the studies included limited parathyroidectomy, subtotal parathyroidectomy, total parathyroidectomy with autotransplantation, and total parathyroidectomy without autotransplantation. The choice of the appropriate surgical technique demands individualization of the treatment and depends mainly on the experience of the surgeon.

Conclusion: The predominant treatment options appear to be subtotal parathyroidectomy and total parathyroidectomy with autotransplantation.

Keywords

autotransplantation, chronic kidney disease, parathyroid hormone, parathyroidectomy, tertiary hyperthyroidism

INTRODUCTION

Patients with long-standing chronic kidney disease (CKD) develop elevated serum parathyroid hormone (PTH) concentrations, which are frequently accompanied by hypercalcemia and cannot be explained by calcium carbonate or calcitriol supplements.[1,2] This condition is defined as tertiary hyperparathyroidism and occurs in patients with secondary hyperparathyroidism that persists even after a successful kidney transplant or in patients who are on chronic dialysis.[1,2] The chronic kidney disease is associated with hyperphosphatemia, calcitriol deficiency, and hypocalcemia. These metabolic disturbances lead to prolonged parathyroid cell stimulation and nodular hyperplasia of the parathyroid gland. Hyperplastic parathyroid glands become autonomous, resulting in PTH hypersecretion. In

this stage, even elevated serum calcium levels cannot prevent the secretion of parathyroid hormone. Indications for treatment are persistent hypercalcemia and/or increased parathyroid hormone levels.[2] As treatments with vitamin D or calcitriol offer low cure rates, usually fail, and are not indicated, the primary treatment is surgery.[2-4] Four are the surgical procedures that are usually performed: total parathyroidectomy (PTX) with autotransplantation, total parathyroidectomy without autotransplantation, subtotal parathyroidectomy, and limited parathyroidectomy.

The benefits of surgery may include improving survival and bone mineral density and alleviating the unpleasant and painful symptoms of tertiary hyperparathyroidism.[5] Because of the diversity of tertiary hyperparathyroidism and the specificities of chronic kidney disease patients, the indications for surgical treatment, the selection of appropriate surgical treatment, and the potential complications that may arise are all subjects of investigation.

AIM

This study aims to present a comparison of the surgical treatments of tertiary hyperparathyroidism.

MATERIALS AND METHODS

This systematic review was conducted using the Preferred Reporting Items for Systematic Review & Meta-Analyses (PRISMA) guidelines. The search was performed using PubMed databases up to 1988. The following Medical Subject Headings terms and keywords were used: "tertiary parathyroidism" and "surgery". The following MeSH terms were used: parathyroidism, chronic kidney diseases, and surgery. After the search, titles and abstracts were screened. Two authors reviewed the full text of potentially selectable articles independently and appropriate studies were selected. The first selection was performed based on the title and abstract. Afterwards, the whole text was reviewed. A study was included when it was a retrospective cohort study that suggested a surgical approach for tertiary hyperparathyroidism. The following criteria were used to exclude studies from consideration: 1) whether it was a letter, review, case report, conference abstract, remark, or discussion; 2) whether surgical treatment was not recommended; 3) studies that were not published in English; 4) studies for which it was not possible to obtain the complete study text online or through a request to the authors. The data extracted from each included publication were first author, publication year, study design, and operations performed.

RESULTS

The literature search yielded over 828 articles. 718 articles were removed before screening by automation tools. After screening the titles and abstracts, 13 articles remained for full-text review. After the review, 13 articles were suitable for qualitative synthesis.

Fig. 1 shows the PRISMA flowchart of the search and selection process and **Table 1** summarizes the final list of included studies. In total, 1705 patients were included in 13 studies.

Subtotal parathyroidectomy was suggested by six studies. In total, it concerned 380 patients. Total parathyroidectomy with autotransplantation was proposed by four studies which included 1112 patients. Total parathyroidectomy without autotransplantation appeared in two studies in which 130 patients participated. Finally, limited parathyroidectomy was supported only by one study with 83 patients.

DISCUSSION

The ideal surgical intervention for the treatment of tertiary hyperparathyroidism has not been established. The adequate surgical treatment of tertiary hyperthyroidism should aim for an appropriate balance between the method of resection, control of recurrences, and prevention of persistent postoperative hypoparathyroidism.[11] Surgical complications are rare, and parathyroidectomy appears to be a safe and feasible treatment option for tertiary hyperparathyroidism. Parathyroidectomy increases bone mineral density and leads to a reduced risk of major cardiovascular events and death compared with conservative treatment.[19]

Confined published studies compare surgical techniques. In their majority, they suggest that a limited or focused PTX should be avoided as it continues to be unclear and controversial. Although, limited resections are recommended because of their high success rates and minimum complications compared to more extensive surgeries, in patients with chronic kidney disease[14] and kidney transplantation, renal graft function deteriorates[2]. Moreover, in limited resections, hypocalcemia, although transient in the postoperative period, could also be detected.[2]

Some authors recommend subtotal PTX with simultaneous thymectomy, and others subtotal PTX without thymectomy. Subtotal parathyroidectomy with the identification of all parathyroids (even supernumerary or ectopic ones) is recognized as a safe and effective method for tertiary hyperparathyroidism. This operation is associated with an acceptably low recurrence rate, long-term correction of hypercalcemia, and rehabilitation of bone disease over extremely long follow-up periods. Furthermore, studies show that the risk of permanent hypoparathyroidism is less likely with subtotal parathyroidectomy. Additionally, subtotal parathyroidectomy does not impair renal graft function and provides long-term correction of hypercalcemia and tertiary hyperparathyroidism. For all these reasons, subtotal parathyroidectomy seems to be preferred in most studies **(Table 1).**[7,10,11,13,17,18]

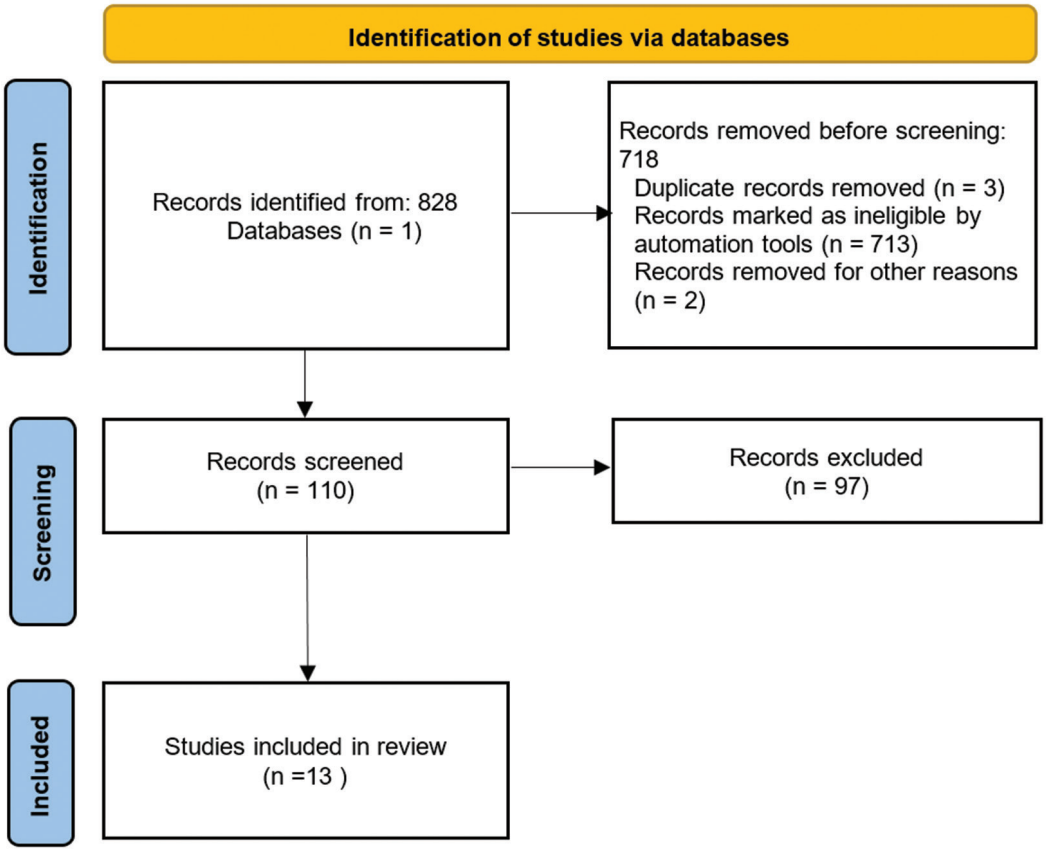

Figure 1. PRISMA 2020 flow diagram of the search and selection process.

Table 1. The results of the search for ideal surgical treatment of tertiary hyperparathyroidism. The names of the first author, the year of publication, the number of patients who participated in each study and underwent parathyroidectomy, and the recommended procedure by each article are listed.[6-18]

Study	Year	Number of patients with tHPT included in the respective study	Subtotal PTX	Total PTX with autotransplantation	Total PTX without autotransplantation	Limited PTX
Alexander PT et al.[6]	1988	20		+		
Punch JD et al.[7]	1995	91	+			
Wheatley TJ et al.[8]	1997	15			+	
Tominaga Y et al.[9]	2001	1053		+		
Triponez F et al.[10]	2005	70	+			
Schlosser K et al.[11]	2007	69	+			
Coulston JE et al.[12]	2010	115			+	
Park JH et al.[13]	2011	15	+			
Jäger MD et al.[14]	2011	83				+
Robin-Lersundi A et al.[15]	2012	13		+		
Sadideen HM et al.[16]	2012	26		+		
Gawrychowski J et al.[17]	2015	30	+			
Choi HR et al.[18]	2021	105	+			
Total number of studies: 13		Total number of patients: 1705				

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Total parathyroidectomy appears to be a safe and effective method for the treatment of hyperparathyroidism in CKD patients who have been transplanted or who are awaiting renal transplantation.[8,12] In renal failure patients who have been transplanted or who are awaiting renal transplantation, a total parathyroidectomy frequently leaves functioning parathyroid tissue behind.[8] Rates of persistent hypoparathyroidism after total parathyroidectomy without autotransplantation have been reported to be low and it appears to be protective against disease recurrence.[8,12] Subsequently, if it is performed early in the course of the disease, total parathyroidectomy is associated with long-term patient survival, based on long-term follow-up of patients population with chronic kidney disease.[8,12] Nevertheless, this surgical procedure is suggested by fewer studies compared to the other surgical methods.

Total parathyroidectomy with autograft has proven to be a satisfactory and commonly performed procedure. PTX should be combined with forearm autotransplantation for easier management of possible recurrence and immediate normalization of serum calcium, phosphorus, and parathormone.[6,9,15,16] It has been proved that this access provides pleasant bone disease rehabilitation. The choice of gland to be transplanted into the forearm, as well as the transplantation procedure used, are critical factors in determining the outcome of the operation.[6,9,15,16] The morphology of the gland to be autotransplanted must be assessed both macroscopically and microscopically. The nodular appearance of the glands on histological examination should be taken into account as nodular hyperplasia is associated with increased rates of postoperative hypertrophy.[6,9,15,16] Thus, total parathyroidectomy with autotransplantation is an effective treatment for tertiary hyperparathyroidism but carries an increased risk of impaired renal graft function as decreased graft perfusion, particularly for patients who already have poor renal function at the time of surgery. Moreover, the chance of complications and reoperation rates are generally increased.[9,20-22] The high rate of recurrence could be attributed to the method's widespread use.

Renal function after parathyroidectomy for tertiary hyperparathyroidism appears to decline transiently or permanently.[2,5,11] The existing literature shows either, no effect of parathyroidectomy on overall renal graft survival. In contrast, others show that subtotal or total parathyroidectomy, based on long-term follow-up of this population of patients with chronic kidney disease, is associated with long-term patient survival if performed early.[2,5,11] The renal function decrease could happen either due to parathyroidectomy or the graft's chronic rejection. This fact can only be determined by controlled, prospective studies investigating the long-term consequences of parathyroidectomies on renal function in patients with tertiary hyperparathyroidism. Currently, there are no such studies.

An accurate and careful parathyroidectomy guided by intraoperative parathyroid hormone measurement should be considered the best surgical option for the definitive treatment of tertiary hyperparathyroidism, avoiding recurrences and ruling out the presence of supernumerary glands. Rapid biopsy could be performed as an adjuvant treatment. The choice of appropriate surgical management strongly depends on the endocrine surgeon's experience which plays a decisive role in the outcome of the operation. Due to the specificity of nephrological patients, the individualization of treatment seems imperative.

CONCLUSION

In conclusion, it appears that subtotal parathyroidectomy and total parathyroidectomy with autotransplantation are safe and efficient methods for the surgical treatment of tertiary hyperparathyroidism. Although a total parathyroidectomy with autotransplantation is the most common method, subtotal parathyroidectomy seems to be a method without complications, without an extremely high occurrence of hypoparathyroidism and it is characterized by good prognosis and survival in transplant patients.

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Competing Interests

The authors have declared that no competing interests exist.

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Сравнение хирургических методов лечения третичного гиперпаратиреоза. Систематический обзор

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Резюме

Введение: Третичный гиперпаратиреоз развивается у пациентов со вторичным гиперпаратиреозом, который сохраняется, несмотря на успешную трансплантацию почки, или у пациентов, находящихся на хроническом диализе.

Цель: Целью данного исследования является представление сравнения хирургического лечения третичного гиперпаратиреоза.

Материалы и методы: Систематический обзор опубликованных на английском языке исследований, в которых сообщалось о хирургическом лечении третичного гиперпаратиреоза, был проведён с использованием баз данных PubMed в соответствии с рекомендациями PRISMA. Два автора независимо друг от друга рассмотрели полный текст статей, которые потенциально можно было бы выбрать, и выбрали соответствующие исследования. Были оценены варианты хирургического лечения.

Результаты: Этот обзор содержит тринадцать соответствующих исследований. Лечение, рекомендованное в исследованиях, включало ограниченную паратиреоидэктомию, субтотальную паратиреоидэктомию, тотальную паратиреоидэктомию с аутотрансплантацией и тотальную паратиреоидэктомию без аутотрансплантации. Выбор подходящей хирургической техники требует индивидуализации лечения и зависит главным образом от опыта хирурга.

Заключение: Преобладающими вариантами лечения являются субтотальная паратиреоидэктомия и тотальная паратиреоидэктомия с аутотрансплантацией.

Ключевые слова

аутотрансплантация, хроническая болезнь почек, паратироидный гормон, паратиреоидэктомия, третичный гиперпаратиреоз

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Review

Managing Polypharmacy through Medication Review Tools – Pros and Cons

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Abstract

Inappropriate polypharmacy is a common occurrence in elderly patients, resulting in increased adverse drug reactions, nonadherence, and increased healthcare costs. Medication review and deprescribing are the primary strategies described in the literature for dealing with problematic polypharmacy. To effectively carry out the medication review, various tools have been developed. These tools can support medication review in a variety of ways. Some tools include a list of medications requiring detailed attention, while others guide medical professionals with principles and algorithms for reviewing and prescribing medicines. A third category of tools focuses on tracking and identifying symptoms that may be due to drug-related problems.

This article aims to present the medication review support tools used in the management of polypharmacy in the geriatric population, emphasizing their advantages and disadvantages.

Keywords

elderly, medication review, pharmacist, polypharmacy, tools

INTRODUCTION

The prevalence of polypharmacy tends to rise with age, reflecting the increased likelihood of individuals having multiple health conditions and requiring various medications.[1] While polypharmacy can be necessary and beneficial for managing multiple health conditions, it presents challenges such as an increased risk of adverse drug reactions, drug interactions, and medication non-adherence.[2,3] Therefore, healthcare providers need to carefully assess the necessity of each medication and consider potential risks and benefits when managing patients with polypharmacy.[4] Patients with complex medical conditions often require specialized care from different healthcare providers, each prescribing medications to address specific aspects of the patient's health.[5] Effective management of polypharmacy requires a systematic approach involving a range of strategies.[6] **(Fig. 1).**

A medication review is a comprehensive assessment of an individual's medications by a healthcare professional.[7] A medication review evaluates the appropriateness, effectiveness, and safety of the medications a person is taking.[8] Key aspects of a medication review may include a Medication List where the healthcare provider will compile a complete and accurate list of all medications the individual is currently taking.[9] This includes prescription medications, over-the-counter medications, and supplements. Secondly, the provider will assess whether each medication is still necessary and whether the medical conditions for which they were prescribed are still relevant.[10] The healthcare professional will evaluate the effectiveness of each medical product. If a medication is not providing the necessary benefits, alternatives should be considered.[11]

The review includes an assessment of potential interactions between medications, as well as any interactions with food or other substances.[12] The healthcare provider will

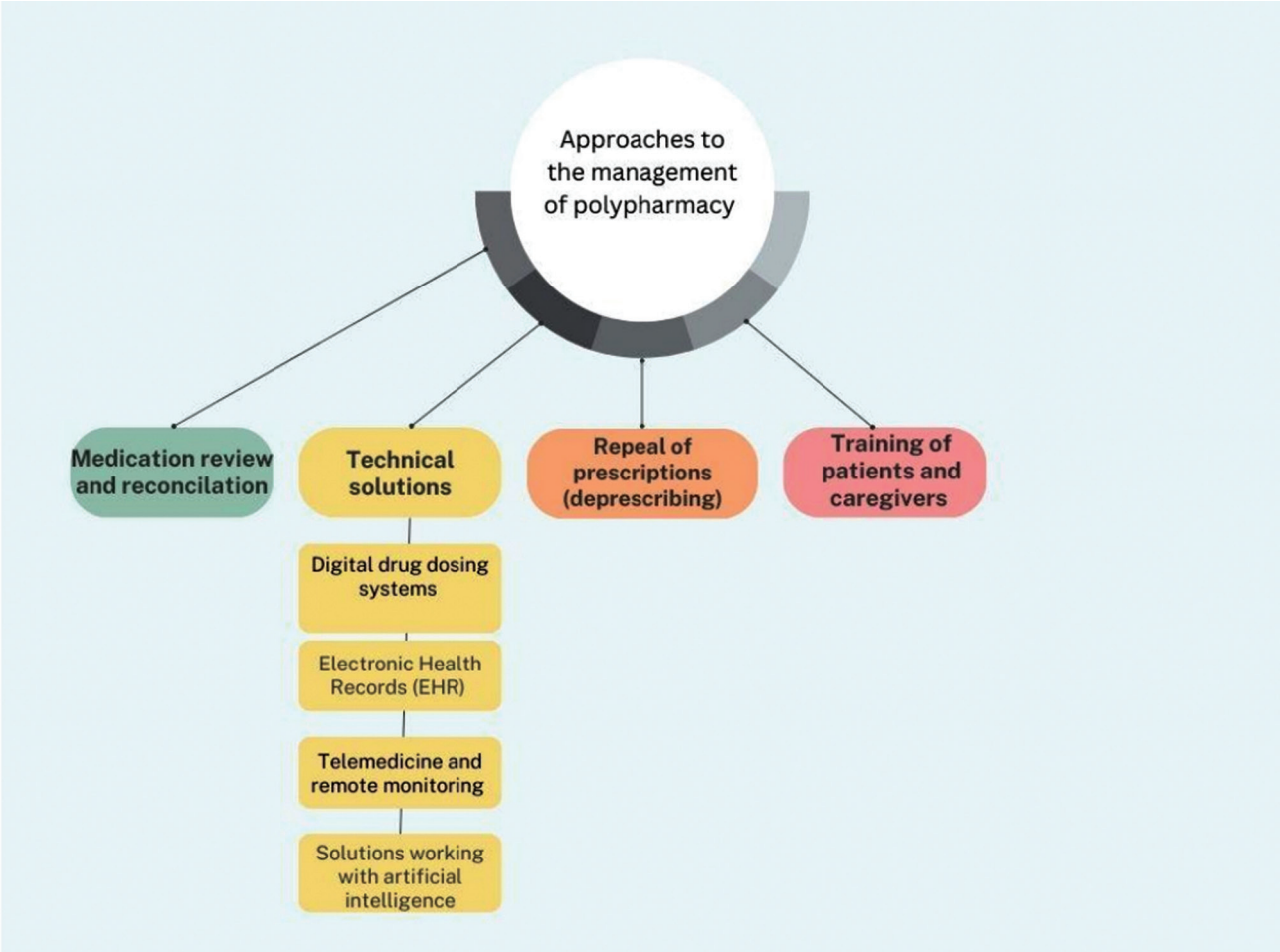

Figure 1. Approaches for managing polypharmacy (created via Canva.com).

inquire about and assess any side effects or adverse reactions experienced by the individual. If the side effects are significant, alternative medications may be considered.[13] The individual's preferences and lifestyle factors are considered. This includes factors such as ease of medication administration, cost, and any challenges the individual may face in adhering to the prescribed regimen.[14] The healthcare provider will work with the individual to develop or adjust a comprehensive treatment plan that optimally addresses their health conditions while minimizing risks and adverse effects.[8]

It is crucial for individuals to actively participate in the medication review process by providing accurate information about all medications, communicating any concerns or side effects, and discussing their preferences and goals with their healthcare providers.[10] Several screening tools are commonly used by healthcare professionals to conduct medication reviews.[15] These tools help identify potential issues related to medication use, including inappropriate prescribing, potential drug interactions, and medication-related problems.[16]

The aim of this narrative review is to outline the main tools recommended in the medication review approach, highlighting their pros and cons.

A comprehensive search of electronic databases (PubMed and Google Scholar), guidelines on polypharmacy management in geriatric patients, and official websites of the national competent authorities in countries implementing the medication review method was carried out. In this narrative review, the following search terms were used alone or in combination: "medication review", "tools", "polypharmacy", "geriatric patients", "multimorbidity", and "pharmacist". Only English-language results were considered for further analysis. In the context of using tools for medication review, we constructed a SWOT analysis. Additionally, a content analysis of available tools was performed, shedding light on the different approaches to medication review and their potential impact on patient care.

Medication review support tools

Medication review tools are often used with clinical judgment and patient input to conduct a comprehensive medication review.[17] They help healthcare providers identify areas for improvement in medication management, reduce the risk of adverse events, and optimize treatment plans.[18] The choice of tool may depend on the specific population being assessed and the goals of the medication review.

Medication review is a continuing process that involves multidisciplinary approaches and continued monitoring of the patient. One of the disadvantages of the more complex types of tools is that in practice the medical specialist does not have more than 10 minutes to conduct a medication review. A tool that provides consultation for 10 minutes is NO TEARS (Need/indication, Open questions, Tests, Evidence, Adverse effects, Risk reduction, Simplification/switches).[19]

As elderly patients are more vulnerable to polypharmacy and drug interactions, many tools have been developed for assessing the medication therapy of this specific patient group[11,16,17,20-35] **(Table 1).**

Table 1. Medication review support tools used in the geriatric population[20]

Tool	Scope
The American Geriatrics Society (AGS) Beers Criteria (the Beers list)[21]	List of medications that are either not appropriate or should be used with caution in adult patients. By focusing on the identification of potentially inappropriate medications and providing evidence-based recommendations, the criteria contribute to promoting safer and more effective prescribing practices for the elderly population.
Screening Tool of Older Persons' Prescriptions (STOPP) and Screening Tool to Alert to Right Treatment (START) STOPP/START criteria[17]	A series of rules and suggestions related to common problems in adult therapy. By offering specific criteria and recommendations, STOPP aims to enhance the quality of prescribing practices for older adults and reduce the risk of adverse drug events in this vulnerable population.
Need/indication, Open questions, Tests, Evidence, Adverse effects, Risk reduction, Simplification/switches. NO TEARS [22]	The structure of NO TEARS offers a means for conducting a swift medication review during a 10-minute consultation, enhancing efficiency. This adaptable system can be customized to align with the unique consultation style of each practitioner.
Drug Burden Index DBI[23]	A method for calculating an index for the risk of falls due to the intake of medications with a sedative and anticholinergic effect
Anticholinergic Cognitive Burden (ACB) scale / Anticholinergic Risk Scale (ARS)[24]	The primary scope of both ACB and ARS is to identify medications that have anticholinergic properties. Anticholinergic drugs block the action of acetylcholine, a neurotransmitter in the central and peripheral nervous systems, and their use has been associated with various side effects, including cognitive impairment.
PRISCUS list (Latin for "old and venerable")[25]	List of recommendations for specific medications developed in Germany. It contains a compilation of medications that are considered potentially inappropriate for use in older adults. The aim of the PRISCUS list is to improve medication safety and quality of care for elderly individuals by identifying and minimizing the use of medications that may pose a higher risk of adverse effects or have limited efficacy in this population.
Medication Appropriateness Index (MAI)[11]	Method for measuring potentially inappropriate prescribing by index for appropriate medications. It is the only implicit tool with validated inter-rater reliability.
Australian prescribing indicators tool[26]	Contains 41 criteria to assess the relationship between medication intake and the most common drug-related problems in Australian adult patients.
NOR-FRAIL tool (Fatigue, Resistance, Aerobic capacity, Illnesses and Loss of weight)[27]	Identifies frailty in older people, including medication-related aspects.
Brown Bag Medication Review[28]	Visual inspection of all medications the patient is taking. Helps identify any discrepancies, duplicate therapies, or potential medication use problems.
Healthcare Effectiveness Data and Information Set HEDIS[29]	Used by health plans to measure performance on various aspects of care, including appropriate medication use. HEDIS encompasses over 90 metrics distributed across six care domains: Efficiency of Care. Accessibility and Availability of Care. Quality of Care Experience. Utilization and Risk-Adjusted Utilization. Descriptive Information about Health Plans. Metrics Reported Utilizing Electronic Clinical Data Systems.

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MedStopper[30]	Deprescribing tool used in Canada. It organizes a patient's medication list, prioritizing drugs from "more likely to discontinue" to "less likely to discontinue." This sequencing is determined by three crucial factors: the drug's potential to alleviate symptoms, its ability to mitigate future health risks, and its likelihood of causing harm.
RxISK Polypharmacy Index[31]	RxISK, established in 2012, is a freely accessible and independent online platform designed to empower individuals to engage in more informed discussions with their healthcare providers about medications. It acknowledges that the person taking a drug holds valuable insights into its effects, emphasizing the importance of individuals actively participating in conversations about their medications with their doctors.
FORTA (Fit for the aged)[30]	An internationally validated tool for managing pharmacotherapy in older adults. The tool combines both negative and positive labelling based on individual indications. It ranks medications into four groups depending on evidence for safety, efficacy, and overall age appropriateness: (**A**) indispensable with obvious benefit; (**B**) proven efficacy but limited effects or possible safety concerns; (**C**) questionable efficacy or safety; (**D**) avoid.
Screening Tool of Older Persons Prescriptions in Frail adults with limited life expectancy STOPPFrail[32]	A list of explicit criteria for potentially inappropriate medication (PIM) use in frail older adults with limited life expectancy.
Improving Prescribing in the Elderly Tool IPET[33]	A list of 14 instances in which inappropriate prescribing may occur for an elderly patient. The tool was developed in 1997 by an expert panel in Canada and has been validated by two studies in acutely hospitalized elderly patients.
Assessing Care of Vulnerable Elders ACOVE[16]	A national project that developed evaluation indicators for the care of elderly patients who suffer from conditions that contribute most to morbidity, mortality, and functional decline.
Geriatric Risk Assessment MedGuide GRAM[34]	Applying the tool in long-term care was proven efficacious in reducing the rate of delirium, hospitalizations, and mortality resulting from adverse drug events.
Specific, Measurable, Acceptable, Realistic, and Time-framed Tool SMART[11]	It consists of 10 questions that draw attention to the appropriateness and safety of the drug plan.
CRIteria to assess appropriate Medication use among complex Elderly patients CRIME[35]	Developed in Italy, this tool represents recommendations for improving the quality of prescribing in geriatric patients with a limited life expectancy, and functional and cognitive impairment.

Beers criteria

The Beers Criteria was developed by the late Mark Beers, MD, and colleagues at the University of California Los Angeles in 1991 to identify medications for which potential harm outweighed the expected benefit and that should be avoided in nursing home residents.[36] The 1997 update, led by Dr. Beers, expanded the criteria to apply to all older adults. The Beers criteria have become a widely accepted standard for assessing the appropriateness of medication use in older adults, and they play a role in promoting medication safety and reducing the risk of adverse events in this population. Healthcare providers may use the Beers criteria as part of a comprehensive medication review process to optimize the care of older patients.[37]

The American Geriatrics Society (AGS) Beers criteria (AGS Beers Criteria) for Potentially Inappropriate Medication (PIM) Use in Older Adults is widely used by clinicians, educators, researchers, healthcare administrators, and regulators. Since 2011, the AGS has been the steward of the criteria and has produced updates on a regular cycle. The AGS Beers criteria is an explicit list of PIMs that are typically best avoided by older adults in most circumstances or under specific situations, such as in certain diseases or conditions.[21]

Key features of the Beers criteria include:

- **Identification of PIMs:** The Beers criteria provide a list of medications that may pose more risks than benefits for older adults. These medications are categorized into different classes, such as sedative hypnotics, nonbenzodiazepine receptor agonists, antipsychotics, and certain antihistamines.[38]
- **Consideration of individual patient characteristics**: The Beers criteria consider specific patient characteristics, such as age, kidney function, and existing medical conditions, as these factors can influence the risk-benefit profile of certain medications.[39]
- **Cautions and Recommendations:** The criteria include information on potential adverse effects and cautions related to the use of specific medications in

older adults. They also provide recommendations for safer alternatives when available.[40]

The Beers criteria is periodically updated to incorporate new evidence and changes in clinical practice. Updates are typically published by expert panels, such as the American Geriatrics Society (AGS). The Beers criteria serves as an educational tool for healthcare providers, helping them stay informed about medications that may be risky for older adults and prompting thoughtful consideration when prescribing for this population.[41] It is important to note that the Beers criteria are not meant to be strict rules but rather guidelines to assist healthcare professionals in making informed decisions.[42] Individual patient factors, preferences, and clinical judgment should always be considered when determining the appropriateness of a medication. This tool has been in use the longest, and its effectiveness has been investigated in a variety of settings.[43]

Pharmacists' role in medication review

Pharmacists play a crucial role in medication reviews, contributing their expertise to ensure the safe and effective use of medications.[28] There are several ways in which pharmacists can participate in the medication review process.[44] Pharmacists can conduct thorough medication reconciliations, comparing the patient's current list of medications with their medical records to identify any discrepancies, duplications, or omissions.[45] Pharmacists are trained to recognize potential drug interactions.[46] They can assess the patient's medication list to identify and manage any interactions that could compromise safety or efficacy.[47,48] Pharmacists can assess the appropriateness of medication dosages, by considering factors such as age, weight, renal function, and other patient-specific characteristics.[49] Pharmacists can provide valuable education to patients about their medications, including proper administration, potential side effects, and the importance of adherence to the prescribed regimen.[50] If a patient is experiencing adverse effects from their medications, pharmacists can work with healthcare providers to explore alternative medications or adjust dosages.[51] Pharmacists can establish monitoring plans to assess the patient's response to medications over time.[52] This may involve a routine follow-up to evaluate the efficacy, adverse effects, and adherence.[53] Pharmacists can collaborate with physicians and other healthcare providers to optimize medication regimens.[54] This may include making recommendations for medication adjustments, substitutions, or discontinuations.[55] Pharmacists can work with patients to address barriers to medication adherence, offering strategies and solutions to help patients take their medications as prescribed.[56]

Pharmacists' expertise in pharmacology and medication management makes them valuable members of the healthcare team, and their involvement in medication reviews helps improve patient outcomes, enhance medication safety, and promote overall health and well-being.[57-64] **(Table 2).**

SWOT analysis for the use of medication review tools

Despite offering numerous benefits, medication reviews also pose potential disadvantages or challenges.[65] Rec-

Table 2. Examples of a pharmacist-led medication review

Country	Author, year	Service	Outcomes
United Kingdom	Krska et al., 2001[57]	Use of medication review in providing pharmaceutical care to adult patients	The effectiveness of pharmaceutical care is improved
Ireland	Riordan et al., 2016[58]	Medication review with feedback to the patient's doctor	Improving prescribing habits
Brazil	Aguiar et al., 2016[59]	Medication review provided by a clinical pharmacist	Improvement of HbA1c values
Spain	Malet-Larea et al., 2017[60]	Medication review provided by a community pharmacist	The service is cost-effective. A cost-utility and cost analysis were performed.
United Kingdom	De Barra et al., 2018[61]	All services provided by pharmacists other than the preparation or dispensing of medications	Improvement of HbA1c values, blood pressure, lipid profile and respiratory function
Turkey	Ertuna et al., 2019[62]	Medication review provided by clinical pharmacists in a geriatric ward.	The process of implementation of clinical pharmacy in Turkey is ongoing and reviews need improvement.
USA	Yates et al., 2020[63]	Comparison between pharmacists' interventions in a cardiological ward and the alternative of no intervention.	The interventions provided by pharmacists lead to fewer drug-related problems.
Iran	Shahrami et al., 2022[64]	Medication review provided by clinical pharmacists in outpatient pharmacotherapy clinic.	High patient compliance with the proposed recommendations.

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ognizing and effectively addressing these factors is crucial to maximizing the benefits of medication reviews. In this regard, employing a SWOT analysis proves to be a valuable tool. A SWOT analysis is a strategic planning tool that assesses the **S**trengths, **W**eaknesses, **O**pportunities, and **T**hreats related to a specific initiative or situation. Conducting a thorough medication review can be time-consuming for healthcare professionals.[9] This may be a challenge in busy clinical settings where time and resources are limited. Additionally, there may be variations in how medication reviews are conducted, and standards for what constitutes a comprehensive review may differ. This lack of standardization could lead to variability in the quality of reviews.[10] Another obstacle is the limited access to patient information.[66] Incomplete or inaccurate patient information may hinder the effectiveness of a medication review. If a healthcare provider does not have access to the patient's complete medical history or medication list, it may be challenging to make informed decisions. Even after identifying issues and recommending changes, patients may not always adhere to the proposed medication regimen. Non-adherence can limit the success of interventions suggested during the review. Some healthcare providers' resistance to change may be a problem when changing established medication regimens, particularly if there is uncertainty about the potential benefits or if they are accustomed to a particular approach. In some cases, healthcare systems may lack effective integration, making it difficult to share comprehensive patient information across different care settings. This can impede coordination during transitions of care. Medication reviews may not always fully consider patient preferences and goals, potentially leading to a mismatch between the prescribed regimen and the patient's lifestyle or values.[67] Changes to medication regimens, even when well-intentioned, can have unintended consequences. For example, discontinuing a medication may result in symptom recurrence or exacerbation of a condition.[68]

Despite these potential disadvantages, the overall goal of medication reviews is to enhance patient safety, optimize therapeutic outcomes, and improve quality of life. Addressing these challenges through collaboration, standardized processes, and patient engagement can help mitigate potential drawbacks associated with medication reviews.

We constructed a SWOT analysis to identify strengths and weaknesses, as well as opportunities and threats related to the implementation of medication review tools **(Fig. 2).**

Medication review tools provide a data-driven approach to assessing medication regimens, helping healthcare providers make informed decisions based on evidence and patient-specific factors. On the other hand, one of the weaknesses is that medication review using tools is resource intensive. Conducting thorough medication reviews can be time-consuming, and implementation may require additional resources, which could be a limitation in busy healthcare settings. There may be variations in how tools are used or interpreted, leading to inconsistencies in the medication review process. Some tools may not fully capture patient preferences or factors affecting medication adherence, potentially overlooking crucial aspects of the patient's experience. Tools that rely on technology may face challenges in terms of accessibility, usability, and integration with existing healthcare systems.

The opportunities are related to advancements in technology. Opportunities exist to leverage advancements in

Figure 2. SWOT analysis of the use of medication review tools for managing polypharmacy (created via Canva.com).

technology, such as electronic health records and clinical decision support systems, to enhance the efficiency and effectiveness of medication review tools. Investing in education and training healthcare professionals on medication review tools can improve their adoption and effectiveness. Integrating tools that promote patient engagement and shared decision-making can enhance the overall success of medication reviews. The increasing use of telehealth provides an opportunity to integrate medication review tools into virtual care settings, improving accessibility and continuity of care.

Threats are in the first place the resistance to change. Healthcare providers may resist adopting new tools or changing established practices, particularly if there is skepticism about the benefits or concerns about disruptions to workflow. The use of technology in medication review tools raises concerns about data privacy and security, especially given the sensitive nature of healthcare information. Reimbursement policies may not adequately support the time and resources required for comprehensive medication reviews, posing a financial challenge for healthcare organizations. Regulatory constraints or changing guidelines may impact the implementation and use of medication review tools.

This SWOT analysis provides an overview of the factors influencing the use of tools for medication review. Addressing weaknesses and threats while capitalizing on strengths and opportunities can help optimize the integration and impact of these tools in healthcare settings.

CONCLUSION

The management of polypharmacy is a systematic approach that optimizes care for multimorbid patients by maximizing benefits while simultaneously reducing patient safety risks. Medication review tools contribute to identifying and mitigating potential risks, improving patient safety by reducing the likelihood of adverse drug events. The use of tools can help healthcare providers identify barriers to medication adherence and develop strategies to enhance patient compliance. Tools facilitate a more efficient and standardized approach to medication reviews, ensuring that healthcare professionals follow evidence-based guidelines and protocols. Tools promote collaboration among healthcare professionals, including pharmacists, physicians, and other team members, fostering a comprehensive and holistic approach to patient care. The extent and nature of medication review services can vary widely even within regions or countries. The adoption of these practices often depends on the specific healthcare policies, regulatory frameworks, and the integration of pharmacists into the healthcare team. The aim, however, is universal: to ensure that patients receive the most appropriate and safe medication regimens tailored to their individual needs.

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Conflict of Interest

We have no conflicts of interest to disclose

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Управление полипрагмазией с помощью инструментов обзора лекарств – плюсы и минусы

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Резюме

Неуместная полипрагмазия является частым явлением у пожилых пациентов, что приводит к увеличению побочных реакций на лекарства, несоблюдению режима лечения и увеличению затрат на здравоохранение. Обзор и отмена назначения лекарств являются основными стратегиями, описанными в литературе для борьбы с проблемной полипрагмазией. Для эффективного проведения обзора лекарств были разработаны различные инструменты. Эти инструменты могут помочь в анализе лекарств различными способами. Некоторые инструменты включают список лекарств, требующих пристального внимания, а другие знакомят медицинских работников с принципами и алгоритмами проверки и назначения лекарств. Третья категория инструментов направлена на отслеживание и выявление симптомов, которые могут быть связаны с проблемами, связанными с наркотиками.

Целью этой статьи является представление инструментов поддержки обзора лекарств, используемых при лечении полипрагмазии у гериатрической популяции, подчёркивая их преимущества и недостатки.

Ключевые слова

пожилые люди, обзор лекарств, фармацевт, полипрагмазия, инструменты

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Original Article

Factors Affecting Prediction Accuracy of Postoperative FEV1 and $D_{L,CO}$ in Patients Undergoing Lung Resection

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Abstract

Introduction: Despite significant development in systemic therapy and radiotherapy, surgery is still the cornerstone for curative lung cancer treatment. Although predicted postoperative function (ppo) somewhat exactly correlates with actual postoperative function bigger differences may be a cause of serious clinical outcome.

Aim: The aim of our study was to identify clinical factors affecting prediction accuracy of postoperative lung function for more careful selection of operable lung cancer patients.

Patients and methods: Seventy patients were studied prospectively. The preoperative lung function tests (FEV1 and $D_{L,CO}$) were performed within a week before surgery, and the follow-up tests were performed 4 to 6 weeks after surgery. Calculation of predicted postoperative values were calculated by three methods: two segment formulas and vibration response imaging (VRI). The correlation between each clinical parameter and accuracy of prediction was screened on univariate analysis of Pearson's correlation coefficient, and significant factors were confirmed by multivariate linear regression analysis applying backward stepwise elimination approach.

Results: Univariate linear regression analysis between the predicted and the actual postoperative values of FEV1% and $D_{L,CO}$ showed the highest prediction accuracy with acoustic mapping (VRI). Multivariate regression analysis showed that prediction accuracy of postoperative lung function is significantly affected by COPD ($p<0.001$) and volume of resection ($p<0.001$).

Conclusion: Vibration response imaging (VRI) is a more accurate method for predicting postoperative lung function than segment method formulas. Anatomical calculation significantly underestimates the postoperative values of FEV1% in patients with COPD. Prediction of FEV1% and $D_{L,CO}$ with segment counting is significantly influenced by the volume of resection.

Keywords

lung cancer, postoperative lung function, prediction accuracy, vibration response imaging

INTRODUCTION

Despite significant development in systemic therapy and radiotherapy, surgery is still the cornerstone for curative lung cancer treatment. Pulmonary lobectomy is the standard operative treatment for primary non-small cell lung cancer (NSCLC). Because of relatively high incidence of postoperative complications, the hospital mortality, as well as disappointing long-term survival after surgical resection of lung cancer, the appropriate selection of patients for pulmonary resection is a continuing challenge. Forced expiratory volume in one second (FEV1) and the diffusing capacity for carbon monoxide ($D_{L,CO}$) are the most commonly used predictors of postoperative outcome.[1-3] Postoperative values of FEV1 and $D_{L,CO}$ are the mainstay for assessing perioperative risk after lung resection. Although predicted postoperative function (ppo) somewhat exactly correlates with actual postoperative function, bigger differences may be a cause of serious clinical outcome, especially in patients with marginal postoperative lung function: someone may undergo life-threatening lung resection, and someone may lose the opportunity to be cured by surgery.

AIM

The aim of our study was to identify clinical factors affecting prediction accuracy of postoperative lung function for more careful selection of operable lung cancer patients.

PATIENTS AND METHODS

Patients

We conducted a prospective cohort study of patients undergoing anatomical lung resection in the Department of Special Surgery at St George University Hospital in Plovdiv and the Department of Thoracic Surgery at Kaspela University Hospital in Plovdiv.

Lung function tests

The preoperative lung function tests were performed within a week before surgery, and the follow-up tests were performed 4 to 6 weeks after surgery. Lung function tests were performed using the MasterScreen Body/Diffusion™ computerized spirometer (Jaeger, Wuerzburg, Germany) with real-time curve drawing and automatic correction (BTPS – body temperature pressure saturated). The postbronchodilator lung function values were used.

Calculation of predicted postoperative values (ppo) of FEV1 and $D_{L,CO}$

Predicted postoperative FEV1% and $D_{L,CO}$%, were calculated by three methods.

Anatomical calculation

SF1

The simple calculation method introduced by Juhl et al.[4] assumes that the right lung is composed of 10 segments (3 segments in the upper lobe, 2 segments in the middle lobe, and 5 segments in the lower lobe), the left lung of 9 segments (5 segments in the upper lobe, 4 segments in the lower lobe), and that all the segments contribute equally to lung function.

SF1–(Juhl & Frost, 1975; Zeiher et al., 1995).[4,5]

$$\text{Number of segments (S): ppoFEV1} =$$

$$\text{preoperative FEV1} \times [1 - (\frac{\text{number of segments to be resected}}{19})]$$

SF2

Modified segment formula introduced by Bolliger that takes into account only functional (none obstructed) segments

SF2–(Bolliger et al. 2002)[6]

$$\text{Number of functional segments (FS): ppoFEV1} =$$

$$\text{preoperative function} \times (1 - \frac{y}{z}),$$

where y is the number of functional segments to be removed and z is the total number of functional segments.

Vibration response imaging (VRI) and O-Plan software

The vibration response imaging system (VRIxp, Deep Breeze, Or-Akiva, Israel) quantifies breath sounds and displays the results as a dynamic image and numerical values. It measures the vibration energy of lung sounds generated during the respiratory cycle. Vibration response imaging (VRI) technology is harmless, non-invasive and does not require the addition of a tracer to the inhaled air or blood stream. The technology and the calculation were described in detail earlier.[7,8]

Clinical parameters affecting prediction accuracy of postoperative lung function

In the literature, we have identified clinical factors with potential impact on the accuracy of prediction of postoperative lung function: sex, body mass index, smoking, preoperative FEV1%, presence of COPD and type of operation.[9-12]

Patients were divided in groups according to the studied factor: male/female; smoker/ex-no smoker; patients with baseline FEV1% ≥ 80% and with FEV1% < 80%; with COPD and without COPD as defined by GOLD 2007 criteria; patients with COPD index ($COPD_I$) <1.5 and those with $COPD_I$ ≥1.5. To investigate the influence of operative intervention on the accuracy of prediction of the post-

operative lung function, we performed a comparison between the results obtained in patients with upper and lower lobectomy, lobectomy and pulmonectomy and removal of >4 and ≤4 functioning lung segments. We compared the prediction accuracy of the three calculation methods. The influence of the clinical parameters on prediction was studied separately for each method.

Statistical analysis

The adjusted coefficient of determination (adjusted R^2) was used to compare the prognostic accuracy of the three methods.

To assess the influence of studied clinical factors on the prediction made by the three different methods used (SF1, SF2 and VRI), the value of relative deviation in percentages was introduced:

$$D\% = \frac{ppo-apo}{apo}.100,$$

where ppo – predicted postoperative values of FEV1 and $D_{L,CO}$; and apo – actual postoperative values of FEV1 and $D_{L,CO}$.

Metric variables were checked for normality of distribution by the Kolmogorov-Smirnov test. An independent sample *t*-test was used to compare means of continuous variables. The Mann-Whitney *U*-test was used for variables not normally distributed. The correlation between each clinical parameter and accuracy of prediction was screened on univariate analysis of Pearson's correlation coefficient, and significant factors were confirmed by multivariate linear regression analysis applying backward stepwise elimination approach. A *p*-value less than 0.05 was considered to be significant in all statistical analyses.

The study design was approved by the Medical University of Plovdiv's institutional review board and is in accordance with the Declaration of Helsinki ethical standards.

RESULTS

Patient characteristics

The baseline characteristics of the patients are summarized in **Table 1**. One hundred and two patients were studied prospectively. Of these, five patients dropped out due to failure to appear for post-operative examination and 27 due to initiation of adjuvant therapy before post-operative examination. Seventy patients remained for the study. There were 52 (74.3%) men and 18 (25.7%) women. The mean age was 61.7±8.0 years in the range between 46 and 78 years.

Prediction accuracy of postoperative lung function

Univariate linear regression analysis between the predicted and the actual postoperative values of FEV1% and $D_{L,CO}$ showed the highest prediction accuracy with acoustic mapping (VRI). The adjusted coefficient of determination for FEV1% was R^2 adj=55.12% for VRI, R^2 adj=46.23% for SF1, and R^2 adj=31.29% for SF2. For $D_{L,CO}$%, VRI showed R^2 adj=64.00%. SF1-R^2 adj=47.85% and SF2-R^2 adj=45.11.

Table 1. Patient characteristics of the studied population

Patient characteristics	n	(%)
BMI		
< 18.5	2	2.9
18.5-24.9	31	44.3
25-29.9	19	27.1
> 30	18	25.7
COPD index		
< 1.5	25	35.7
> 1.5	45	64.3
GOLD		
Without COPD	51	72.9
COPD	19	27.1
Type of surgery		
Pneumonectomy	17	24.3
Upper lobectomy	34	48.6
Lower lobectomy	19	27.1
Smoking status		
smoker	38	54.3
no+ex smoker	32	45.7
Number of resected functional segments		
≤ 4	30	42.9
> 4	40	57.1
Preoperative FEV1%		
> 80%	48	68.6
< 80%	22	31.4

Clinical parameters affecting prediction accuracy of postoperative lung function

Univariate analysis of BMI and smoking status showed no statistically significant influence on prediction accuracy with all three methods.

When examining the influence of gender on the determination of ppo FEV1.0% and $D_{L,CO}$, a difference was found. In men, the values were negative, indicating that the predicted values were lower than those measured postoperatively. For women, the values were higher than actually measured. Statistically significant difference was found only for the prediction of ppo FEV1% with acoustic mapping. A similar trend was observed for $D_{L,CO}$(%), but the sex difference did not reach significance.

To investigate the influence of baseline preoperative FEV1 (%), patients were categorized into two groups: FEV1

(%) >80% and FEV1 (%) ≤80%. All three methods reported an overestimation of functional loss. When using the SF1 segment formula, we observed a significantly greater value of relative deviation (D%) of FEV1 (%) in the FEV1 (%) ≤80% group with a median of −25.58% compared to median −4.97% in patients with FEV1 (%) >80%, p=0.001. With the SF2 formula, a significantly higher D% value was also found in the FEV1 (%) ≤80% group with a median of −7.68% compared to a median of −3.03% in patients with FEV1 (%) >80%, p=0.012. D% showed no significant difference between the groups when calculated with VRI, p=0.256. No statistically significant difference was observed between the two groups in the calculation of $D_{L,CO}$% with all the three methods.

In patients with COPD, all methods reported an underestimation of postoperative FEV1 (%). Significantly greater values were found in patients with COPD predicted with SF1 formula – median −20.68% compared to median −5.69% in patients without COPD, p=0.002. The difference was significant with SF2 formula, with a median of −12.4% in the COPD group and 4.00% in the non-COPD group, p=0.005. The relative deviation for diffusion capacity (D%) $D_{L,CO}$% showed no significant differences between patients with and without COPD in all three prognostic methods.

In patients with $COPD_I$ ≥1.5 and $COPD_I$ <1.5, a significant difference was found in the prediction of FEV1 (%) with SF1 segment formula, with a significantly greater value in the group with $COPD_I$ <1.5 (-18.65±15.7%) compared to those with $COPD_I$ ≥1.5 (−2.81±18.8%), p=0.001.

Lobectomy was performed in 53 patients, in 34 of whom with upper lobectomy and in 19 with lower lobectomy. Pulmonectomy was performed in 17 patients. Comparative analysis of the relative deviation between lobectomy and pulmonectomy patients was preceded by an intergroup comparison between upper and lower lobectomy patients. Based on the intergroup analysis, upper and lower lobectomy patients were pooled into one lobectomy group, due to lack of significant difference between upper and lower lobectomy patients in all indices and methods

When predicting FEV1 (%), the D% values, predicted with either of the three methods, were negative as a result of the greater underestimation of postoperative FEV1 (%) in the pneumonectomy patients compared to the lobectomy patients. The difference was significant in patients with pulmonectomy calculated with the first variant of the segment method SF1 (p<0.001). Significantly higher negative D% $D_{L,CO}$% values were found in the pneumonectomy patients predicted with all three methods.

A significant difference was observed in the prediction of $D_{L,CO}$% with both segment formulas when comparing patients with more than 4 segments removed to patients with 4 or less removed segments. A significantly higher relative deviation of $D_{L,CO}$% was observed with SF1 method in the >4 segments removed group (−12.94±18.4%) compared to the ≤4 segments removed group (−2.14±16.3%), p=0.015. For SF2, the relative deviation D% $D_{L,CO}$% was (6.94±14.3%) in the ≤4 segments removed group, while in those with >4 segments removed, D% $D_{L,CO}$% had a negative value (−8.52±18.6), p<0.001. In SF2, the same dependence was observed in D% FEV1 (%), where in the group of patients with ≤4 segments removed, the relative deviation had a positive value (8.22±24.1%) and negative in the group with >4 segments removed (−6.31±17.8%), p<0.001.

Sex and operative intervention (pulmonectomy/lobectomy) were identified by univariate analysis as factors affecting the accuracy of VRI to predict postoperative FEV1 (%) and $D_{L,CO}$%. The results of the multivariate regression analysis showed that they do not contribute significantly to improvement of the prediction accuracy

For the first variant of the segment method (SF1), multivariate regression analysis found out that two of the six variables included in the prognostic model have a significant prognostic role: COPD (p=0.019), in the prediction of postoperative FEV1% and the type of operation (lobectomy/pulmonectomy) in the prediction of $D_{L,CO}$% (p<0.001).

For the second variant of the segment method (SF2), COPD (p=0.004) and number of removed segments (p=0.049) were found to be significant factors in prediction accuracy of FEV1%.

DISCUSSION

Medical operability of lung cancer has been frequently determined based on FEV1, D_{LCO}, and VO2max. Accurate prediction of postoperative residual lung function is mandatory to minimize postoperative morbidity and mortality. Although predicted postoperative function (ppo) somewhat exactly correlates with actual postoperative function bigger differences may be critical in the patients with marginal lung function after lung resection. We should consider that the accuracy can be affected not only by the technique to measure the regional lung function, but also several clinical factors

In our study, we found that VRI-based prediction was a more accurate method than anatomical calculation irrespective of the extent of resection. This result is in consistency with Berreta et al.[13], and Detterbeck et al.[14] The prediction accuracy of VRI was confirmed by the studies of Comce et al.[15], Jimenez et al.[16], Morice et al.[17], and Kim et al.[18] in comparison with perfusion scintigraphy, which is considered the gold standard in determining the predicted postoperative values of FEV1 and $D_{L,CO}$.

We observed a higher overestimation of functional loss in patients with FEV1%<80%, (postoperatively measured values were higher than predicted) in all three methods. Boushy et al.[19] first reported that the decrease in FEV1 after lung resection was inversely related to the preoperative FEV1% and that patients with better function had a greater decrease in FEV1. Pierce et al.[20] found a significant relationship between percent change in FEV1.0 after pulmonary resection and baseline FEV1%, indicating that functional loss was proportionally less in patients with worse baseline function. Santambrogio et al.[21] observed a con-

sistent decrease in post-operative FEV1% in patients with FEV1% more than 80% and slight decrease in the post-operative FEV1% in patients with FEV1% less than 80% and the difference was highly statistically significant.

This smaller difference in the degree of functional loss in individuals with poor baseline lung function has also been observed by Baldi et al.[22], Bobbio et al.[23], and Edwards et al.[24] Current experience with lung volume reduction surgery suggests that predicted postoperative FEV1 may be underestimated in COPD patients undergoing lobectomy for lung cancer. In addition, COPD patients with lower FEV1 may have less loss of lung function after lobectomy.[22,25,26]

COPD is defined in several ways, and differences in definitions and diagnosis affect estimates of disease severity. The Global Initiative for Chronic Obstructive Lung Disease (GOLD) 2007 defined the disease in degrees of clinical severity based on FEV1 and FEV1/FVC (forced vital capacity) from post-bronchodilator spirometry. To define airflow limitation, the fixed ratio FEV1/FVC <0.7, measured after the bronchodilator, is recommended, despite the risk of overdiagnosis.[27]

Using this definition, we found a significantly less volume loss in COPD patients when comparing predicted versus actual postoperative FEV1 (%) values calculated with the segmental (SF1 and SF2) formulas, and no significant differences in $D_{L,CO}$%. Sekine et al.[12] also found minimal change in postoperative pulmonary function in patients with COPD. The ratio of actual postoperative forced expiratory volume in one second to the predicted postoperative forced expiratory volume in one second (apo/ppo FEV1) was higher in the chronic obstructive pulmonary disease (COPD) group than in the non-COPD group.

A study by Pompili et al.[28] 2010 showed that patients with COPD had a lower reduction in FEV1 (6% vs. 13%, p=0.0002) compared to patients without COPD after lobectomy for lung cancer. The studies by Baldi et al.,[22] Kushibe et al.[29], and Liao et al.[30] confirm these results.

Another way to define and evaluate chronic obstructive pulmonary disease in the practice of thoracic surgery is the so-called "COPD index". To classify patients according to severity and purity of obstructive pulmonary disease, Korst et al.[25] defined a "COPD index" (COPDI) and calculated it for each patient as the sum of the preoperative FEV1 (% of predicted in decimal form) to the preoperative FEV1/FVC (forced vital capacity) ratio. For example, if a patient has an FEV1 of 60% and the FEV1/FVC ratio is 0.5, the COPD index would be 0.6 plus 0.5, or 1.1. The COPD index, defined in this way, is an attempt to identify those patients with the most severe and pure obstructive pulmonary disease. Therefore, the patients with the lowest COPD index were those with the purest and most severe obstructive disease.

We found underestimation of predicted postoperative FEV1 (%) and $D_{L,CO}$% in patients with COPD index <1.5. Baldi et al.[22] similarly observed a better than predicted postoperative FEV1% when the COPD index was less than 1.5. Santambrogio et al.[21], in their study, applied the Korst index and divided COPD patients into two groups. The authors found that in the subgroup with a strong decrease in FEV1 (%), the COPD index was 1.35, and in the other with a smaller decrease, it was 1.15 and the difference was statistically significant.

When examining the effect of the volume of resection (lobectomy/pulmonectomy) on the accuracy of prediction of ppo FEV1, we found greater underestimation in pneumonectomy patients compared with lobectomy patients. The difference was statistically significant only with the SF1 segment formula. This underestimation is statistically significant for $D_{L,CO}$% in all three methods. Similar to our results, Bolliger et al.[6] found that anatomical calculations had significantly reduced correlation coefficients after pulmonectomy, the lowest when using equation SF1 (segments), which did not take into account the function of the parenchyma to be removed (SF2). This formula is consistently worse than all other methods because it significantly overestimates functional loss, especially after pneumonectomy. The authors suggest that anatomically calculated scores should only be used for resections that do not exceed one lobe. Beccaria et al.[31] reported that a simple calculation of ppoFEV1 correlated well with the actual value of apoFEV1 six months after surgery in all patients who underwent lobectomy. However, this is not the case in patients who have undergone pneumonectomy; in fact, in these patients, ppoFEV1 consistently underestimated actual apoFEV1 by an average of 500 ml. These results are consistent with data previously presented by Zeiher et al.[5] They found that in individuals with atelectasis, hilar involvement, or endobronchial involvement with radiologic evidence of dysventilation, simple calculation of ppoFEV1 is not reliable. In this group of patients, ppoFEV1 did not correlate with the actual postoperative value in patients who underwent pulmonectomy. The bias was always in the direction of underestimation of the actual apoFEV1.[32]

Kim et al.[10] performed multivariate linear regression analysis to identify clinical parameters influencing the accuracy of prediction. They found the number of resected lung segments and the preoperative FEV1 to be significant factors. The smaller the preoperative FEV1 and the more lung segments resected, the more the postoperative FEV1 (apoFEV1) tends to be greater than the predicted ppoFEV1. Apo FEV1 was closest to ppoFEV1 when four segments were resected.

We found an underestimation of the postoperative indicators in the group of patients with more than 4 segments resected. This difference was significant when predicting $D_{L,CO}$(%) and FEV1 (%) with the SF2 formula and for $D_{L,CO}$ (%) calculated with the SF1 formula.

In our study, we found no influence of the type of lobectomy (upper/lower) on the accuracy of prediction of postoperative lung function. Accurate assessment of anatomic-functional loss after lung lobectomy is also complicated by the fact that damaged lung areas, especially emphysematous areas, are often distributed heterogeneously in the upper or lower lobe, changing the functional roles of these

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lobes. Data are conflicting in the literature. Kim et al. found no influence of the type of lobectomy on the prediction of ppoFEV1 (%) and $D_{L,CO}$ (%).[10] Kushibe et al.[26] studied 178 lobectomy patients and found that upper lobectomy resulted in less than predicted loss of FEV1 (%) and may have an effect similar to volume reduction surgery. Sekine et al.[12] reported that the presence of COPD and resection of the lower part of the lung (lower lobectomy or mid-inferior bilobectomy) were significantly associated with minimal deterioration of lung function after lobectomy. Minimal change in postoperative lung function was confirmed to be associated with COPD (vs. non-COPD) and lower lung resection (vs. upper lung) in multivariate analysis.

The theory of volume reduction surgery may explain the minimal change of apoFEV1 in patients with COPD. For patients without COPD, the authors speculated that accidental anatomical repositioning after upper lobectomy, which causes narrowing of the opening of the lower or middle lobe of the bronchi, and different movement and elevation of the diaphragm between upper lobectomy and lower lobectomy may be the potential reason for the minimal change in the cases of resection of the lower part of the lung.[12,33,34]

Sengul et al. found that in lower lobectomy, volume recovery is mainly due to expansion of the contralateral lung along with increase in the volume of ipsilateral remaining lung, especially after right lower lobectomy.[35]

CONCLUSION

We should consider that the accuracy of prediction can be affected not only by the technique to measure the regional lung function, but also several clinical factors such as preoperative FEV1%, the presence of obstructive lung disease, extent of lung volume resection

Vibration response imaging (VRI) is a more accurate method for predicting postoperative lung function than the segment method formulas.

Anatomical calculation significantly underestimates the postoperative values of FEV1% in patients with COPD.

Prediction of FEV1% and $D_{L,CO}$ with segment counting is significantly influenced by the volume of resection and should not be used alone to determine the postoperative values of FEV1% and $D_{L,CO}$% in patients scheduled for an operative intervention greater than lobectomy.

Author contributions

A.C.: design, collection of data, operative task completion and critical review of manuscript; M.T.: design, collection of lung function and VRI data and critical review of manuscript; N.C.: collection of data, statistical analysis, and critical review of manuscript; B.M.: design, collection of lung function and VRI data and critical review of manuscript. All authors have contributed to the tasks of the study and critically reviewed the final version of the manuscript.

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Competing Interests

The authors have declared that no competing interests exist.

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Факторы, влияющие на точность прогнозирования послеоперационного FEV1 и $D_{L,CO}$ у пациентов, перенёсших резекцию лёгкого

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Резюме

Введение: Несмотря на значительное развитие системной терапии и лучевой терапии, хирургия по-прежнему остается краеугольным камнем радикального лечения рака лёгких. Хотя прогнозируемая послеоперационная функция (ППОФ) точно коррелирует с фактической послеоперационной функцией, большие различия могут быть причиной серьёзного клинического исхода.

Цель: Целью нашего исследования было выявление клинических факторов, влияющих на точность прогнозирования послеоперационной функции лёгких, для более тщательного отбора операбельных больных раком лёгких.

Пациенты и методы: Проспективно исследовано 70 пациентов. Предоперационные тесты функции лёгких (FEV1 и $D_{L,CO}$) проводились в течение недели до операции, а последующие тесты проводились через 4–6 недель после операции. Расчёт прогнозируемых послеоперационных значений осуществлялся тремя методами: двухсегментной формулой и визуализацией вибрационного отклика (VRI - Vibration Response Imaging). Корреляция между каждым клиническим параметром и точностью прогноза проверялась с помощью одномерного анализа коэффициента корреляции Pearson, а значимые факторы были подтверждены с помощью многомерного линейного регрессионного анализа с применением подхода обратного пошагового исключения.

Результаты: Одномерный линейный регрессионный анализ между прогнозируемыми и фактическими послеоперационными значениями FEV1% и $D_{L,CO}$ показал самую высокую точность прогнозирования с помощью акустического картирования (VRI). Многофакторный регрессионный анализ показал, что на точность прогнозирования послеоперационной функции лёгких существенное влияние оказывают ХОБЛ (p<0.001) и объём резекции (p<0.001).

Заключение: Визуализация вибрационного отклика (VRI) является более точным методом прогнозирования послеоперационной функции лёгких, чем формулы сегментного метода. Анатомический расчёт существенно занижает послеоперационные значения FEV1% у больных ХОБЛ. На прогноз FEV1% и $D_{L,CO}$ при подсчёте сегментов существенное влияние оказывает объём резекции.

Ключевые слова

рак лёгких, послеоперационная функция лёгких, точность прогнозирования, виброотдача

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Original Article

Complications Due to Ultrasound Transthoracic Cutting Biopsy of Peripheral Pulmonary Lesions and Lesions in the Chest Wall and Mediastinum

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Abstract

Introduction: Evaluation of patients with peripheral lung lesions and lesions of the chest wall and mediastinum is challenging. The nature of the lesion identified by imaging studies can be determined by histological evaluation of biopsies. An important place in this direction is the ever-increasing popularity among thoracic surgeons of the transthoracic biopsy with a cutting needle under ultrasound control (US-TTCNB).

Aim: This article aims to outline potential complications that may arise from transthoracic incisional biopsy performed under ultrasound guidance, along with a percentage reduction algorithm and treatment approaches.

Materials and methods: The present study is based on 264 patients with CT-detected peripheral lesions of the lung, chest wall, and the mediastinum performed in the Department of Thoracic Surgery of Kaspela University Hospital in Plovdiv over a period of 2 years (January 2020 – December 2021).

Results: Complications were found in 11 (4.17%) patients.

Conclusion: Our established biopsy technique achieves a sufficient amount of biopsy material with a low rate of post-biopsy complications.

Keywords

CT, pulmonary lesions, ultrasound-guided transthoracic incisional biopsy, ultrasound

INTRODUCTION

Worldwide, lung carcinoma is the most common cause of death from malignancy in both men and women. In most cases, early diagnosis is difficult and requires the use of a number of diagnostic methods with varying degrees of invasiveness. Currently, both smaller and larger tumor masses can be detected due to improvements in scanning resolution[1,2], and lung cancer screening with CT is gaining in popularity. Histological verification is essential for correct

diagnostic determination of the pathology of these masses, as the distinction between malignant and benign is essential for treatment planning.[3,4]

Thoracic surgeons have become increasingly interested in US-guided transthoracic biopsy in recent years because of its advantages, which include being available at the patient's bedside, requiring no radiation, being low cost, providing precise real-time control, and allowing targeted percutaneously guided biopsy.[5-13] The technique is relatively safe with a very low risk of complications (below 0.5%)[6] and is preferable, especially if the alternative is diagnostic surgery. In the hands of a thoracic surgeon, the ultrasound-guided transthoracic cutting needle biopsy of peripherally located tumor masses of the chest wall, lung, and mediastinum is a sufficiently reliable invasive procedure allowing the collection of sufficient material for histologic verification.[6,14]

AIM

This article aims to outline potential complications that may arise from transthoracic incisional biopsy performed under ultrasound guidance, along with a percentage reduction algorithm and treatment approaches.

MATERIALS AND METHODS

Between January 2020 and December 2021, 281 patients were referred to us for verification of one or more peripheral lung lesions. Such lesions are defined as lesions that not only border the pleura but also have an accessible ultrasound window. 264 of these patients underwent US-TTCNB according to the criteria for inclusion in the study. In the remaining 17 patients, it was not performed either because of small, insufficient contact of the formation with the chest wall and related to this insufficient window for transthoracic biopsy or because of general contraindications.

The inclusion criteria were: a) peripheral lung masses that rest on the pleural surface; b) pleural mass or diffuse pleural thickening; c) patients with a mediastinal tumor anteriorly, posteriorly, or in a location that touches the chest wall; e) tumor masses on the chest wall, ribs and vertebrae; f) acoustic window for penetration of the ultrasound beam; and g) patients with normal coagulation status or achieving such during the hospital stay.

Exclusion criteria: a) patients who do not meet the inclusion criteria; b) hemodynamic instability; c) arteriovenous malformation or aneurysm; d) unstable bronchial asthma, severe impairment of lung function; and e) patients with uncontrolled convulsions.

Ultrasound control was carried out using a HITACHI ALOKA f37 device, equipped with a convex biopsy transducer with a side attachment for biopsy with two angles of projection with a frequency in the range of 3.0-5.0 MHz and with the possibility of Doppler ultrasound examination **(Fig. 1)**.

We used GEOTEK Maxicor 16G × 20 cm cutting needles **(Fig. 2)**.

All patients were followed-up after the manipulation for the presence of a complication, and adequate measures were taken in the event of such a complication. Results, discussion, and primary conclusions are based on 264

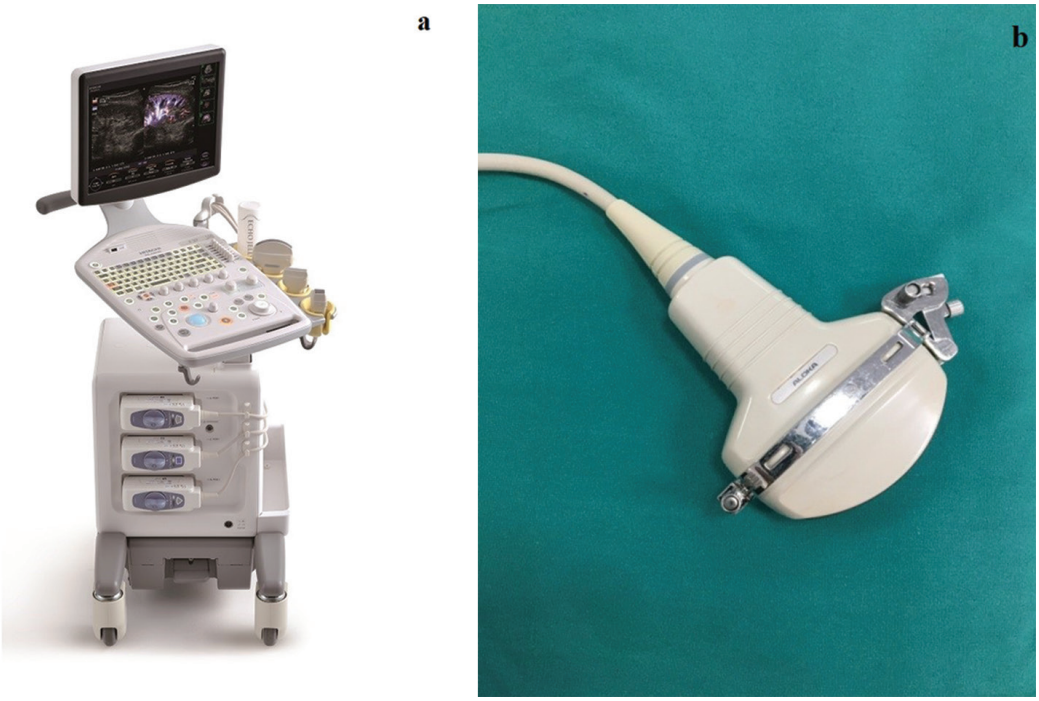

Figure 1. a) Ultrasound device HITACHI ALOKA f37; **b**) convex transducer with biopsy attachment.

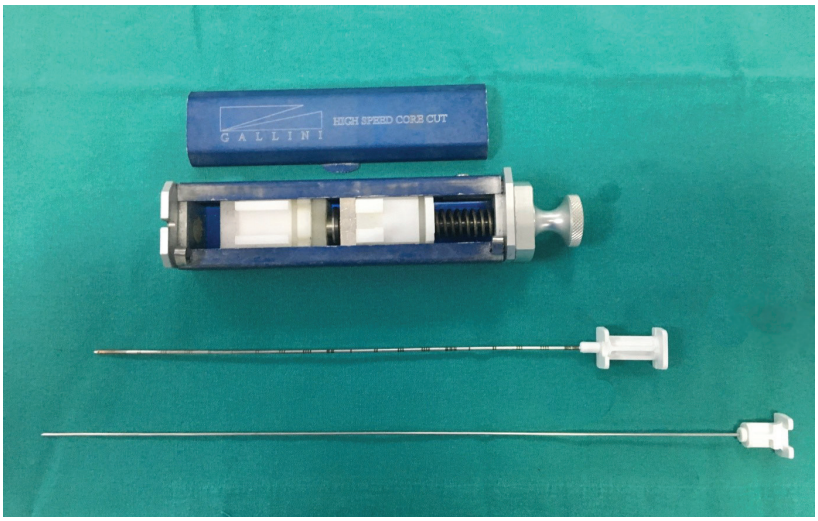

Figure 2. Segmented biopsy needle 16G × 20 cm and a semi-automatic cutting biopsy gun.

patients. In 9 of the cases, it was necessary to repeat the invasive manipulation due to unsatisfactory quality and/or quantity of the initially taken material.

Of the 264 patients included in the study, 188 were men and 76 were women **(Fig. 3)**. The age of the examined patients varied from 13 to 90 years.

The distribution of patients according to the size of peripherally located formations in the chest shows the highest relative share of patients with formations larger than 5 cm, constituting 54.20% (n=143) of the studied group. The difference with the other two categories was statistically significant (p=0.001). One hundred and four (39.40%) patients were categorized with formations between 2 cm and 5 cm. This size formations significantly prevailed (p<0.001) compared to formations smaller than 2 cm, which were found in 17 patients (6.40%) **(Fig. 4)**. The mean value of the tangent to the chest wall was 2.25±069 cm, with a median of 2 cm and a range between 1 cm and 3 cm.

As to the location of tumors, we found 67 (29.70%) tumors in the upper right, 62 (27.40%) in the lower right, 57 (25.20%) in the upper left, and 40 (17.70) in the lower left. The total number amounted to 226 and exceeded the total number of lung neoplasias (n=200) because in some of the cases two locations were found **(Fig. 5)**.

Fig. 6a illustrates the relative proportion of patients with carcinoma in the chest wall and mediastinum. **Fig. 6b** shows the relative proportion of patients with carcinoma in the chest wall. The relative proportion of patients with carcinoma in the mediastinum is illustrated in **Fig. 6c**.

RESULTS

Complications were found in 11 (4.17%) out of a total of 264 patients who underwent US-TTCNB. Seven cases of pneumothorax (2.66%), hemoptysis in two (0.76%), hemorrhage in one (0.38%), and vasovagal attack in one (0.38%) were recorded **(Table 1)**.

Because pain at the biopsy site was reported by 49 patients (18.50% of the study group), we examined this sub-

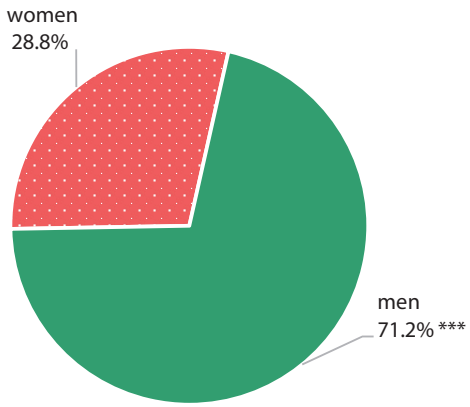

Figure 3. Distribution of patients by sex. All patients underwent a preliminary computed tomography diagnosis.

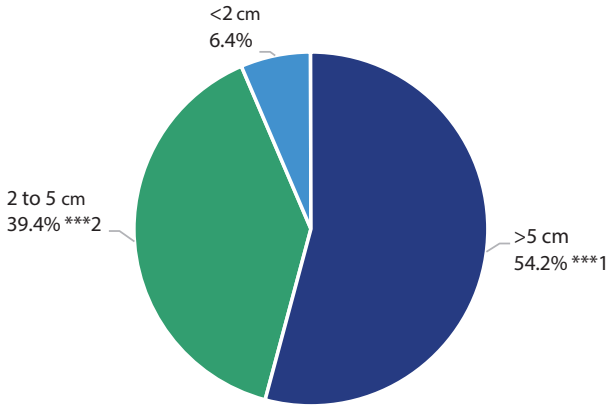

Figure 4. Distribution by size of the peripherally located formation in the chest. ***1 – Significantly higher relative share compared to other sizes (p=0.001); ***2 – Significantly higher relative proportion to tumors smaller than 2 cm (p<0.001).

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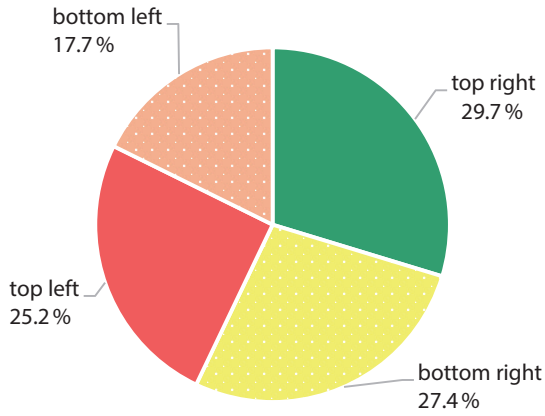

Figure 5. Distribution of tumors by location.

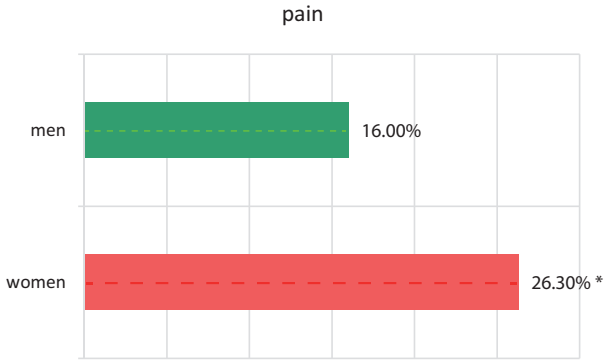

Figure 7. Marginally significant difference between female and male patients regarding pain complaints at the biopsy site after US-TTCNB. * – Marginally significant difference at p=0.05.

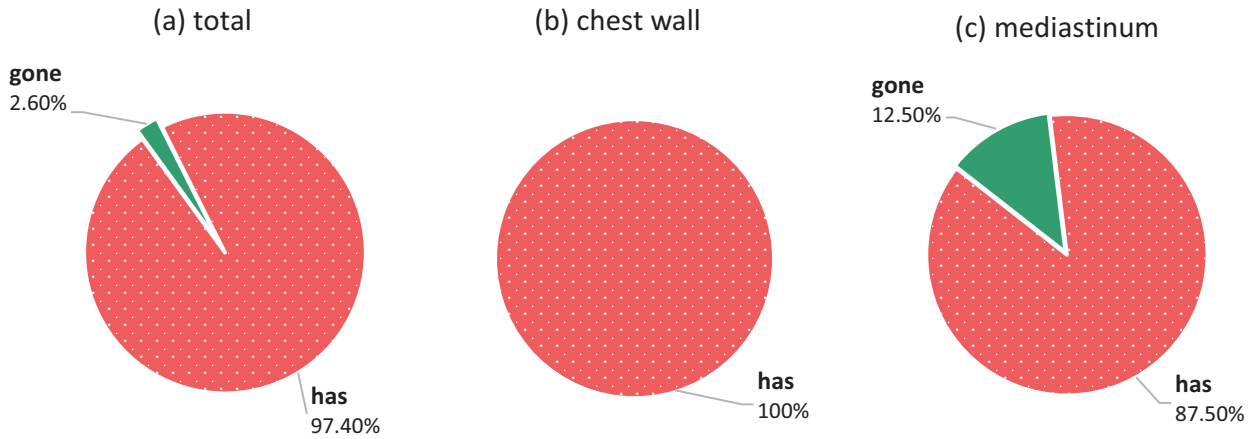

Figure 6. Relative proportion of patients with carcinoma in the chest wall and mediastinum (**a**); Relative proportion of patients with carcinoma in the chest wall (**b**); Proportion of patients with mediastinal carcinoma (**c**).

Table 1. Complications

Complications	Number	Percentage
Pneumothorax	7	2.66%
Hemoptysis	2	0.76%
Hemorrhage	1	0.38%
Vasovagal attack	1	0.38%
Total	11	4.17%

jective factor in our study and correlated it with the patients' sex. A marginally significant difference was found between male and female patients. In particular, 26.30% of the female patients complained of pain at the biopsy site, compared to 16% of the male patients (p=0.05) **(Fig. 7)**. No relationship was found between the presence of pain and the size of the tangent to the chest (p=0.870).

We also analyzed the relationship between the age of patients and the incidence of complications. For this purpose, age was coded into three categories: up to 50 years (n=16), from 50 to 65 years (n=105), and over 66 years (n=143). Results showed no significant association between age groups and complication rates **(Table 2)**.

Pneumothorax was the most common complication, but it did not occur in the youngest age group, appearing in 2.90% of the 50-to-65-year-old group and 2.80% of the over-65-year-old group, with no significant difference (p=0.793).

Hemoptysis was observed in two patients, one in the age group from 50 to 65 years (1.0%) and the other in the group over 65 years (0.70%).

Hemorrhage in the pleural cavity was found in one patient in the age group of 50-65 years (1.0%, significantly small).

Overall, patients without any complications constituted the highest relative proportion of the youngest group (100%) followed by the 50-to-65-year group (95.23%) and had the lowest relative proportion in the over 66 group (96.5%), but the differences did not reach statistical significance (p=0.106).

Table 2. Complications versus age groups

Complications	<50 yrs (n=16)	50 to 65 yrs (n=105)	>65 yrs (n=143)	*p*
Pneumothorax	0 (0.0%)	3 (2.90%)	4 (2.80%)	0.793 *
Hemoptysis	0 (0.0%)	1 (1.0%)	1 (0.70%)	0.913 *
Hemorrhage	0 (0.0%)	1 (1.00%)	0 (0.0%)	0.468 *
Vasovagal attack	0 (0.0%)	0 (0.0%)	1 (0.70%)	0.654 *
Total No complications	16 (100%)	100 (95.23%)	138 (96.5%)	

* The chi-square test was used

We also checked for a potential relationship between the size of the tumor mass and the incidence of complications using the total number of all complications **(Table 3)**.

Table 3. Complications vs. size of tumor mass

Complications	<2 cm (n=13)	2-5 cm (n=102)	>5 cm (n=149)
Pneumothorax	1 (7.69%)	3 (2.94%)	3 (2.01%)
Hemoptysis	1 (7.69%)	0 (0.0%)	1 (0.67%)
Hemorrhage	0 (0.0%)	0 (0.0%)	1 (0.67%)
Vasovagal attack	0 (0.0%)	0 (0.0%)	1 (0.67%)
Total Complications	2 (15.38%)	3 (2.94%)	6 (4.02%)

Pain at the biopsy site, as a subjective factor, was observed in 6.30% of the patients under 50 years of age, in 18.10% of patients aged 50 to 65 years, and in 21% of patients over 66 years of age.

The percentage difference is small and does not reach statistical significance (p=0.347).

DISCUSSION

Based on 264 patients in our study, iatrogenic complications were found in 11 patients (4.17%).

Pneumothorax

Pneumothorax is the most common complication, with a reported frequency ranging from 0 to 61%, with an average of about twenty percent.[15-23] In our study, it is 2.66% (n=7). Previous research on this relationship has identified factors that contribute to the complication's high frequency rate. The size of the needle is important, as is the researcher's judgment and familiarity with this technique. Older patients (>70 years)[24,25] are more likely to have COPD and/or emphysema[26,27], which increases their risk of complications during biopsy. The same is true for people suffering from pulmonary bullous changes. In patients who underwent surgery of the lung on the same side, the frequency of pneumothorax is significantly lower due to the presence of pleural adhesions occurring after the surgery. Fibrous changes and pleural adhesions "fix" the lung parenchyma and help self-limit the pneumothorax. The patient's lifestyle, age, and comorbidities, the experience of the examiners and the institution in which they work should be considered before resorting to ultrasound-guided biopsy.

If a pneumothorax develops after the surgery, there are a few things that need to be done. Physical observation, vital sign monitoring, appropriate radiographic management, and oxygen administration as needed can all help manage a small, partial pneumothorax (decreased saturation below 90%). The pneumothorax can be manually aspirated[28] after the intervention with an aspiration system, if it is partial and does not grow (the procedure was performed in one patient). It is also recommended that the patient be placed biopsy-side down and receive oxygen with a nasal catheter. Pneumothorax unchanged after 4 hours under X-ray control are unlikely to grow.[16,17,22,29] A large pneumothorax with signs of shortness of breath require urgent intervention – the placement of a thin thoracolumbar tube and switching to active aspiration. A tension pneumothorax is a medical emergency: it is a condition that should be treated immediately with needle decompression followed by pleural drainage without waiting for further X-ray and other investigations.

In our study, the pneumothorax in 2 cases (28.57%) was partial, without pronounced clinical and physiological manifestations, which is why it did not require the placement of a thoracic drain. The patients recovered quickly, without the occurrence of new additional complications. In 5 patients (71.42%), the pneumothorax was more significantly manifested with shortness of breath, which is why it was necessary to insert a thin thoracic drain and switch to active aspiration. Our analysis of patients with pneumothorax found that the frequency of this complication is the greatest when attacking changed areas with a small area of stenosis to the parietal pleura, especially those that have no adhesion to it and are mobile during breathing. With area-specific stenting, we have not observed these complications in any case. The presence of bullous changes and emphysematous lung structure increases the risk of pneu-

mothorax. Fibrous changes and pleural adhesions limit the size of the pneumothorax. Forced cessation of respiratory excursions of the lung may sometimes be a reasonable factor in reducing the complication.[23] The occurrence of pneumothorax is considered risky when the biopsy needle passes through the pleural groove. Biopsied foci with cavitation are also risky due to possible communication of the latter with a bronchus.`

Hemorrhage and hemoptysis

Hemorrhage in the pleural cavity after US-TTCNB has an average frequency of 11% according to literature.[16,17,19,28] In our interventions, we observed hemorrhage in only one patient (0.38%), but it was an insignificant and clinically undetermined hemorrhage. The same one established at video-assisted thoracoscopic surgery was necessary in order to perform an atypical resection of the tumor mass assessed histologically with immunohistochemical examination from the material of the previously performed ultrasound-guided biopsy as a metastasis from colon carcinoma. Bleeding into the lung parenchyma can be seen in approximately 5% to 15% of lung biopsies. Pulmonary parenchymal bleeding after transthoracic biopsies can be diagnosed by lung ultrasound and may present as hemoptysis (seen in 1%-5%)

We observed hemoptysis in two (0.76%) of our patients. It appeared immediately after the intervention, already on the operating table, accompanied by a cough. In one of the patients, it continued over the next 24 hours with decreasing intensity against the background of hemostatic therapy.

Hemorrhages and hemoptyses are considered self-limiting and usually do not require intervention. However, patients should be placed biopsy-side down to prevent aspiration of blood into the contralateral lung.[30] It is imperative to initiate hemostatic therapy. Massive hemorrhages are very rare but may require anesthesia consultation for intubation with a double-lumen endotracheal tube.[21] Of course, patients who are taking anticoagulants or have a bleeding diathesis are contraindicated for the intervention until their coagulation status is normalized.

These complications can be prevented by using color Doppler to avoid taking material from areas of the lesion with a rich blood supply and/or suspicion of necrotic changes, as well as a proper assessment of the coagulation status.

We did not observe a hematoma in the area of the chest wall at the biopsy site in our study. We believe that in compliance with the algorithm of behavior when performing the procedure, the risk of hematoma is negligibly small and does not require significant therapeutic measures. The biopsy site should be pressed for a certain time and subsequently treated with an anticoagulant cream.

Vasovagal attack

Vasovagal attack is a rare complication: we observed it in one of our patients (0.38%). It started immediately during the procedure, after the second biopsy, and continued the next day. It manifested with cyanosis of the face and extremities, hypotension to collapse, tachypnea, and dyspnea. The ECG showed no significant deviations, except for tachycardia. The emergency echocardiography (due to suspicion of a pericardial lesion – the target area was in the immediate vicinity, located in the upper lobe on the left) showed no deviations from the norm. Saturation dropped by 10 units from baseline. After resuscitation, sedatives, and nasal oxygen 3 l/h, the condition was controlled by the next day.

We did not observe hematoma and tumor implantation at the biopsy site as well as air embolism described in the available literature as complications after the procedure.

Pain at the biopsy site

Pain at the biopsy site as a subjective factor during and after US-TTCNB is relevant and, therefore, we analyzed it as well (n=49; 18.50%). Some patients reported pain during the biopsy, which was associated with insufficient analgesia or insufficient waiting time to start the procedure when the anesthetic had not worked. In general, the pain is transient, appears after the analgesia wears off at the intervention site, rarely during the biopsy and very rarely the next day (according to literature data 3.1%). Biopsy is relatively well tolerated by patients and is easily controlled with the administration of analgesics. The difference in the percentages of the data reported in the literature and our study is most likely due to the criteria of the pain reports and whether they occur during the intervention or because of it, after the end of the effect of the infiltrative anesthesia, which, with good infiltration, is negligibly small and can be coped with.

CONCLUSION

Data analysis shows that transthoracic ultrasound biopsy is a method proven to be safe, effective, easy, affordable, real-time traceable and highly accurate for biopsy diagnosis. During the intervention, the actual progress of the needle is monitored. A real assessment of its position relative to the target lesion is made in the different phases of breathing when there is no adhesion with the parietal pleura. This is of great importance in small and/or hard-to-reach outbreaks. Reliable images made by CT within a month, with good resolution and the image reconstruction programs used, allow us to estimate with great accuracy the location of the target lesion and is a first step in conducting the study. Important in our practice regarding the size of the lesion is not its actual size, but the area of stenting. The results show that small lesions under 1.5 cm, with overlying bone structures, regardless of the obtained ultrasound image, are difficult to biopsy due to the impossibility of directing the cutting needle, regardless of a change in trajectory angle. Patient positioning and breathing management, if possible, greatly facilitate the accuracy of the procedure. Precisely planned manipulation in advance reduces the risk of complications

and taking material from an area with necrotic changes. For physically active and fit patients, the time for correct positioning is not missed time. Adherence to the principles of asepsis reduces the occurrence of inflammatory changes in the biopsy area to zero.

Potential errors when performing US-TTCNB can be grouped into several groups:

a) Patient-related factors

The lack of cooperation on his part, as well as some physiological needs, spontaneous coughing, sneezing, occurring at the time of the intervention can prevent its successful implementation and even lead to unwanted complications, especially if the pathological focus is located next to vitally important structures – pericardium, vena cava, aorta, etc.

b) Factors related to the performance technique

It depends largely on the experience of the surgical team performing the intervention. Accurate knowledge of the topographical anatomy of the underlying structures, as well as mastery of the echographic image, ensures the avoidance of these errors. In our case, the use of a 2-cm-launched cutting needle at the time of biopsy should be considered when marking the biopsy trajectory.

c) Factors related to the characteristics of the focus and the surrounding parenchyma

Needle control and positioning in small lesions and hidden under overlying bony structures (ribs, scapula, clavicle) often make ultrasound-guided biopsy difficult and can lead to potential errors. We mentioned that this problem is largely solvable with appropriate positioning of the patient, adjustment of breathing phases, and adjustment of the projection angle of the biopsy attachment.

The performance of US-TTCNB by a surgical team with an acquired thoracic specialty with sufficient experience is essential and a guarantee for successful management of any complications that may arise at the time of the biopsy. The main disadvantage of the intervention is its strong dependence on the experience and qualification of the operator.

Based on our analysis of patients with biopsy-related complications, we recommend the following treatment-diagnostic algorithm:

- Correct and in-depth assessment of the patients undergoing US-TTCNB regarding the specificities of the target lesion, the general and local status, the images from the X-ray examinations and the mental behavior.
- Appropriate positioning of the patient and correctly selected angle and trajectory of the biopsy course.
- Strict laboratory and hematological control and measures for its correction within the hospital stay before the diagnostic manipulation or in home conditions when the intervention is implemented as an outpatient procedure.

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Competing Interests

The authors have declared that no competing interests exist.

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Осложнения вследствие ультразвуковой трансторакальной режущей биопсии периферических поражений лёгких и поражений грудной стенки и средостения

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Резюме

Введение: Оценка пациентов с периферическими поражениями лёгких, грудной стенки и средостения является сложной задачей. Природу поражения, выявленного при визуализационных исследованиях, можно определить с помощью гистологической оценки биопсий. Важное место в этом направлении занимает всё возрастающая популярность среди торакальных хирургов трансторакальной биопсии режущей иглой под контролем УЗИ (US-TTCNB).

Цель: Целью данной статьи является описание потенциальных осложнений, которые могут возникнуть при трансторакальной инцизионной биопсии, выполняемой под ультразвуковым контролем, а также алгоритм процентного снижения и подходы к лечению.

Материалы и методы: Настоящее исследование основано на данных 264 пациентов с периферическими поражениями лёгких, грудной стенки и средостения, выявленных с помощью КТ, выполненной в отделении торакальной хирургии Университетской больницы „Каспела" в Пловдиве в течение 2 лет (январь 2020 г. – декабрь 2021 г.).

Результаты: Осложнения выявлены у 11 (4.17%) пациентов.

Заключение: Наша общепринятая методика биопсии позволяет получить достаточное количество биопсийного материала при низкой частоте постбиопсионных осложнений.

Ключевые слова

КТ, поражения лёгких, трансторакальная инцизионная биопсия под контролем УЗИ, УЗИ

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Original Article

Prognostic Models of Drug-Induced Neutralizing Antibody Formation in Patients with Rheumatoid Arthritis, Psoriatic Arthritis, Ankylosing Spondylitis Treated with TNF-α Blockers

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Abstract

Aim: This study aimed to construct prognostic mathematical models utilizing multifactorial regression analysis to assess the risk of developing drug-induced neutralizing antibodies in patients with rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis treated with tumor necrosis factor alpha blockers.

Materials and methods: Over a four-year period, we enrolled 213 patients in this study and divided them into three groups: the rheumatoid arthritis group (n=121), the ankylosing spondylitis group (n=50), and the psoriatic arthritis group (n=42). The study included also a group of healthy controls consisting of 31 healthy subjects who matched the patient groups in age, sex, body mass index, and conditions typical for rheumatology patients. Prognostic mathematical models based on statistically significant factors determined through univariate correlation and regression analyses incorporated patient medical history and serological and immunological data.

Results: The study encompassed all 213 patients and 31 healthy controls. Data analysis was conducted at 12 and 24 months after commencing treatment. During this follow-up, the patients exhibited the highest percentage of antidrug antibodies. At 6 months, 6.57% of patients had confirmed neutralizing antibodies, which increased to 12.69% at 12 months and 17.72% at 24 months. Multivariate logistic regression analysis revealed that factors such as age over 55 years, excess weight, smoking, and absence of methotrexate treatment at a dose less than 7.5 mg per week had the highest predictive value.

Conclusions: Investigating clinical and biological markers with predictive value for individual patients' therapeutic responses is a complex task. This complexity arises from the interplay of at least three distinct parameters: the patient's disease state, drug bioavailability, and pathophysiological changes within the patient's body, all of which are influenced by various factors.

Keywords

anti-drug antibodies, prognostic model

INTRODUCTION

The increasing prevalence of rheumatoid arthritis (RA), psoriatic arthritis (PsA), and ankylosing spondylitis (AS) has led to a growing reliance on tumor necrosis factor alpha (TNF-α) blockers for the management of these conditions. While these drugs have demonstrated efficacy in ameliorating symptoms and improving quality of life for patients, the emergence of drug-induced neutralizing antibodies poses a significant concern. These antibodies can compromise the effectiveness of TNF-α blockers, leading to diminished therapeutic outcomes and potential complications in patients undergoing treatment.

In light of this, there is a pressing need to develop robust prognostic models that can assess the risk of patients developing drug-induced neutralizing antibodies. To address this concern, our study employed multifactorial regression analysis to construct mathematical models for evaluating the risk in patients with RA, PsA, and AS. This involves an in-depth examination of various demographic, clinical, and immunological factors, identified through univariate correlation and regression analyses, to ensure the models are based on statistically significant variables.[1-3]

Existing literature underscores the importance of considering patient medical history, serological, and immunological data in constructing predictive models for drug-induced neutralizing antibodies.[4] Despite the acknowledged significance of these factors, there remains a dearth of comprehensive studies that integrate them into a unified prognostic framework. Our research seeks to bridge this gap by providing a thorough exploration of the interplay between demographic, clinical, and immunological factors, emphasizing their collective impact on the development of neutralizing antibodies in patients treated with TNF-α blockers.

AIM

This study aims to contribute not only prognostic models but also a deeper understanding of the intricate relationships between patient characteristics and the risk of developing neutralizing antibodies. By addressing this gap in the literature, we strive to enhance the precision of risk assessment and, subsequently, the effectiveness of therapeutic interventions in patients undergoing TNF-α blocker treatment.

MATERIALS AND METHODS

This prospective, longitudinal, open-label study spanned a four-year period, involving a comprehensive assessment of 213 patients diagnosed with rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis. The study also included a control group consisting of 31 healthy individuals. The patient groups were further divided into two categories based on the type of biological agent used: adalimumab and etanercept.

A cross-sectional, multicenter observational approach was employed for data collection. After obtaining informed consent, patients were interviewed, examined, and their information was collected and entered into files for subsequent statistical analysis. Self-assessment questionnaires were voluntarily completed by patients, and blood samples were taken for the assessment of cytokines (TNF-α and IL-6) and the detection of drug-induced neutralizing antibodies. Some patients, particularly those treated with adalimumab, were additionally tested for drug bioavailability six months after treatment initiation.

Participant groups

The patients included 121 with RA, 50 with AS, and 42 with PsA, while the control group comprised 31 healthy individuals. The demographic characteristics, disease-specific details, and medication information for each group were meticulously recorded **(Table 1)**.

These categorizations were implemented to allow a comprehensive analysis of the impact of demographic, clinical, and immunological factors on the development of

Table 1. Number of individuals monitored – patients and healthy controls by number, age, sex, underlying disease, and disease duration (x±Sx)

Indicators	Patients treated with adalimumab	Patients treated with etanercept	Healthy control individuals
Number	121	92	31
Main disease, number of patients			
Rheumatoid arthritis	79	42	
Ankylosing spondylitis	16	34	
Psoriatic arthritis	26	16	
Age, years	49.8±10.8	45.6±13.4	48.7±2.2
Sex			
Men, n (%)	43 (35.54%)	47 (51.08%)	15 (48.28%)
Women, n (%)	78 (64.46%)	45 (48.02%)	16 (51.62%)

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drug-induced neutralizing antibodies in patients treated with TNF-α blockers. The rationale for grouping patients based on both disease type and medication is to account for potential variations in treatment response and antibody development among different rheumatological conditions and specific medications.

Hypotheses

The null hypothesis (H0) posited that the presence of neutralizing antibodies to TNF-α blockers used in the treatment of severe forms of RA, AS, and PsA is clinically relevant, affecting the course of the disease. Conversely, the alternative hypothesis (H1) proposed that these antibodies do not influence the disease's progression.

Inclusion and exclusion criteria

Patients meeting the established diagnostic and classification criteria for RA, PsA, and AS and undergoing treatment with adalimumab or etanercept were included. Exclusion criteria were carefully defined to ensure the selection of eligible participants.

Study procedures

This study utilized a variety of clinical and laboratory methods **(Table 2)**, including comprehensive medical history and examination, pain assessment using a visual analogue scale (VAS), disease activity evaluations specific to each condition, and assessment tools such as Disease Activity Score in 28 joints (DAS-28), Bath Ankylosing Spondylitis Disease Activity Index (BASDAI), Disease Activity in Psoriatic Arthritis (DAPSA), and Health Assessment Questionnaire – Disability Index (HAQ-DI).

Table 2. Clinical and laboratory studies performed during patient visits

Procedure	0 mos	6 mos	12 mos	24 mos
Medical history	•	•	•	•
Physical examination	•	•	•	•
Pain assessment (VAS)	•	•	•	•
Disease activity indices	•	•	•	•
CRP	•	•	•	•
ESR	•	•	•	•
Serum levels of drug induced neutralizing antibodies	•	•	•	•
Bioavailability of the drug		•	•	•

Self-assessment questionnaires

Patients voluntarily filled out two key self-assessment questionnaires to provide information about their subjective experiences and perceptions of their rheumatological conditions.

Visual Analogue Scale (VAS)

Patients used a VAS to subjectively indicate their pain intensity at the time of examination. This scale allowed patients to express their pain levels on a continuous scale, providing valuable information on pain perception.

Health Assessment Questionnaire Disability Index (HAQ-DI)

HAQ-DI was employed to assess patients' quality of life by examining their ability to perform daily activities. Higher HAQ-DI values indicated greater disability, allowing for an evaluation of the impact of rheumatological conditions on daily functioning.

Laboratory investigations

These included:

Complete blood count (CBC): assessing the number and types of blood cells to evaluate general health and detect abnormalities.

Erythrocyte sedimentation rate (ESR): a non-specific measure of inflammation, indicating the presence of an inflammatory condition.

C-reactive protein (CRP): another marker of inflammation often used to monitor disease activity.

Cytokine assays: TNF-α and IL-6: these were assayed using ELISA methodology with specific kits to measure the levels of these proinflammatory cytokines.

Determination of neutralizing antibodies

Antidrug antibodies

Assayed for etanercept (Enbrel) and adalimumab (Humira) using ELISA with Immundiagnostik kits.

Determination of drug bioavailability

Assessed in two independent laboratories using ELISA methodology from Immundiagnostik.

Observation parameters

Patient visits at 0, 6, 12, and 24 months included a thorough examination of medical history, physical examination, pain assessment, disease activity indices, and relevant laboratory tests.

Study locations

The research was conducted at the Department of Propaedeutics of Internal Medicine, Medical University of Plovdiv, as well as in outpatient clinics and rheumatology departments. Quantitative analyses of cytokine levels, drug bioavailability, and drug-induced neutralizing antibodies were conducted in specified laboratories under the supervision of qualified professionals.

Statistical analysis

Data analysis involved a rigorous process, including verification, coding, and entry into a computer database. SPSS v. 24 was employed for statistical analyses, encompassing descriptive statistics, parametric and non-parametric tests, linear regression, binary logistic analysis, and ROC analysis. The homogeneity of variations was assessed using the Levene equality test, and correlations in non-normally distributed data were explored using Spearman rank analysis. Multivariate regression analysis was utilized to calculate the relative risk (RR) for disease development. The inclusion of a wide array of clinical and laboratory variables allowed for a comprehensive evaluation of the impact of TNF-α blockers on patients with RA, AS, and PsA.

RESULTS

The process of constructing prognostic models for the emergence of drug-induced neutralizing antibodies in patients with RA, PsA, and AS treated with TNF-α blockers within the analyzed patient group followed these steps:

- Identifying a sample of 213 patients to isolate independent, statistically significant prognostic factors for the emergence of drug-induced neutralizing antibodies in these patients.
- Analyzing all factors proven to be statistically significant in univariate analysis through multivariate analysis, employing a regression model with a stepwise selection procedure.
- Ensuring the proportionality of all covariates included in the regression model.
- Investigating the existence of individual data instances with notably deviating values for the model-influencing covariates.
- Repeating a multifactorial analysis involving all 213 patients.
- Evaluating the goodness-of-fit of the model, which assesses its adequacy in describing the resulting variable.

The significant variables identified through unifactorial regression analysis or correlation analysis were tested to establish a predictive mathematical model for the emergence of drug-induced neutralizing antibodies in patients with RA, PsA, and AS treated with TNF-α blockers. These variables included gender, age, education, excess weight, smoking, alcohol intake, concomitant pathology, dosage regimen of methotrexate below 10 mg per week, disease severity, ESR ≥28 mm, CRP ≥10 mg/L, VAS for pain as assessed by the patient, scales and activity indices such as HAQ-DI, DAS-28 for patients with RA, BASFI, ASDAS, BASDAI for patients with AS, and DAPSA for patients with PsA. Additionally, the levels of TNF-α and IL-6, as determined through ROC analysis, played a role in distinguishing patients from healthy controls, as well as the bioavailability of adalimumab exceeding the specified laboratory threshold value.

The equation used to calculate the risk likelihood estimate is as follows:

Z – linear combination
Z = Vo+B1o1+B2o2+B3o3+... In Hoch
E – Non-zero number – 2.71.
Vo – represents the constant unique to each mathematical model of probabilities
B1o1 – constant of the first indicator
B2o2 – constant of the second indicator, and so forth

High risk of drug-induced neutralizing antibodies in patients with RA, PsA, and AS treated with TNF-α blockers was associated with factors including age over 55 years, BMI over 25, smoking, methotrexate dosage below 10 mg per week, and disease duration exceeding 12 years, as shown in **Table 3**.

Table 3. A prognostic mathematical model for the emergence of drug-induced neutralizing antibodies in patients with RA, PsA, AS treated with TNF-α blockers by demographic and patient history evaluation – multivariate regression analysis

Indicators	Regression coefficient (B±SE)	Relative risk	95% CI for EXP (B)		R
			Lower	Upper	
Age 55+	4.869±1.038	3.0166	1.005	9.383	0.000
BMI over 25.2770	0.802±0.264	2.229	1.328	3.741	0.002
Smoking more than 15 cigarettes a day	4.772±1.037	1.182	1.1820	6.8208	0.000
Use of methotrexate below 7.5 mg/week	1.342±0.321	5.6201	3.541	7.228	0.000
Disease duration over 15 years	1.868±0.229	1.154	1.154	1.154	0.000
Constant	−4.519±1.069	0.001			0.000

χ^2 =61.34, K=5, p<0.001

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Our mathematical models are based on patient history information and the medical documentation provided by patients at their initial appointments with the physician. These models encompass a comprehensive range of sequential serological and immunological parameters.

A heightened risk of developing drug-induced neutralizing antibodies in patients with RA, PsA, and AS treated with TNF-α blockers is associated with specific criteria, including:

- Elevated ESR ≥28 mm
- Elevated CRP ≥10 mg/L
- Significantly different levels of TNF-α and IL-6 compared to the control group of individuals corresponding to the respective disease
- Evaluation of adalimumab drug bioavailability conducted prior to treatment initiation **(Table 4)**.

These criteria aid in identifying patients with a higher risk of developing drug-induced neutralizing antibodies.

A simplified predictive mathematical model, information on disease limitation, morning stiffness, and disease activity indexes were obtained in Steps 3 and 4 by multivariate regression analysis **(Table 5)**.

This model is preferable because it only collects information from the patient without the need for clinical laboratory tests.

The following mathematical model was obtained with the serological indicators included, drug bioavailability of adalimumab (model for RA patients only), and activity indices **(Table 6)**.

Table 4. A prognostic mathematical model for the emergence of drug-induced neutralizing antibodies in patients with RA, PsA, AS treated with TNF-α blockers by evaluation of serological and immunological parameters – multivariate regression analysis (RR)

Factors	Regression coefficient (B±SE)	Relative risk	95% CI		*P*
			Lower	Upper	
ESR >48 mm	4.137±0.909	3.627	1.542	7.202	<0.0001
CRP >48 mg/L	4.305±0.797	4.085	3.559	13,520	<0.0001
TNF-α for					
RA=40.8000 pg/ml	2.330±0.947	2.120	2.067	13.781	<0.0001
AS=42.1200 pg/ml	0.394±0.559	1.775	1.994	11.223	<0.0001
PsA=53.500 pg/ml	6.123±1.048	5.762	3.341	13.440	<0.0001
IL-6 ng/ml for					
RA=14.2150 ng/ml	1.378±1.019	3.969	0.542	9.412	<0.0001
AS=13.8600 ng/ml	1.322±0.992	2.004	1.116	4.443	<0.0001
PsA=13.5500 ng/ml	6.455±2.058	5.391	5.922	12.390	<0.0001
Medicinal bioavailability of adalimumab – below 3.3250 ng/ml	2.682±0.758	4.9182	3.312	8.5616	<0.0001

χ^2 =53.29, K=5, p<0.001; RA: rheumatoid arthritis; AS: ankylosing spondylitis; PsA: psoriatic arthritis

Table 5. A prognostic mathematical model for the occurrence of drug-induced neutralizing antibodies in patients with RA, PsA, AS treated with TNF-α blockers by scale evaluation and disease activity indices – multivariate regression analysis (RR)

Factors	Regression coefficient (B±SE)	Relative risk	95% CI for EXP (B)		R
			Lower	Upper	
Morning stiffness over 58 minutes	1.819±0.343	3.164	1.147	6.076	<0.0001
HAQ-DI >2.3239	2.246±0.316	4.453	3.099	7.973	<0.0001
Duration of the disease over 15 years	3.401±0.657	3.526	2.930	9.257	<0.0001
Activity indices					
RA – DAS-28 >6.10	2.633±0.393	3.918	1.9946	10.096	<0.0001
AS – BASDAI >5.1400	3.981±1.221	4.9221	3.772	7.248	<0.0001
PsA – DAPSA >38.0	1.665± 0.779	1.955	1.008	3.883	<0.0001
Constant	−3.221±0.546	0.004			<0.001

χ^2 (chi-square)=34.12, K=4, p<0.001; RA: rheumatoid arthritis; AS: ankylosing spondylitis; PsA: psoriatic arthritis

Table 6. A prognostic mathematical model for the emergence of drug-induced neutralizing antibodies in patients with RA, PsA, AS treated with TNF-α blockers by serological evaluation, drug bioavailability for adalimumab and disease activity indices – multifactorial regression analysis (RR)

Factors	Regression coefficient (B±SE)	Relative risk	95% CI for EXP (B)		R
			Lower	Upper	<0.0001
ESR >48 mm	1.856±0.354	2.397	1.992	5.198	<0.0001
CRP >48 mg/L	2.063±0.328	3.869	2.303	8.135	<0.0001
Drug bioavailability of adalimumab – below 3.3250 ng/ml (PA)	2.303±0.389	5.003	4.665	11.391	<0.0001
VAS – for pain over 70.16 mm	2.302±0.391	3.990	1.646	7.480	<0.001
Activity indices					
RA – DAS-28 >6.10	1.503±0.347	4.494	2.278	8.867	<0.0001
AS – BASDAI >5.14	2.345±0.925	3.0661	1.899	8.072	
PsA – DAPSA >38.09	1.503±0.347	2.774	1.404	5.553	
Constant	−6.243±0.597	0.002			<0.0001

χ^2 =31.44, K=5, p<0.001; RA: rheumatoid arthritis; AS: ankylosing spondylitis; PsA: psoriatic arthritis

DISCUSSION

The pursuit of identifying predictors of a favorable clinical response to TNF-α blockers in patients with inflammatory joint diseases, such as RA, AS, and PsA, has been acknowledged in numerous scientific studies.[5-8] According to Hyrich et al.[5], they found a correlation between higher HAQ scores and reduced therapy efficacy, while the concomitant use of NSAIDs and MTX intake was associated with improved clinical responses in patients with RA. Interestingly, this study also revealed that smokers exhibited a less favorable response to therapy, while factors like patient age, disease duration, and previous DMARD use did not significantly impact therapy effectiveness.[5] However, it is worth noting that despite discussing risk factors, these authors did not consolidate them into predictive mathematical models.

In our research, we have constructed several mathematical models aimed at predicting the development of drug-induced neutralizing antibodies in patients with RA, PsA, and AS undergoing treatment with TNF-α blockers. These models incorporate a wide array of demographic, serological, immunological, and disease activity index data. It is important to recognize that in practical clinical settings, physicians may not always be able to assess cytokine levels, drug bioavailability, or antibodies to the medication used. Furthermore, we did not include sex as a predictive element in our models, as our studies indicated that sex does not hold predictive value. Interestingly, these findings differ from those reported by Arends et al.[9], who suggested that younger age, male sex, higher ASDAS values, lower ESR and CRP values, and elevated PtGADA values hold prognostic value for the effectiveness of TNF-α blocker treatment.

In our evaluation of the prognostic value of serum TNF-α and IL-6 levels, we sought to create specific predictive models for each disease, incorporating only these two cytokines. While the medical literature recommends the study of the Cytokine Activity Index, which includes an array of cytokines, such as TNF-α, IL-1, IL-6, GM-CSF, IL-4, IL-5, IL-13, and others[10,11], practical considerations led us to propose predictive mathematical models that focus on the two cytokines most relevant to our study.

Our analysis considered both groups of patients, those with and without serum-neutralizing antibodies treated with TNF-α blockers, as a unified cohort. This approach was adopted because, despite variances in the etiology of RA, AS, and PsA, the ultimate outcome of treatment, as indirectly measured by the presence of neutralizing antibodies, exhibited no substantial difference at 12 and 24 months from the initiation of therapy. These findings diverge from those of Maneiro et al.[12], who suggested that factors like younger age, male sex, high baseline BASDAI, low baseline ESR, low baseline CRP, and HLA-B27 positivity are predictive of a better response to TNF-antagonist treatment in AS patients but not in those with PsA.

Each of the prognostic mathematical models that integrated pharmacovigilance data related to adalimumab's bioavailability yielded highly significant results. This underscores the findings of Jani et al.[13,14], who reported that adalimumab's bioavailability is the most reliable predictor of changes in DAS-28 at the 12-month mark in RA patients monitored over a year. They found a strong regression coefficient of 0.060 (95% CI 0.015, 0.10, p=0.009). These authors, like us, concluded that pharmacological assessments of patients receiving TNF-α blockers offer clinical utility, even in the absence of drug-induced antibodies.

Despite the numerous predictive factors identified for response to biological therapies, consensus among research-

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ers is only shared for a select few. Predicting outcomes in severe rheumatic diseases, indicated by a high HAQ score and prior treatment failure, remains a challenging endeavor, as a high baseline DAS-28 predicts a stronger DAS-28 in a follow-up, irrespective of the type of treatment received. Conversely, younger age, smoking cessation, alcohol abstinence, lower body weight, and a negative serological status prove to be reliable predictors of TNF-α blocker treatment efficacy.

CONCLUSION

The pursuit of clinical and biological markers with predictive value for therapeutic responses in individual patients remains a formidable task. The complexity arises from the fact that treatment responses depend on at least three distinct parameters: the disease's state, drug bioavailability, and the pathophysiological changes occurring in the affected organism. Moreover, each of these parameters is influenced by a myriad of other factors.

Ethics Committee Approval

This study was approved by the Ethics Committee of the Medical University of Plovdiv (approval No. DP- P-8278 /02/13/2018)

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Competing Interests

The authors have declared that no competing interests exist.

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Прогностические модели лекарственно-индуцированного образования нейтрализующих антител у больных ревматоидным артритом, псориатическим артритом, анкилозирующим спондилитом, получающих блокаторы TNF-α

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Резюме

Цель: Целью данного исследования было создание прогностических математических моделей с использованием многофакторного регрессионного анализа для оценки риска развития лекарственно-индуцированных нейтрализующих антител у пациентов с ревматоидным артритом, псориатическим артритом и анкилозирующим спондилитом, получавших альфа-блокаторы фактора некроза опухоли.

Материалы и методы: В течение четырёх лет мы включили в исследование 213 пациентов и разделили их на три группы: группу ревматоидного артрита (n=121), группу болезни Бехтерева (n=50) и группу псориатического артрита (n=42). В исследование была включена также контрольная группа из здоровых людей, состоящая из 31 здорового человека, соответствующих группам пациентов по возрасту, полу, индексу массы тела и состояниям, типичным для больных ревматологией. Прогностические математические модели, основанные на статистически значимых факторах, определённых с помощью одномерного корреляционного и регрессионного анализа, включали историю болезни пациента, а также серологические и иммунологические данные.

Результаты: В исследование были включены все 213 пациентов и 31 здоровый человек из контрольной группы. Анализ данных проводился через 12 и 24 месяца после начала лечения. Во время этого наблюдения у пациентов наблюдался самый высокий процент антилекарственных антител. Через 6 месяцев у 6.57 % пациентов были подтверждены нейтрализующие антитела, которые увеличились до 12.69 % через 12 месяцев и 17.72 % через 24 месяца. Многофакторный логистический регрессионный анализ показал, что наибольшую прогностическую ценность имели такие факторы, как возраст старше 55 лет, избыточный вес, курение и отсутствие лечения метотрексатом в дозе менее 7.5 mg в неделю.

Заключение: Исследование клинических и биологических маркеров, имеющих прогностическую ценность для терапевтического ответа отдельных пациентов, является сложной задачей. Эта сложность возникает из-за взаимодействия по крайней мере трёх различных параметров: состояния заболевания пациента, биодоступности лекарства и патофизиологических изменений в организме пациента, каждый из которых находится под влиянием различных факторов.

Ключевые слова

антилекарственные антитела, прогностическая модель

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Original Article

Can FDG-PET Assess the Response to Chemotherapy and Predict Tissue Necrosis in Osteosarcoma and Ewing Sarcoma?

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Abstract

Introduction: Osteosarcoma (OS) and Ewing sarcoma (ES) represent the pediatric population's most common malignant bone tumors. 18-Fluorodeoxyglucose positron emission tomography has been shown to be effective in both the diagnostic and staging phases of cancer treatment. In recent years, some studies have also explored the possibility that FDG-PET could have a prognostic role.

Aim: Our research aimed to evaluate if maximum standardized uptake value (SUVmax) variations after chemotherapy could be correlated with tissue necrosis and be linked with patients' survival rates.

Materials and methods: This observational retrospective study included all cases treated for skeletal OS or ES in our institution between 2006 and 2018. We recorded patients' SUVmax values before and after chemotherapy, the necrosis grade (for those who received surgery), and survivorship. Forty-one cases (17 OS and 24 ES) were included. Among the 36 cases that received surgery, 15 were responders, and 20 were non-responders.

Results: Our data suggested a statistically significant correlation between tumor necrosis and differential SUVmax after neoadjuvant treatment (p=0.007). In particular, cases with differential SUVmax higher than 4.7 or a variation higher than 63% had better oncological outcomes.

Conclusion: Our study testifies to the effectiveness of FDG-PET in predicting tissue necrosis on ES and OS, thereby representing a promising prognostic factor.

Keywords

Ewing sarcoma, necrosis, osteosarcoma, PET, prognosis

INTRODUCTION

Osteosarcoma and Ewing sarcoma represent two main challenges in oncologic orthopedics, the two most frequent forms of sarcomas in the pediatric population.[1,2] The survival rate has been significantly improved thanks to a better understanding of the disease within the context of a multidisciplinary approach.

The introduction of adjuvant and neoadjuvant chemotherapy helped raise the survival rate from 15% to 60%.[3] For this reason, being able to evaluate a good response to therapy has been proven to be one of the main prognostic factors.

In modern oncology, fluorine-18-fluorodeoxyglucose positron emission tomography combined with computed tomography (FDG-PET/CT) plays a pivotal role in assessing the diagnosis and staging both Ewing sarcoma and osteosarcoma.[4-9] In recent years, some authors suggested that this exam could also potentially evaluate patients' early response to neoadjuvant chemotherapy, thereby representing a prognostic indicator.[10]

AIM

In this study, we evaluated the evolution of maximum standardized uptake value (SUVmax) values before and after chemotherapy in patients with Ewing sarcoma and osteosarcoma, investigating whether variations of carbohydrate metabolism were associated with the share of tissue necrosis on the surgical specimen.

MATERIALS AND METHODS

This single-center retrospective study was performed following the ethical standards of the 1964 Declaration of Helsinki and its later amendments.

Our study consisted of an observational retrospective study of cases treated for skeletal osteosarcoma or Ewing sarcoma in our institution between 2006 and 2018. An inclusion criterion was a multidisciplinary approach, combining neoadjuvant chemotherapy, surgical resection, and adjuvant chemotherapy. Another criterion was the execution of FDG-PET/CT scans at the moment of diagnosis (t0) **(Fig. 1)**, before surgery (t1), and at the end of the therapeutic path (t2).

Time t0 was established as the date of diagnosis before the patient had begun any treatment. T1 represents the end of the neoadjuvant chemotherapy and before the day of surgery. T2 expresses the date on which the last PET-CT scan was performed, approximately one month after the end of the adjuvant chemotherapy. An FDG-PET-TB scanner and a CT system were used in parallel for this study. Before performing the PET-CT, each individual was prepared by monitoring their blood glucose levels. The procedure was carried out exclusively if values were below 150 mg/dL. Image acquisition took place 60 minutes after intravenous administration of the radiopharmaceutical. PET data were obtained in a Whole-Body 3D mode and then corrected for tissue attenuation by low-dose CT acquisition without MDC administration. The uptake of FDG was defined as pathological when it was greater than the adjacent normal bone tissue. Areas with abnormal FDG uptake and their extension were assessed with SUV considering the following parameters: the amount of FDG injected, patient body weight, and regional uptake. The SUVmax of the region of interest was calculated using the following equation: (metabolic activity/volume unit) / (injected dose of radiopharmaceutical / patient body weight).

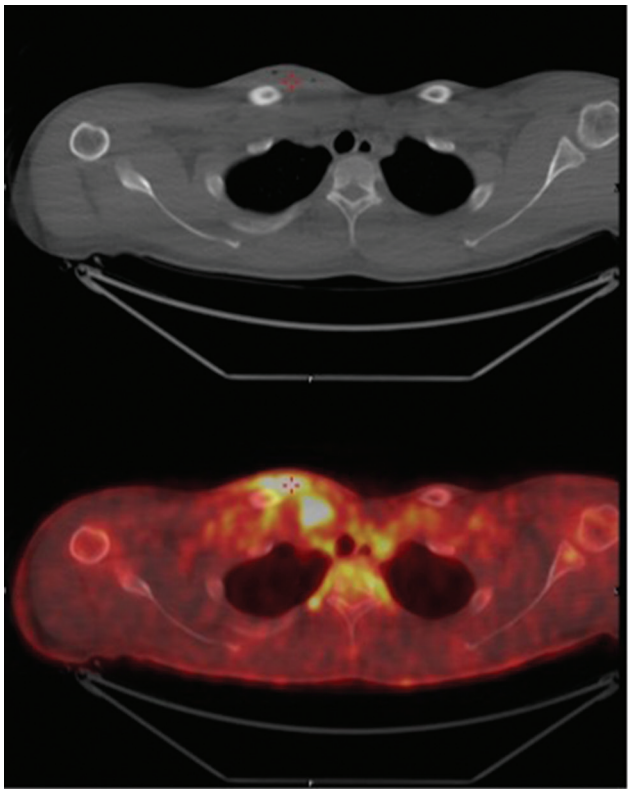

Figure 1. FDG-PET/TC scans of a case with Ewing sarcoma of the left clavicle before chemotherapy (t0).

For each patient, personal data, such as age, sex, and clinical presentation, were collected. The diagnosis was established after a biopsy, according to the findings of our anatomical pathology unit. Cases were divided into subgroups according to their histopathological diagnosis, and we investigated whether the lesion treated was solitary or if the patient already had the disease in other anatomical districts. After surgery, the resected tissue was set to pathological analysis to confirm the previous diagnosis and estimate the necrosis grade, which was classified according to the Huvos or Picci criteria for osteosarcomas and Ewing's sarcomas. Those cases involving 90% or more of the neoplastic volume were considered good responders, whereas those whose necrosis was lower than 90% were considered poor responders. All patients were treated according to the latest ESMO guidelines.[11]

Local SUVMax at t0, t1, and t2 were recorded and compared.

Patients' follow-ups were performed both in oncological and orthopedic units. Survival was noted throughout the whole follow-up period. Local recurrences and metastatic lesions diagnosed during the post-operative intercourse were reported, as well as their subsequent treatment.

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Statistical analysis

Statistical analysis was performed using Stata SE 13 (StataCorp LLC, College Station, TX). Statistical significance was set at 0.05 for all endpoints.

RESULTS

Our review consisted of 41 cases, seven females and 34 males. Their mean age at diagnosis was 21.7 years (range, 3-44 years). Seventeen patients suffered from osteosarcoma: 10 of them were diagnosed with osteoblastic osteosarcoma, 5 had chondroblasts or fibroblastic lesions, whereas a telangiectasic lesion was diagnosed in the remaining 2 cases. Among those 17, eleven had a single localized lesion at the moment of diagnosis, while the other five already had at least one metastasis. Twenty-four patients were diagnosed with Ewing sarcoma. The disease was localized in 13 cases and spread systemically in 11. Femur was the most frequent localization (16 cases, 39.0%), followed by the pelvis (8, 19.5%), humerus (5, 12.2%), and tibia (4, 9.8%).

Among our 41 cases, 36 were able to complete their therapeutic path. The other 6 had a rapid progression of the disease and died before they could undergo surgery. Of those 36 patients, 15 responded to chemotherapy, and 20 were non-responders. The mean necrosis percentage on the surgical specimen was 68.3% (9-100%).

The mean SUVmax was 10.1 at t0, 5.0 at t1 and 3.2 at t2. The mean differential between SUVmax at t0 and t1 was 5.56, and none of our cases increased their SUVmax value between t0 and t1. In particular, responders (tumor necrosis >90%) had a mean SUVmax variation between t0 and t1 of 8.6, whereas the same differential in non-responders was only 3.0. These findings were supported by the Student's *t*-test, which assessed that cases with a good response regarding tumor necrosis had a significantly higher SUVmax variation between t0 and t1 compared to poor responders (p=0.014).

Our data suggested a statistically significant correlation between tumor necrosis and SUVmax variation between t0 and t1 (p=0.007) and between t0 and t2 (p=0.006) according to Pearson correlation tests. This result suggests a strict link between necrosis and FDG-PET results. A linear regression analysis identified a linear correlation between the percentage of tissue necrosis and the reduction of SUVmax values. Inside our population, the mean variation between t0 and t1 could be approximated using the equation ΔSUVmax [t0;t1] = −1.60+(0.10 × necrosis percentage) **(Fig. 2)**, whereas the one for the variation between t0 and t2 would be ΔSUVmax [t0;t1] = −1.05+(0.13 × necrosis percentage).

Conversely, those who had a persistent SUVmax between the three PET evaluations (ΔSUVmax [t0;t1] <1.0 and ΔSUVmax [t0;t1] <2.0) had mean necrosis at the surgery of 55%, whereas those who saw a remarkable reduction in their SUVmax values (ΔSUVmax [t0;t1] >1.0 and ΔSUVmax [t0;t1] >2.0) had mean necrosis of 85%.

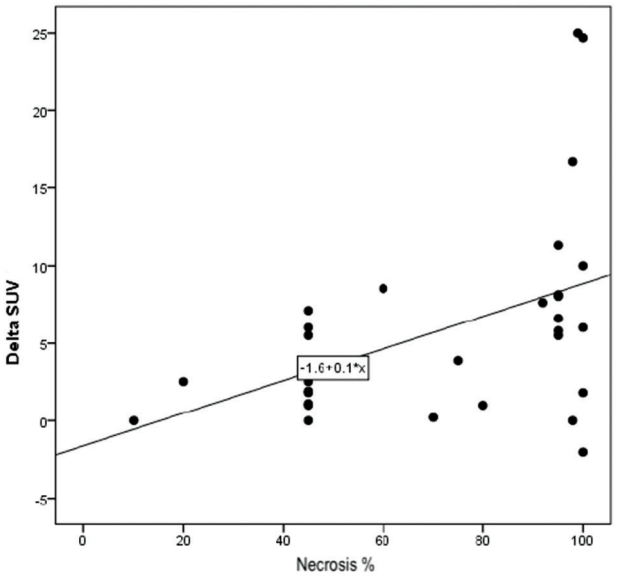

Figure 2. Linear approximation of cases' distribution in terms of differential SUVmax and percentual tissue necrosis.

Evaluating the correlation between SUVmax differentials and surgical necrosis, we searched for a cut-off value that could predict whether the patient would respond to systemic treatment. In our series, establishing the cut-offs for the periods t0-t1 and t0-t2 at 4.7 (or a reduction of 63% of the previous value) and 7.5, respectively, the results were good sensibility (80-78%) and specificity (72-77%) values for assessing patients' response to chemotherapy. In particular, cases with differential SUVmax between t0 and t1 lower than 4.7 or a percentage reduction higher than 63% had a mean event-free survival of 15.6 months and an overall survival of 34.4 months. The remaining patients, instead, had significantly higher values of event-free survival (30.6 months) and overall survival (63.3 months).

Cases with a t1 value equal to or lower than 2.5 had a significantly lower incidence of local recurrence at the patient's latest follow-up (exact Fisher's test: p=0.038).

The Event-Free Survival (EFS) at five years was 73% for the responders and 22% for the non-responders, testifying to the role of tissue necrosis as a statistically significant predictive factor (exact Fisher's test, p=0.009) **(Fig. 3)**.

DISCUSSION

The fluorine-18-fluorodeoxyglucose positron emission tomography combined with computed tomography (FDG-PET/CT) is an imaging tool that measures a tumor's metabolic activity.[12]

Over the last decades, the importance of this exam for diagnosing and staging malignant tumors has been progressively increasing. In fact, since its introduction in clinical practice, the FDG-PET/CT has been appreciated for its capacity to dovetail imaging evidence of the tumoral masses with an assessment of their metabolic activity. It is,

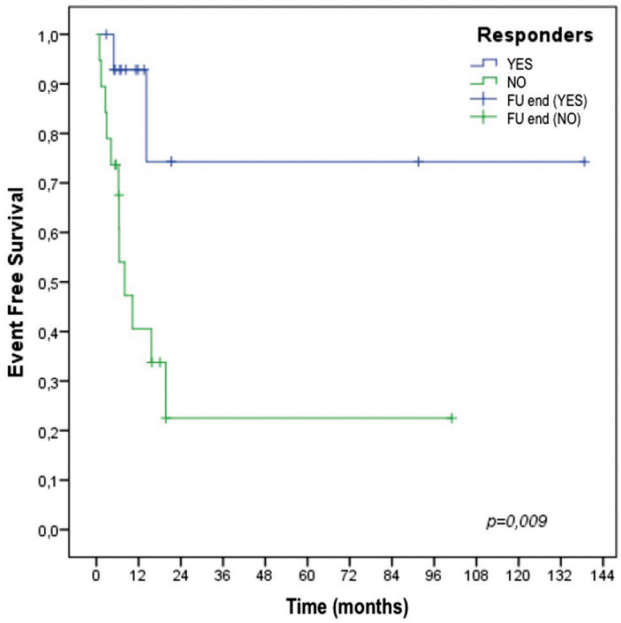

Figure 3. Kaplan Meier curves of patient's Event-Free Survival through their follow-up. The population was divided into responders (blue line) and non-responders (green line).

therefore, a common-use exam for improving the staging accuracy for malignant bone and soft tumors due to its high sensitivity.[13,14]

However, the information provided by the exam is not limited to recognizing areas with increased glucosidic uptake with a reasonable neoplastic nature. It also often allows a more profound and better comprehension of its characteristics. High-grade tumors should have higher standard uptake values (SUV) than low-grade tumors, allowing them to distinguish between different malignancy histologic grades and act as a surrogate marker for tumor grade.[15-20] It has been demonstrated that upregulation of glucose transporter type 1 (GLUT-1) in cancer cells, an increased tendency to cellular glucose intake, and 18F-FDG avidity represent a prognostic factor in various malignancies.[21-23] These findings could explain the relationship between FDG uptake on PET and tumor aggressiveness and suggest PET values as direct prognostic factors in cancer prognosis.[24-26]

FDG-PET/CT has been proven to be a useful tool to evaluate interim response to antibiotic therapy in patients affected by hematogenous spondylodiscitis[27], and others investigated whether it could represent a prognostic factor also in malignant bone or soft tissue sarcomas treatment[26].

In 2017, Bailly et al. analyzed a large cohort of 62 pediatric patients with ES and OS the potential prognostic role of PET/CT parameter, including SUVmax, but they could not find histological response to therapy or overall survival.[28]

In the same year, Palmerini et al. published their experience with a population of 77 cases suffering from Ewing sarcoma or osteosarcoma. In their cases with Ewing sarcoma, the SUVmax recorded at the moment of diagnosis was the only independent pretreatment prognostic factor to retain statistical significance. On the other hand, the same statement was not valid for their cases with osteosarcoma, and neither SUVmax at the end of chemotherapy nor the differential between the two values appeared as statistically significant prognostic factors in their casuistry.[29]

Hawkins et al. had more encouraging results in their population of 36 Ewing sarcomas. Their mean SUVmax value before chemotherapy was 7.9, while the same value decreased to 2.1 at the end of the medical treatment. Patients' SUVmax after chemotherapy was also significantly associated with progression-free survival. The authors proposed a value of 2.5 or lower to represent a positive prognostic factor for disease control and survival.[30] Raciborska et al.[31] obtained comparable results, which found a significant correlation between SUVmax and clinical outcomes after chemotherapy. The median value of those who experienced a disease progression was significantly higher than those who had a better outcome. Furthermore, the study confirmed a positive predictive value of 2.5 or lower for a favorable response to the therapeutic approach as a whole.[31]

Our results corroborate the idea that cases with an SUVmax value below 2.5 after chemotherapy suggest lower risks of local recurrence.

We could also identify a strict link between necrosis and FDG-PET results. Cases with a necrosis percentage above 90% had significantly higher reductions of their SUVmax values after chemotherapy (Student's *t*-test; p=0.014). Furthermore, we detected a statistically significant linear correlation between percentual tumor necrosis on surgical specimens and SUVmax variation (Pearson correlation test; p=0.007). In line with our outcomes, we suggest an overall reduction of 4.7 or a cut of 63% between SUVmax values at the diagnosis and after chemotherapy in order to assess the response to treatment: cases in which chemotherapy induced a SUVmax reduction equal or greater than 4.7 overall or higher than 63% of the first record should theoretically be considered as good responders, whereas lower values should orientate toward a negative response. This cut-off was associated with good sensibility (80%) and specificity (72%) in our population.

Furthermore, the necrotic response was a statistically significant predictive factor for patients' EFS, supporting the idea that SUVmax and overall survival might be indirectly but strictly correlated.

This correlation between necrosis and SUVmax variation could also be helpful for those cases that could not be treated with surgery and whose necrotic percentage is not given. In these patients, the differential SUVmax could even replace necrosis as one of the main prognostic factors after the administration of chemotherapy.

Our study is not free of limitations. One of them is represented by the retrospective nature of our study, which did not allow the complete standardization of the postoperative follow-up procedures for each patient. Another limitation is the wide period covered by our study.

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Between 2006 and 2018, surgical technologies and chemotherapy, radiation therapy, and imaging technologies developed, which have had innovations for more than ten years. These changes inevitably reduced the grade of standardization in our cohort.

Another limitation is represented by the diversity of the patients we analyzed regarding age and histological diagnosis. Due to the relatively limited number of available patients, osteosarcomas and Ewing sarcomas were included despite their differences in histological nature and treatments. These variabilities further reduced the grade of standardization in our cohort. These issues could be overcome in the future by performing similar evaluations, on a prospective basis, on broader populations and performing separate evaluations for Ewing sarcomas and osteosarcomas.

Beyond these limitations, our study provides evidence that the variation of SUVmax values could represent a reliable prognostic factor for patients with osteosarcoma or Ewing sarcoma undergoing multidisciplinary therapeutic approaches.

CONCLUSION

Although further studies with larger cohorts of patients and a prospective nature would still be necessary and should be encouraged, our study testifies to the effectiveness of FDG-PET/CT (in terms of SUVmax variation after chemotherapy) in order to predict tissue necrosis on target Ewing sarcomas and osteosarcomas, thereby representing a promising prognostic factor for patients' outcome. Physicians should pay attention to the SUVmax values' differential since it could represent a pivotal predictive factor, allowing an earlier and more customized cure standard for each case.

Author contributions

All authors contributed equally to the preparation of the study

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Competing Interests

The authors have declared that no competing interests exist.

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Может ли FDG-PET оценить ответ на химиотерапию и предсказать некроз тканей при остеосаркоме и саркоме Юинга?

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Резюме

Введение: Остеосаркома (ОС) и саркома Юинга (СЮ) представляют собой наиболее распространённые злокачественные опухоли костей в педиатрической популяции. Позитронно-эмиссионная томография с 18-фтордезоксиглюкозой (FDG-PET) показала свою эффективность как на диагностическом, так и на стадийном этапе лечения рака. В последние годы в некоторых исследованиях также изучалась возможность того, что FDG-PET может иметь прогностическую роль.

Цель: Наше исследование было направлено на то, чтобы оценить, могут ли изменения максимального стандартизированного значения поглощения (SUVmax) после химиотерапии коррелировать с некрозом тканей и могут ли быть связаны с выживаемостью пациентов.

Материалы и методы: В это обсервационное ретроспективное исследование вошли все случаи, проходившие лечение по поводу скелетной ОС или СЮ в нашем учреждении в период с 2006 по 2018 год. Мы регистрировали значения SUVmax пациентов до и после химиотерапии, степень некроза (для тех, кто перенёс операцию) и выживаемость. Был включен 41 случай (17 OS и 24 СЮ). Среди 36 пациентов, перенёсших операцию, 15 реагировали на лечение, а 20 не реагировали на лечение.

Результаты: Наши данные свидетельствуют о статистически значимой корреляции между некрозом опухоли и дифференциальным SUVmax после неоадъювантного лечения (p=0.007). В частности, случаи с дифференциалом SUVmax выше 4.7 или вариацией выше 63% имели лучшие онкологические исходы.

Заключение: Наше исследование свидетельствует об эффективности FDG-PET в прогнозировании некроза тканей при СЮ и ОС, тем самым представляя собой многообещающий прогностический фактор.

Ключевые слова

Саркома Юинга, некроз, остеосаркома, PET, прогноз

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Original Article

Evaluation of Oral Health Status in Pregnant Women and its Correlation with Calcium and Phosphate Levels

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Abstract

Aim: This study aimed to analyze the oral health conditions of pregnant women. The analysis involves evaluating two key indices: the decayed, missing, and filled teeth (DMFT) index and the basic erosive wear examination (BEWE) index. Furthermore, this study investigated potential correlations between calcium (Ca) and phosphate (P) levels within specific time intervals and the aforementioned oral health indices.

Materials and methods: This study included 66 women. The examination consisted of assessing the condition of the teeth by using the DMFT index. Additionally, the erosive decay of the teeth was evaluated according to the BEWE index. Salivary concentrations of Ca and P were determined using a colorimetric method. These measurements were performed during the first (T1) and third (T3) trimesters of pregnancy.

Results: The study results showed that the DMFT index value in T3 was significantly higher compared to that in T1. However, there were no significant differences in BEWE index values between the two time intervals. Furthermore, notable differences were observed in the levels of Ca and P between T1 and T3 ($Z=4.87$, $p=0.000$ and $Z=2.95$, $p=0.003$, respectively). Nevertheless, the analysis of the relationship between DMFT/BEWE indices and Ca and P levels found no significant correlation.

Conclusions: The results suggest that the third trimester poses a greater oral health burden. Additionally, there were notable fluctuations in the Ca and P levels during pregnancy. These findings shed light on the correlation between pregnancy phases and oral health indicators, emphasizing the significance of the salivary composition.

Keywords

calcium, caries, erosion, phosphate, tooth

INTRODUCTION

Oral health is a crucial aspect of the overall well-being and should be maintained during pregnancy and throughout a woman's lifetime. Maintaining proper oral hygiene can have a positive impact on the progression of general ailments such as cardiovascular diseases and diabetes.[1] Recent research indicates that 35% of American women have not undergone a dental check-up, while 56% have not visited the dentist at all during pregnancy.[2]

Hormonal and nutritional changes during pregnancy render pregnant women particularly vulnerable to tooth

decay, while dental hygiene also plays a significant role.[3] The oral cavity is frequently exposed to stomach acidity during pregnancy, which causes tooth enamel erosion. Additionally, acid reflux from the stomach caused or exacerbated by morning sickness in early pregnancy, a weakened esophageal sphincter, and pressure from the growing uterus can further contribute to dental erosion development.[4]

Various biochemical changes occur during pregnancy. Salivary calcium levels decrease during the third trimester, whereas salivary phosphate reduction begins in the second trimester, according to some studies. A decrease in phosphate concentration, which acts as a remineralizing agent, affects the remineralization process. Subsequently, the buffering capacity of saliva decreases from the first to the third trimester, resulting in an increase in the acidity of the oral environment.[5]

Numerous studies have examined the oral health status of pregnant women. Both Cordero et al. and Fakheran et al. discovered a substantial impact of oral health on the quality of life of pregnant women, with dental and gingival diseases hurting their self-perception and overall well-being.[6,7] However, Gambhir et al. pointed out a lack of knowledge and awareness about oral health among pregnant women, indicating the need for education and motivation.[8] Vamos et al. emphasized the significance of oral health promotion interventions during pregnancy, particularly those that address oral-related symptoms, hygiene behaviors, and potential oral-systemic implications.[9] The variables that contribute to this condition are complicated and interconnected, requiring a comprehensive understanding of the underlying factors.[10] However, advanced therapies hold promise for oral tissue regeneration, potentially providing a future solution for pregnant women with dental issues.[11,12]

Although various studies have been conducted[13], it is evident that there exist contradictory findings concerning the association between the decayed, missing, and filled teeth (DMFT) index and the basic erosive wear examination (BEWE) index among pregnant women. Additionally, the available literature lacks a connection between these indices and the levels of calcium and phosphorus despite the pivotal roles these minerals play in the development of tooth structure.

AIM

This study aimed to assess the oral health of pregnant women during the first (T1) and third (T3) trimesters of pregnancy by employing two indices: DMFT and BEWE. Additionally, the study intended to investigate potential correlations between Ca and P levels and the aforementioned indices.

MATERIALS AND METHODS

This cohort study was conducted at the Gynecology Clinic of the University Clinic Center of Kosovo between December 2019 and July 2020. The study was approved by the Ethics Committee of the Faculty of Medicine, University of Prishtina, Kosovo (reference number 4096/2019) and involved 66 pregnant women who provided consent and information following the Declaration of Helsinki. The patients were briefed on the research, willingly participated, and signed an informed consent form.

The inclusion criteria comprised pregnant women with good systemic and oral health mucosa during their first pregnancy. The exclusion criteria were as follows: diabetes, xerostomia, hypertension, preeclampsia, eclampsia, stomatitis aphthosis, viral infections, ulcerative changes in the oral cavity, dental fluorosis, use of chlorhexidine gluconate in the last three months, antibiotics, or other antibacterial agents.

A sample size of 66 individuals was determined in consultation with a statistical specialist, considering the number of women receiving initial pregnancy treatment at the Gynecology Clinic and the inclusion and exclusion criteria for the research. The sample size was calculated using the Cohran formula.[14] To prevent confounding effects during the study design and analysis phases, pregnant women in their first and third trimesters were selected for this study.

Clinical examination for scoring of DMFT and BEWE indexes

The assessment of caries involved the application of the DMFT indices in accordance with the criteria laid out by the World Health Organization. The DMFT score is derived by tallying the number of permanent teeth affected by caries, with 'D' representing decayed, 'M' indicating missing teeth due to caries, and 'F' denoting filled teeth. The determination of DMFT scores for the samples relied on the findings of clinical examinations, in which the number of D, F, and M teeth resulting from caries was calculated. Capturing the necessary data involved the use of questionnaires and direct examination of the teeth in the samples.[15]

The BEWE index is a scoring system utilized to assess the most severely affected area within a sextant, providing practitioners with a cumulative score to aid in the management of this condition. It classifies the appearance and severity of tooth wear into four levels: 0 = indicates no damage to the enamel on the tooth surface; 1 = signifies only superficial loss of tooth enamel; 2 = indicates enamel loss and exposure of dentin on less than one third of the tooth surface; 3 = indicates enamel loss, exposed dentin, and loss of more than one third of the tooth surface without pulp exposure; 4 = represents a complete loss of enamel and exposed dentin.[16]

Determination of calcium and phosphorus values in saliva

Ca and P values in saliva were determined by examining mineral excretion in unstimulated saliva. Saliva samples were immediately examined to avoid blood contamination, placed in a refrigerator at a temperature of 4°C (not more than a few hours), and then frozen at −20°C. The samples

were collected in high-quality thermopropylene vials that could withstand temperatures of −80°C. Saliva samples, totaling 2-3 milliliters, were collected from the pregnant women in the morning. Ca concentration was determined using the colorimetric method with Arsenazo III Randox, whereas the P concentration was determined using the UV photometric method with human molybdenum.[17]

Statistical analysis

Data analysis was performed using SPSS Statistics for Windows (IBM, version 23.0, Armonk, NY, IBM Corp) and Excel 2016 (Microsoft Corporation, Redmond, WA, USA), and statistical significance was set at $p<0.05$. The results were presented in tabular and graphical formats. The Fisher Exact/Monte Carlo Sig test (two-sided, *p*-value) was used to compare the attribute-based series between the T1 and T3 trimesters. For numerical values such as BEWE, DMFT indices, Ca, and P, descriptive statistics were calculated, including mean, standard deviation (SD) with a 95% confidence interval (±95% CI), minimum, and maximum. Data distribution was evaluated using the Kolmogorov-Smirnov, Lilliefors, and Shapiro-Wilk tests (*p*). The variance in the parameter values analyzed in the T1 and T3 trimesters was examined using the Wilcoxon matched-pairs test (Z/p). Correlations between the index values and Ca and P levels were examined using Spearman's rank order R (R/p).

RESULTS

The values associated with caries (D), missing (M), filled (F), and DMFT index during the first (T1) and third (T3) trimesters of pregnancy are shown in **Table 1**.

In the T3 trimester, the value of D for Z=3.14 and $p<0.01$ ($p=0.002$) was significantly higher than that of T1. However, for T=0.00 and $p>0.05$, there were no significant differences in the M ratio between T1 and T3. Furthermore, the values of F in T3 for Z=2.80 and $p<0.01$ ($p=0.005$) were significantly greater than those in T1. The DMFT index value as a whole in T3 for Z=4.37 and $p<0.001$ ($p=0.000$) was significantly greater than that in T1 **(Table 1a)**.

During the T1 trimester of pregnancy, tooth erosion was not observed in 65 of 66 pregnancies (98.50%). Only one pregnancy (1.50%) during this stage showed significant enamel loss. In the T3 trimester, out of 66 pregnant women, 42 (63.60%) did not have any signs of erosive tooth wear. Among the remaining 24 women, 12 (18.20%) experienced tooth erosion, seven (10.60%) had pronounced enamel loss, and five (7.60%) experienced severe enamel loss **(Table 2a)**.

The BEVE index values in relation to T1 and T3 were analyzed through cross-tabulation, yielding the following results: out of 66 pregnant women (100.00%) without recorded enamel damage at T1, 42 (64.60%) did not have any enamel damage on the tooth surface during T3. Additionally, in 12 (18.50%) pregnant women, only surface wear of the tooth enamel was observed, while in 6 (9.20%) pregnant women, enamel loss was less than one-third of the tooth surface with dentine exposure. Moreover, five (7.70%) pregnant women experienced enamel loss with dentine exposure, and another five (7.70%) had a loss of more than one-third of the tooth surface. One (100%) pregnancy that showed pronounced enamel loss during T1 also had complete enamel loss and pulp exposure during T3. The cross-tabulation for the BEVE index values during the first and third trimesters of pregnancy revealed no significant differences (Fisher's exact test =6.000 and $p>0.05$, respectively ($p=0.180$) / Monte Carlo Sig. / 0.170 – 0.190) **(Table 2)**.

Table 1. Descriptions of parameter values for the DMFT index in the first and third trimesters

Trimester	Parameters	Number	Mean	Confidence −95%	Confidence +95%	Min.	Max.	SD
T1	D	66	3.29	2.7	3.88	0	8	2.41
	M	66	3.33	2.82	3.85	0	12	2.1
	F	66	2.12	1.42	2.82	0	14	2.84
	DMFT	66	8.76	7.95	9.56	2	18	3.27
T3	D	66	3.74	3.17	4.31	0	9	2.32
	M	66	3.3	2.85	3.75	0	8	1.82
	F	66	2.48	1.78	3.19	0	14	2.87
	DMFT	66	9.53	8.65	10.41	2	20	3.57

T1: first trimester; T3: third trimester; D: decayed; M: missing; F: filled; SD: Standard deviation

Table 1a. Comparison of DMFT index parameters between the two trimesters

Parameters	Number	T	Z	*p*-level
D	66	17	3.14	0.002
M	66	0		
F	66	0	2.8	0.005
DMFT	66	0	4.37	0

D: decayed; M: missing; F: filled; T and Z: statistical variables

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Statistical analysis revealed that calcium values in the T3 trimester of pregnancy exhibited a significant increase compared to those in the T1 trimester, with a Z-score of 4.87 and $p<0.001$ ($p=0.000$) **(Table 3)**. Similarly, phosphorus values in the T3 trimester of pregnancy also displayed a significant increase as compared to the T1 trimester, with a Z-score of 2.95 and $p<0.01$, respectively ($p=0.003$) **(Table 3a)**.

Furthermore, **Fig. 1** shows the correlation between the DMF index and Ca values in the saliva of pregnant women during the T1 trimester of pregnancy. A Spearman rank order correlation coefficient (R) of −0.11 and $p>0.05$, establishes a small, negative, and non-significant correlation between the two variables. It was observed that with an increase in Ca values in the T1 trimester, the DMF index values tended to decrease.

Table 2. BEVE index values during the first and third trimesters

			BEWE index / Third trimester				Total
			No erosion	Initial erosion	Clear dentin loss	Hard enamel loss	
BEWE index First trimester	No erosion	Count	42	12	6	5	65
		%	64.60%	18.50%	9.20%	7.70%	100.00%
	Clear dentin loss	Count	0	0	1	0	1
		%	0.00%	0.00%	100.00%	0.00%	100.00%
Total		Count	42	12	7	5	66
%			63.60%	18.20%	10.60%	7.60%	100.00%

Table 2a. Descriptions of parameter values for the BEWE index in the first and third trimesters

Trimester	Parameters	Frequency	Percent	Valid Percent	Cumulative Percent
T1	No erosion	65	98.5	98.5	98.5
	Clear dentin loss	1	1.5	1.5	100
	Total	66	100	100	
T3	No erosion	42	63.6	63.6	63.6
	Initial erosion	12	18.2	18.2	81.8
	Clear dentin loss	7	10.6	10.6	92.4
	Hard dentin loss	5	7.6	7.6	100
	Total	66	100	100	

Table 3. Descriptions of parameter values for calcium and phosphate in the first and third trimesters

Trimester	Parameters	Number	Mean	Confidence	Confidence	Min.	Max.	SD
				−95%	+95%			
T1	Ca	66	2.22	2.2	2.24	2.01	2.55	0.09
	P	66	1.21	1.18	1.23	1.01	1.5	0.1
T3	Ca	66	2.32	2.28	2.36	1.31	2.57	0.17
	P	66	1.29	1.23	1.34	0.79	2.2	0.23

T1: first trimester; T3: third trimester; Ca: calcium; P: phosphate; SD: standard deviation

Table 3a. Comparison of Ca and P parameters between the two trimesters

Parameters	Number	T	Z	*p*-level
Ca	66	343.5	4.87	0
P	66	599	2.95	0.003

Ca: calcium; P: phosphate; T and Z: statistical variables

Fig. 2 shows the correlation between the DMF index and Ca values in the saliva of pregnant women during the T3 trimester of pregnancy. The Spearman rank order correlation coefficient (R) of 0.05 and $p>0.05$ reveal a small, negative, and non-significant correlation between the two variables. However, with an increase in the Ca values in the saliva during the T3 trimester, the DMF index tended to increase.

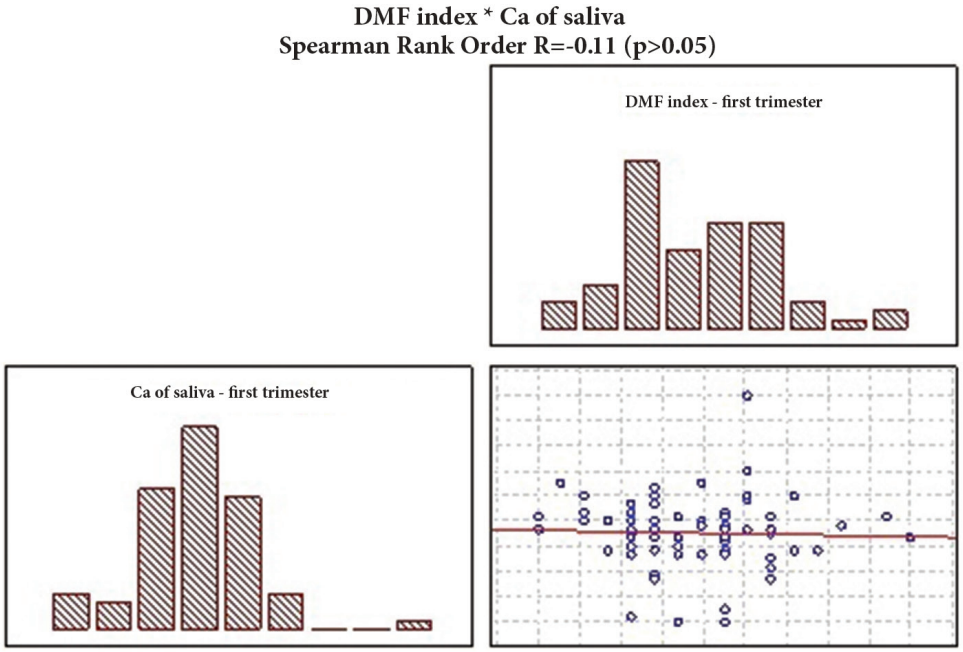

Figure 1. The relationship between DMF index and Ca values in the first trimester.

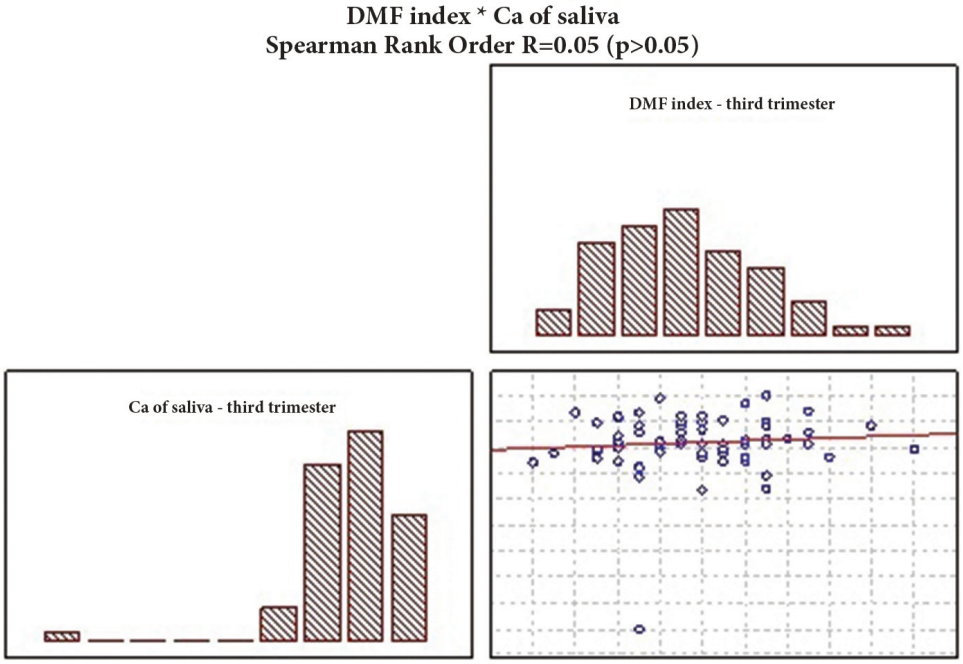

Figure 2. The relationship between the DMF index and Ca values in the third trimester.

Fig. 3 shows the correlation between the BEWE index and Ca level in the saliva of pregnant women during the T1 trimester of pregnancy. A Spearman rank order correlation coefficient (R) of −0.04 and $p>0.05$, indicates a very low, negative, and non-significant correlation between the two variables. Nonetheless, an increase in the level of Ca in saliva leads to a decrease in the BEWE index values.

Fig. 4 shows the correlation between the BEWE index and the level of Ca in the saliva of pregnant women during the T3 trimester of pregnancy. A Spearman rank order correlation coefficient (R) of −0.11 and $p>0.05$, suggests a slight, negative, and non-significant correlation between the two variables. However, an increase in the level of Ca in saliva during the T3 trimester led to a decrease in BEWE index values.

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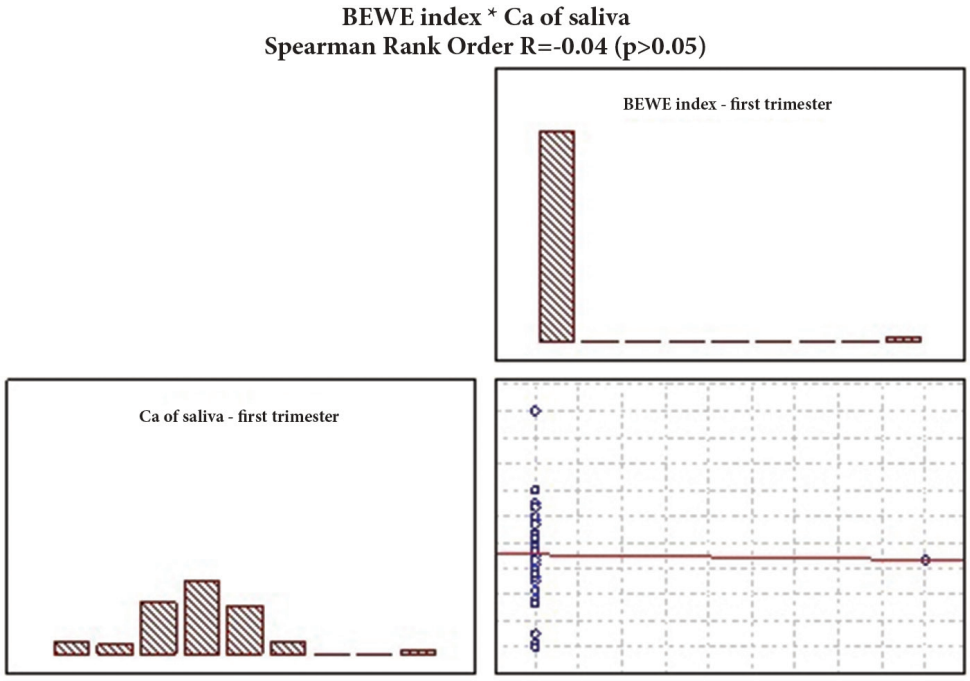

Figure 3. The relationship between the BEWE index and the level of Ca in the first trimester.

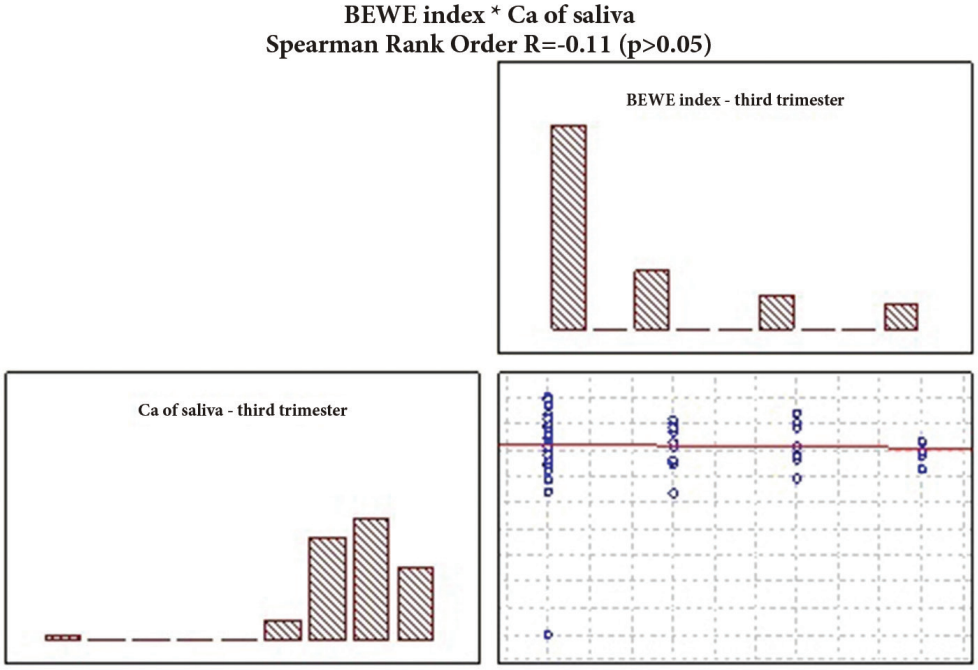

Figure 4. The relationship between the BEWE index and the level of Ca in the third trimester.

DISCUSSION

The objective of the present study was to evaluate the oral health of pregnant women across various trimesters using the DMFT and BEWE indices. The study also aimed to establish a correlation between the levels of calcium and phosphorus in patients and these indices, given the role of these minerals in the remineralization process.

During the monitoring period, an increase in the DMFT index was observed in the T3 trimester of pregnancy. The increase in these values was not unexpected. Velosa-Porras et al. assessed the prevalence of caries in various stages of pregnancy and found that more than 50% of the participants developed new decay during pregnancy.[18] Furthermore, Yousef et al. discovered that during the T3 trimester, several salivary factors associated with caries undergo

changes, which can heighten the likelihood of future caries development.[19] Therefore, initiatives and assessments for caries prevention during pregnancy should commence in the initial or second trimesters.

The increase in DMFT index can also be attributed to the stress experienced during pregnancy. Existing literature suggests that stress during this period affects the growth of cortisol, leading to a decrease in immunoglobulin levels and saliva flow.[20] Consequently, these changes results in a decline in the oral immune system, dental plaque formation, and an increase in bacterial activity.

The participation of pregnant patients for the first time was also a significant factor that may have influenced the increase in DMFT values. Hom et al. discovered that first-time pregnant patients typically possess limited health knowledge, categorizing them as a vulnerable group of women.[21] This lack of awareness can contribute to various oral health issues, such as the development of tooth decay. Additionally, studies by Thomas et al. and Shamsi et al. revealed that over half of the pregnant women did not visit a dentist during their last pregnancy. On the other hand, healthcare providers often hesitate to provide dental services.[22,23] Da Costa et al. pointed out that general dentists, despite offering dental services to pregnant women, are often reluctant to perform this service because of concerns about fetal harm, patient entrapment, and potential legal issues.[24] These data indicate a poor oral health culture among pregnant women and underscores the need for appropriate action.

The elevated DMFT level during the monitoring period of this study is a cause for concern, especially considering that the participants were experiencing their first pregnancy. This is due to the fact that untreated caries contributes to an increased presence of bacteria in saliva, subsequently leading to potential transmission of these bacteria to infants and influencing the development of early childhood caries.[25] Hence, in order to mitigate this risk, it is recommended that pregnant women receive appropriate dental care, including the adoption of preventive measures such as reducing carbohydrate intake, regular dental plaque examinations performed by a dentist, as well as utilizing oral rinses that lower the acidity levels in saliva.[26]

The BEWE index was assessed in this study. Throughout the follow-up period, an upward trend in erosion values was noted, although no statistically significant differences were observed between T1 and T3. Al-Sultani evaluated the oral condition of soft and hard tissues during different trimesters of pregnancy. His findings indicated a significant relationship between dental erosion and the stage of pregnancy.[27] This correlation can be attributed to the specific periods of pregnancy when women experience more frequent episodes of vomiting, resulting in an increased risk of dental erosion due to repeated exposure to acidic stomach contents.[28] Additionally, studies have suggested that the elevated acidity in saliva during pregnancy may be caused by higher levels of progesterone and estrogen, which can impact the demineralization process and protective properties of tooth enamel.[29,30] Consequently, it is crucial for pregnant women to be aware of the potential risk of erosive teeth and to take proactive measures to maintain good oral hygiene.

Regarding the concentration of Ca and P ions, the study findings indicated an increase in the saliva of pregnant women during the T3 trimester compared to the T1 trimester. These results align with those of the recent studies conducted by Sultana et al. whereas other studies have reported conflicting outcomes.[31] Salvolini et al. discovered a decrease in the levels of Ca and P during pregnancy and attributed this decline to the increased demand for these minerals in fetal bone ossification.[32] Moreover, Breslau et al. found that elevated estrogen levels during pregnancy tended to reduce Ca levels.[33] The elevation in Ca and P concentrations observed in our study could be attributed to the possibility that some participants consumed food prior to saliva collection despite being advised not to do so. However, this aspect did not compromise the reliability and validity of our research.

Additionally, this study aimed to establish a correlation between DMFT and BEWE indices and the concentrations of Ca and P ions. Our findings are consistent with those of a recent study by Ghasemi et al., which revealed no significant relationship between DMFT and Ca and P.[34]

Lastly, it is important to note that the physiological and hormonal changes that occur during pregnancy can significantly affect a woman's health. Since the oral cavity is particularly vulnerable to these changes, it is important to take into account any potential pathological and physiological changes.[35] Consequently, maintaining a well-planned diet prior to conception, during pregnancy, and while breastfeeding is crucial in ensuring optimal health for both mother and child.[36]

Limitations of the study

One limitation of the study pertained to the absence of data regarding oral hygiene performance, including the frequency of tooth brushing and regular visits to dental care professionals. These variables have the potential to influence DMFT and BEWE indices. Additionally, the study did not account for the current condition of the periodontium, the levels of estrogen and progesterone hormones in the participants, or the impact of the COVID-19 pandemic on the research. Recent studies have indicated that these factors can significantly affect the Ca and P levels, as well as the oral health of pregnant women.[37-39] Additionally, the small number of participants should be considered when interpreting our findings. Notwithstanding these constraints, our study provides compelling evidence of the correlation between oral health status and mineral levels in pregnant women over time.

CONCLUSIONS

Considering the limitations of the present study, these findings indicate a progressive increase in DMFT values during

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pregnancy. Notably, Ca and P levels significantly increased in the T3 trimester. No significant correlation was observed between the DMFT/BEWE indices and mineral levels.

In light of these results, it is recommended that preventive measures for caries be initiated as early as possible during pregnancy to ensure the oral health of both mother and child. These measures encompass maintaining proper oral hygiene, including regular brushing and flossing, attending dental check-ups, and adopting a balanced diet low in sugary foods. By adhering to these practices, the likelihood of developing tooth decay can be considerably reduced. Additionally, seeking advice from a healthcare professional specializing in dental care during pregnancy is advisable, as they can provide further guidance and support regarding preventive measures.

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Competing Interests

The authors declare that they have no competing interests.

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Оценка состояния здоровья полости рта у беременных и его корреляция с уровнем кальция и фосфата

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Резюме

Цель: Целью данного исследования было проанализировать состояние полости рта беременных женщин. Анализ включает оценку двух ключевых индексов: индекса разрушенных, отсутствующих и запломбированных зубов (DMFT) и индекса основного эрозионного износа (BEWE - basic erosive wear examination). Кроме того, в этом исследовании изучались потенциальные корреляции между уровнями кальция (Ca) и фосфата (P) в течение определённых интервалов времени и вышеупомянутыми показателями здоровья полости рта.

Материалы и методы: В исследование были включены 66 женщин. Обследование заключалось в оценке состояния зубов по индексу DMFT. Дополнительно оценивали эрозивный кариес зубов по индексу BEWE. Концентрацию Ca и P в слюне определяли колориметрическим методом. Эти измерения проводились в течение первого (T1) и третьего (T3) триместров беременности.

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Результаты: Результаты исследования показали, что значение индекса DMFT в T3 было достоверно выше по сравнению с таковым в T1. Однако существенных различий в значениях индекса BEWE между двумя временными интервалами не было. Кроме того, заметные различия наблюдались в уровнях Ca и P между T1 и T3 (Z=4.87, *p*=0.000 и Z=2.95, *p*=0.003 соответственно). Тем не менее, анализ взаимосвязи между индексами DMFT/BEWE и уровнями Ca и P не выявил значимой корреляции.

Заключение: Результаты показывают, что третий триместр представляет собой большую нагрузку на здоровье полости рта. Кроме того, во время беременности наблюдались заметные колебания уровней Ca и P. Эти результаты проливают свет на корреляцию между фазами беременности и показателями здоровья полости рта, подчёркивая важность состава слюны.

Ключевые слова

кальций, кариес, эрозия, фосфат, зуб

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Original Article

Incidence of Breast Cancer in Egyptian Females in Correlation to Different Mammographic ACR Densities

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Abstract

Introduction: The density of breast tissue, radiologically referred to as fibroglandular mammary tissue, was found to be a predisposing factor for breast cancer (BC). However, the stated degree of elevated BC risk varies widely in the literature.

Aim: The purpose of this study was to determine the relationship between different breast mammography densities and the risk of breast cancer in Egyptian women..

Patients and methods: An analytical cross-sectional prospective study was conducted at Ain Shams University Hospital and Private Centre between December 2020 and December 2021. The study included 814 asymptomatic females 40 years old or above, who came for BC screening using full-field digital mammography.

Results: The incidence of BC was found in 84 females (10.3%). Breast cancer incidence was 6.3% in females with ACR-A density, 8.5% in women with ACR-B density, 16.3% in women with ACR-C density, and 27.8% in women with ACR-D density ($p<0.001$). Using logistic regression analysis, we showed that three types of breast density increased the risk of BC, where patients with type B, type C, and type D breast density had a 1.39, 2.92, and 3.12 times more risk for BC, respectively ($p=0.010$, $p=0.003$, and $p=0.036$, respectively).

Conclusion: Among Egyptian females, the incidence of BC is 10.3%. Our data revealed that the greater the breast density, the higher the incidence of BC, and affirmed that breast density is a risk factor for BC development.

Keywords

breast cancer, breast density, mammography

INTRODUCTION

Breast cancer is a malignant tumor that originates in the cells of the breast tissue. It is the most common cancer in women worldwide, accounting for 24.2% of all cancer cases among women. According to the latest World Health Organization statistics, there were an estimated 2.3 million new cases of breast cancer in 2020; it is the second leading cause of cancer death in women after lung cancer. The risk of developing breast cancer increases with age, and most cases occur in women over the age of 50.[1]

The presence of breast cancer can be detected through various methods, including mammography, ultrasound, and magnetic resonance imaging (MRI). If breast cancer is suspected, a biopsy may be performed to remove a sample of tissue for further examination.[1]

The mortality rate of breast cancer varies depending on the stage at diagnosis and the availability of treatment.

According to the American Cancer Society, the 5-year relative survival rate for women with breast cancer is 90%. However, this rate drops significantly for women with advanced stage breast cancer.[2]

Recent statistics show that the incidence of breast cancer is increasing worldwide. This is thought to be due to a combination of factors, including increased awareness and screening, as well as changes in reproductive and lifestyle factors. However, mortality rates have been decreasing in many countries due to improvements in early detection and treatment.[2]

Despite these advances, breast cancer remains a significant public health concern. According to the WHO, there were an estimated 685,000 deaths from breast cancer worldwide in 2020. Continued research and investment in prevention, early detection, and treatment are essential to reducing the burden of this disease.[1,3]

A large Egyptian systematic review and meta-analysis revealed that BC was the most common cancer, accounting for 42% of all cancer cases in Egyptian females, with the majority presenting in advanced stages.[4]

The World Health Organization's principal objective is to reduce the BC-related burden and BC-related mortality which is suggested to be achieved by early detection and proper management of BC.[1]

BC screening contributes considerably to the early detection of asymptomatic cancer, which leads to less intrusive therapies and better outcomes since the disease is found before the progression of the tumor. The most effective imaging method that is still advised for all women to use for BC screening is mammography.[5]

Accordingly, a number of nations have put in place national programs for BC screening, wherein women undergo routine mammography screenings. Women between the ages of 50 and 69 are screened in the majority of nations since it is thought that they are the most suitable population to gain advantages from this screening.[6]

The density of breast tissue, radiologically referred to as fibroglandular mammary tissue, was found to be a risk factor for BC in the early twentieth century. It was reported that dense breasts were more likely to develop BC than fatty breasts.[6] However, the stated degree of elevated BC risk varies widely in the literature, from a one- to a six-fold greater risk.[5,8,9]

AIM

The present study aims to detect the relationship between different breast mammographic densities and the risk for BC in Egyptian females.

DESIGN

Analytical cross-sectional prospective study.

PATIENTS AND METHODS

Asymptomatic females who are 40 years of age or older and eligible for screening mammography were included in the study which was approved by the institutional Ethics Committee.

The study included 814 asymptomatic females who came for BC screening. After the study was approved by the Ethics Committee of the university, asymptomatic females of 40 years of age or older who were eligible for screening mammography were included into our study. Any female who was contraindicated for mammography was excluded, such as a pregnant female or a female under the age of 40, for whom ultrasound (US) is the preferred method.

All females were submitted to demographic and clinical data collection and imaging procedures using full-field digital mammography, a device developed by GE Healthcare (Senographe 2000 D full-field digital mammography Essential GE Healthcare). For all patients, two standard views medio-lateral-oblique (MLO) and cranio-caudal (CC) views were obtained.

Then, according to the Breast Imaging Reporting and Data Systems (BI-RADS) Atlas 5th Edition 2013[10], all females were classified into four density categories based on mammographic findings: excessively fatty (ACR-A), dispersed density (ACR-B), heterogeneous density (ACR-C), and extremely dense (ACR-D).

On the basis of the BI-RADS lexicon[10], females were classified as BI-RADs 0 (need additional evaluation), 1 (negative), 2 (benign), 4 (suspicious), and 5 (highly suggestive of malignancy). Patients with BI-RADs 0, 4, and 5 underwent complementary US using GE LOGIQ P6 XD clear (GE health, Chicago, USA), using a high-frequency linear probe (9-12 MHZ) to scan the entire breast and axilla using combined techniques (radial, axial, and longitudinal). Cases with BI-RADs 4 and 5 underwent US-guided biopsy. Patients with BI-RADs 0 were categorized to BI-RADS 3, 4, or 5 according to the result of complementary US.

Statistical analysis

The statistical software for social sciences, version 23.0 (SPSS Inc., Chicago, Illinois, USA), was used to analyze the recorded data. The ranges and mean ± standard deviation (SD) were displayed for the quantitative statistics. Additionally, percentages and numbers were used to present qualitative factors. To compare the qualitative data, the chi-square test was applied, and to determine the independence of breast density as a predisposing factor for BC, multiple logistic regression was used. The confidence interval was set to 95% and the margin of accepted error was agreed to be 5%. Accordingly, p-value <0.005 reflects significant results.

RESULTS

Among the 814 females, the age ranged from 40 to 79 years

(mean age 53.78±10.22). Most of the females were between 40 to 50 years of age (323 females; 39.7%). According to the BI-RADS 5th edition, ACR-A density was found in 176 females (21.6%), ACR-B density in 424 females (52.1%), ACR-C density in 196 females (24.1%), and ACR-D density in 18 females (2.2%) **(Fig. 1)**.

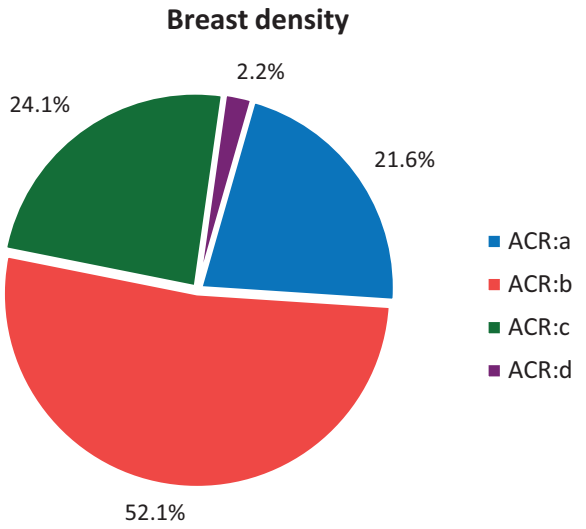

Figure 1. Pie chart breast density distribution among study group.

The age of the females and the type of their breast density showed a statistically significant relation (p<0.001), where the oldest females were found to have breast density type A (64.34±5.83 years) and the youngest had breast density type D (43.33±4.51 years) **(Table 1)**.

The incidence of BC was found in 84 females (10.3%). The BC incidence in females with ACR-A was 6.3%, ACR-B was 8.5%, ACR-C was 16.3% **(Fig. 2)** and ACR-D was 27.8% **(Fig. 3)** (p<0.001) **(Table 2)**.

Fig. 2 illustrates the case of a 43-year-old woman who underwent routine breast cancer screening through both mammography and ultrasound imaging, revealing high breast density according to the American College of Radiology's classification system (ACR-D). **Figs 2A, 2B** display the mammography images obtained from this examination, with (A) showing a dense, irregular mass located in the upper outer quadrant (UOQ) region of the right breast that is most apparent when viewed from below (MLO position). This finding is further highlighted in **Fig. 2B**, where it appears as an irregular shape with indistinct margins. **Fig. 2C** displays an accompanying ultrasound image, which depicts a similarly shaped, but now hypoechoic and irregular mass situated within the same area. Based on these findings, the radiologist assigned a Bi-RADS assessment score of category 5, indicating a highly suspicious malignancy. Subsequent diagnostic confirmation via US-guided core needle biopsy performed outside the screening context uncovered a pathological diagnosis of invasive ductal carcinoma (IDC), classified as grade II based on histopathologic evaluation.

Fig. 3 depicts a 48-year-old woman who underwent regular breast cancer screenings. During her examinations, we observed elevated levels of dense tissue in her breasts, categorized by the American College of Radiology as type C. We employed mammography with complementary ultrasonography to visualize any potential abnormalities. **Fig. 3** showcase the results of our mammography scans taken during the craniocaudal (CC) and medial-lateral oblique (MLO), respectively, highlighting a partial yet undefined mass formation in the Rt upper inner quadrant (UOQ) zone. Compensatory ultrasound shows hypoechoic irreg-

Table 1. Relation between age of females and their type of breast density (n=814)

	Type A	Type B	Type C	Type D	*P*-value
Age (mean ± SD)	64.34±5.83)	53.18±7.95	45.02±3.72	43.33±4.51	<0.001* [a]

[a] Kruskal-Wallis test

Table 2. Association between breast density and BC among study groups (n=814)

Breast density	Breast cancer				Total	
	Negative		Positive			
	No.	%	No.	%	No.	%
ACR-A	165	93.8%	11	6.3%	176	100.0%
ACR-B	388	91.5%	36	8.5%	424	100.0%
ACR-C	164	83.7%	32	16.3%	196	100.0%
ACR-D	13	72.2%	5	27.8%	18	100.0%
Total	730	89.7%	84	10.3%	814	100.0%
Chi-square test	18.252					
p-value	<0.001**					

χ^2: chi-square test for number (%). **p-value <0.001 is highly significant

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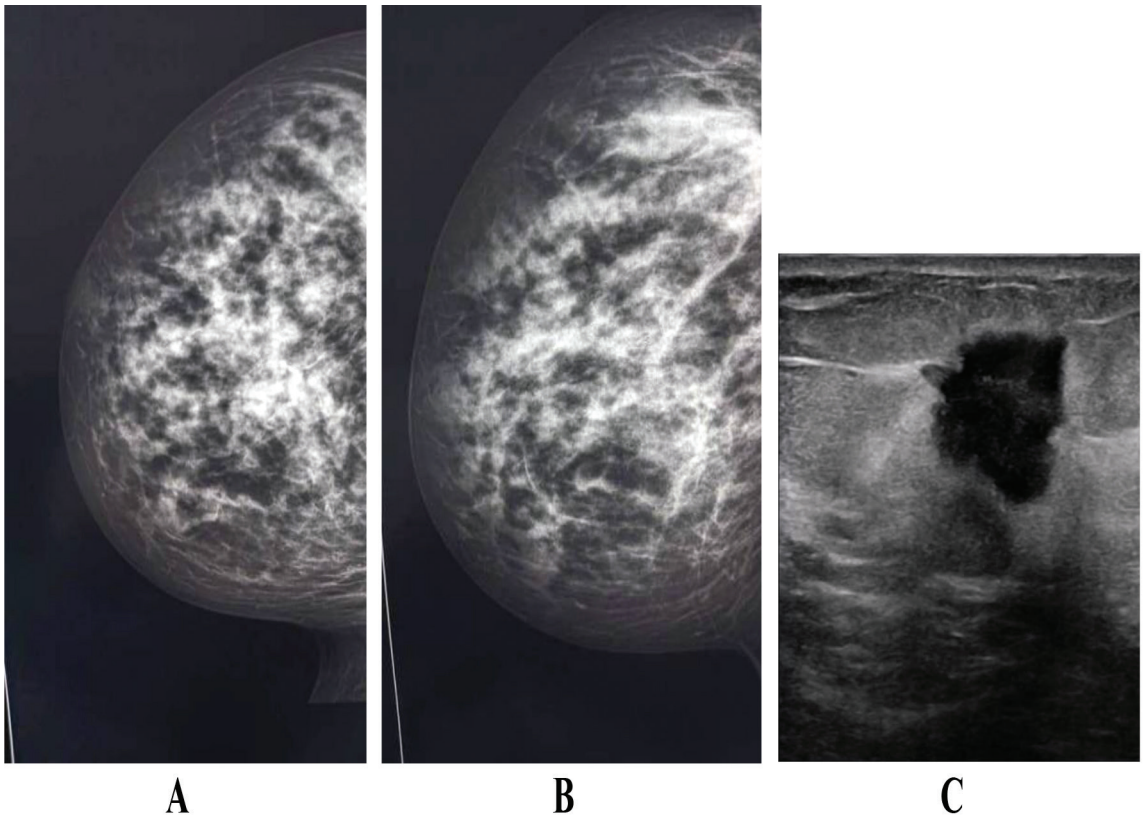

Figure 2. Detection of highly suspicious mass lesions in a patient with ACR-C breast density using mammography and ultrasound imaging biopsy revealed IDC-II.

ular mass and ultrasound guided biopsy taken with pathology revealed IDC-II.

As risk factors for BC, three types of breast densities increased the risk of BC, where females with type B breast density had 1.39 times more risk of breast cancer, while type C patients had 2.92 times more risk, and finally, type D females had 3.12 times more risk for breast cancer (p=0.010, p=0.003, and p=0.036, respectively) **(Table 3)**.

DISCUSSION

Currently, the most widely used tool available for clinically categorizing mammographic density is the BI-RADS, which was released in 2013. Four types of breast density are defined by this system: excessively fatty (ACR-A), dispersed density (ACR-B), heterogeneous density (ACR-C), and highly dense (ACR-D).[10] Using BI-RADS 5th edition, we aimed in our study to find the relationship between different breast mammographic densities and risk for BC in Egyptian females.

Table 3. Logistic regression for the breast density for BC among the study groups (n=814)

Characteristics	OR[a]	CI	*P* value
Type-A	0.54	1.740 – 19.125	0.068
Type-B	1.39	1.399 – 12.286	0.010*
Type-C	2.92	1.323 – 3.410	0.003*
Type-D	3.12	0.169 – 1.522	0.036*

[a] Multiple logistic regression

In this work, according to the BI-RADS 5th edition, among women who were screened for BC, group ACR-B is the most common type which was consistent with previous works.[9,11] We found that more than half of the females were categorized as ACR-B (52.1%), followed by ACR-C (24.1%), ACR-A (21.6%), and ACR-D (2.2%).

This was also similar to a previous Egyptian study by Ali and Raafat[12] which included 49,409 women who presented for screening mammography. The study showed that the majority of Egyptian females were categorized as ACR-B (49%), followed by ACR-C, ACR-A, and ACR-D, representing 49%, 25%, 23%, and 3%, respectively based on the BI-RADS.[12]

In another Egyptian study by Ahmed et al. that included 40 women with confirmed BC, based on the BI-RADS 5th edition, more than half of females were categorized as ACR-B (52.5%), followed by ACR-C (30%), ACR-A (15%), and ACR-D (2.5%).[13]

We found an inverse significant relationship regarding the age of the females and the type of their breast density (p<0.001), where the oldest females were found to have breast density type A (64.34±5.83 years) and the youngest had breast density type D (43.33±4.51 years). These results agreed with Al-Mousa et al., who concluded that increased

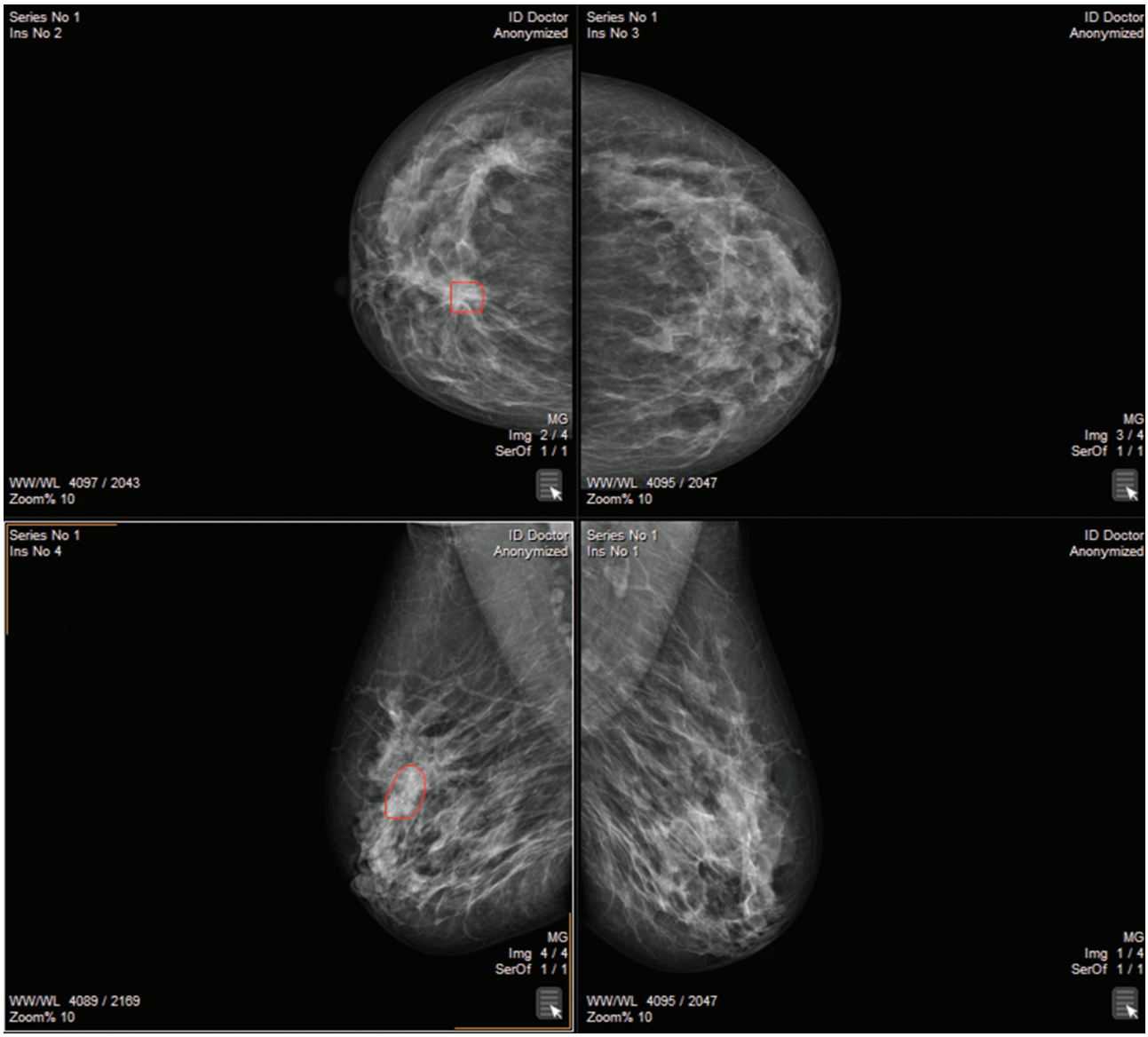

Figure 3. IDC III in a female undergoing routine breast cancer screening with increased dense tissue (ACR-C Type) visualized through mammography images.

breast density on mammography was inversely related to age.[14]

Likewise, Checka et al. observed a significant inverse relation between female ages and mammographically determined breast density (p<0.001); however, they added that age was not a reliable indicator of breast density as they observed a significant percentage of postmenopausal women still having dense breasts on mammography.[15]

These results contradict the fact that BC is more frequent among older females and in dense breasts. However, this may be explained by the low mammographic sensitivity in young women with more dense breasts[16] which may obscure the early detection of BC among young females which delays the presenting age. This emphasizes the need for individual-based screening program taking into consideration females' ages and breast densities.

In the present study, the incidence of BC among our population was found in 84 females (10.3%). This was higher than that reported in a recent Egyptian study performed by Ali and Raafat, who found that among 49,409 women presented for screening mammography 1500 had BC representing only 3%.[12] Different sample sizes may be the cause for this variation.

The BC incidence in females with ACR-A was 6.3%, ACR-B was 8.5%, ACR-C was 16.3%, and ACR-D was 27.8% (p<0.001), indicating a significant relation between the incidence of BC and breast density, in which the females with extremely dense breasts (ACR-D) are more susceptible to develop BC. This result was confirmed by logistic regression analysis which showed that three types of breast densities increased the risk of BC, where patients with type B, type C, and type D breast density had a 1.39, 2.92, and 3.12 times more risk for BC, respectively (p=0.010, p=0.003, and p=0.036, respectively). These find-

ings settle breast density as a predisposing factor for BC development, showing that the greater the breast density, the higher the incidence of BC.

Our findings support the results of many previous studies. Cohort studies have shown a significant correlation between increasing breast mammographic density and the increased incidence of BC in women.[7,12,17]

As mentioned in our study, Ali and Raafat showed that the incidence of BC in females with ACR-D (13.7%) was the highest followed by that in females with ACR-C (3.3%), ACR-B (2.7%), and ACR-A (2.2%).[12]

It was demonstrated by Ahmadinjad et al. that BC growth was more likely to occur in breasts with high densities, more than in breasts with low densities (p=0.007).[18]

Contrary to our findings, Ahmed et al. reported no relation between BC and breast density in a study conducted on a total of 40 females who were radiologically and histopathologically diagnosed with BC. The authors found that the percentage of cases with BC was the highest among females with intermediate breast density (ACR-B), then increased with ACR-C and ACR-A, respectively, suggesting that dense breast tissue should not be regarded as a risk factor for BC.[13]

Also, Kamal et al. studied the role of breast density as one of the BC risk factors in accordance with menopausal status. In contrast to our findings, they reported that in premenopausal women, breast density was insignificantly related to BC, whereas breast density showed an inverse significant relationship to BC in postmenopausal women. After a logistic regression analysis, Kamal et al. found that decreased breast density is a significant independent predisposing factor for BC (p=0.009).[19]

This discrepancy may be related to some factors, including variations in the breast density assessment technique, adjustments made during the analyses, and, to some extent, variations in the demographics of the population being studied, such as menopausal status, ethnicity, and lifestyle variations; however, these reasons are still debatable.

The fact that our study is prospective in design and conducted at one of the largest Egyptian centers for BC screening strengthens our work. However, our study is limited by the relatively small sample size from a single Egyptian center, accordingly, a larger multicentric study is needed to be more representative of the Egyptian female population. Additionally, the study sample was not evenly distributed throughout the ACR and age groups, which might have skewed the findings. Furthermore, the study did not include other parameters that are proposed to be linked to an increased risk of breast density and BC in the past, such as parity, menopausal status, and hormonal use.

We acknowledge that these factors should be included in future research. Finally, we did not report the clinical-histopathological data as it was away from our aim.

CONCLUSION

Among Egyptian females, the incidence of BC is 10.3%. Females with extremely dense breasts (ACR-D) are more susceptible to developing BC. Overall, our data showed that the greater the breast density, the higher the incidence of BC, and affirmed that breast density is a risk factor for BC development. We recommended a nationwide comprehensive program for BC screening in the whole of Egypt not only for the major government and private hospitals and in screening programs, but breast density estimation should also be considered as a tool in BC prediction.

Conflict of interest

None.

Availability of data and materials

Available on request.

Consent for publication

All authors have approved the manuscript for submission.

Funding

None.

Author contributions

M.R.H.S.: conceptualization, methodology, data collection, data analysis, manuscript writing; N.A.T.C.: methodology, data collection, data analysis, manuscript writing; A.A.B.M.: data collection, data analysis, manuscript writing; G.E.E.Y.: methodology, data collection, manuscript.

Ethical considerations

The study took place at Ain Shams University Hospital and Private Centre in the period from December 2020 to December 2021 after obtaining the preliminary approval of the study protocol and the completion of case collection. The study outcomes were approved by the local Ethics Committee with code FMASU M D 236/2020.

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Заболеваемость раком молочной железы у египетских женщин в зависимости от различной плотности маммографических ACR

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Резюме

Введение: Плотность молочной железы, радиологически называемая фиброзно-железистой тканью молочной железы, оказалась предрасполагающим фактором развития рака молочной железы (РМЖ). Однако заявленная степень повышенного риска РМЖ широко варьируется в литературе.

Цель: Найти взаимосвязь между различной плотностью маммографии молочной железы и риском рака молочной железы у египетских женщин.

Пациенты и методы: Аналитическое поперечное проспективное исследование было проведено в университетской больнице и частном центре Айн-Шамс в период с декабря 2020 по декабрь 2021 года. В исследование были включены 814 бессимптомных женщин в возрасте 40 лет и старше, пришедших на скрининг РМЖ с использованием полноформатной цифровой маммографии.

Результаты: Заболеваемость РМЖ выявлена у 84 женщин (10.3 %). Заболеваемость РМЖ у женщин с ACR-A составила 6.3 %, ACR-B - 8.5 %, ACR-C - 16.3 % и ACR-D - 27.8 % со значением $p<0.001$. Используя анализ логистической регрессии, мы показали, что три типа плотности груди увеличивают риск РМЖ, при этом пациентки с плотностью груди типа B, типа C и типа D имели риск развития РМЖ в 1.39, 2.92 и 3.12 раза соответственно ($p=0.010$, $p=0.003$ и $p=0.036$ соответственно).

Заключение: Среди египетских женщин заболеваемость РМЖ составляет 10.3 %. Наши данные показали, что чем больше плотность молочной железы, тем выше заболеваемость РМЖ, и подтвердили, что плотность молочной железы является фактором риска развития РМЖ.

Ключевые слова

рак молочной железы, плотность молочной железы, маммография

folia medica

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Original Article

Emerging Pattern of Asymptomatic Hyperparathyroidism in South India – a Six-Year Retrospective Study

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Abstract

Introduction: Primary hyperparathyroidism (PHPT) is a common endocrine disease with a variable presentation. There is a recent increase in the number of asymptomatic cases due to the use of multichannel automated analyzers.

Aim: To analyze the changing trend of PHPT patients from South India.

Materials and methods: We collected the data on clinical presentation, biochemistry, radiological features, and operative findings of patients with PHPT treated in our hospital over a period of six years and looked at the differences between symptomatic and asymptomatic PHPT.

Results: Our study included 80 patients. A significant proportion (~41%; n=33) of the patients were asymptomatic. Fifty-seven percent of asymptomatic patients were females. Mean age at presentation of asymptomatic patients was 50.58 (±14.67) compared to 47.28 (±14.78) for the symptomatic group, which was not statistically significant (p=0.34). The mean levels of serum calcium, phosphorous, 25(OH)D, iPTH, and 24-hour urinary calcium in symptomatic vs. asymptomatic patients were 12.47 (±2.26) mg/dl vs. 12.27 (±1.82) mg/dl (p=0.70), 2.59 (±0.74) mg/dl vs. 2.38 (±0.77) mg/dl (p=0.27), 12 (±1.2) ng/ml vs. 10.85 (±1) ng/ml (p=0.78), 1212.5 pg/ml vs. 678.5 pg/ml (p=0.31), and 292.6 mg/day vs. 262 mg/day (p=0.64), respectively. When Ca and gland weight were compared with variations in the iPTH levels, there was a significant positive correlation with PTH >600 pg/ml (p=0.001) with no between-group differences. The adenoma weight increased by 0.5291 mg for every unit increase in iPTH in the entire cohort, with no between-group differences (p=0.52).

Conclusion: Asymptomatic hyperparathyroidism is increasingly being identified in clinical practice and constitutes a significant proportion of primary hyperparathyroidism. Though asymptomatic PHPT is expected to be milder, such a difference in presentation was not obvious in our study.

Keywords

asymptomatic hyperparathyroidism, parathyroid gland weight, trend

INTRODUCTION

Primary hyperparathyroidism (PHPT) is a common endocrine disease with a variable clinical presentation. PHPT is usually symptomatic at presentation in majority of the patients, especially in developing countries.[1,2] As the accessibility to investigations, advanced imaging methods and surgical procedures are improving, the clinical profile of the patients with PHPT has undergone a palpable change compared to the earlier description.[3,4] There is a recent increase in number of asymptomatic cases due to the use of multichannel automated analyzers. We have previously reported that among our cohort of PHPT, 38.9% of patients were asymptomatic and were detected incidentally.[5]

AIM

We decided to analyze our cohort to look for any differences in the clinical profile and surgical outcomes between symptomatic and asymptomatic PHPT patients who underwent surgery.

MATERIALS AND METHODS

Subjects

Our study was conducted at St John's National Academy of Health Sciences, Bengaluru. All patients who underwent parathyroid surgery between January 2011 and December 2016 were included in this study. Patients were diagnosed as having hyperparathyroidism based on the following criteria: 1) Elevated serum calcium level of more than 10.5 mg/dl, and an inappropriately low phosphorous level; 2) Inappropriately high intact parathyroid hormone (iPTH) level.

Measurement of parameters

Serum calcium assay was done using the modified ortho-cresolphthalein complexone method on the Beckman Coulter AU analyzer; the reference range for normal serum calcium in our laboratory is 8.5 to 10.5 mg/dl. Serum phosphate was assessed using modified phosphomolybdate method on the Beckman Coulter AU analyzer; the reference range for normal serum phosphate in our laboratory is 2.5 to 4.5 mg/dl. Intact parathyroid hormone (iPTH) was measured by electrochemiluminescence (ECLIA) sandwich assay (Elecsys system, Roche Diagnostics), with the normal reference range being 15 to 65 pg/ml. 24-hour urinary calcium was measured by a modified ortho-cresolphthalein complexone method; values above 4 mg/kg/24 hours were considered as evidence of hypercalciuria. Urine was collected in a container containing 10-20 ml of 6 N (M) hydrochloric acid (HCl). 25 hydroxy vitamin D [25(OH)D] was measured by competitive immunoassay (ADVIA Centaur XP system Siemens healthcare diagnostics), with a value below 20 ng/ml being considered as evidence of deficiency.

Data collection

Medical records of these patients were retrospectively reviewed for age, sex, previous medical history, presenting symptoms and signs, routine biochemical investigations and histopathological diagnosis, operative and peri-operative findings. Patients were categorized into symptomatic and asymptomatic hyperparathyroidism based on these findings and compared on various variables. Secondary and tertiary hyperparathyroidism cases were excluded. Institutional Ethics Committee clearance was sought for conducting the study.

Statistical analysis

SPSS 21 (Statistical Package for Social Sciences 21, USA) was used for data analysis. The data are expressed as mean ± standard deviation (SD); data that did not have a normal distribution are also expressed as median (range). Student's *t* test or Mann-Whitney U test (skewed data) was applied for comparing two groups. A p-value of ≤0.05 was considered statistically significant.

RESULTS

Our study identified 80 patients with ages ranging from 16 to 76 years. A significant proportion (~41%; n=33) of the patients were asymptomatic. Subjects in our study showed a female-to-male ratio of 1.43 to 1. Symptomatic group was defined by their clinical presentation.

In the symptomatic group, 15 patients (31.9 %) presented with recurrent renal calculi, 10 patients (14%) presented with musculoskeletal pains, 6 (12%) presented with fractures, 5 (10.6%) presented with pancreatitis, and 3 (6%) presented with neuro-psychiatric symptoms. In addition, 4 patients (8.5%) had a palpable neck nodule, 3 (6%) had evidence of brown tumor, and 3 (6%) had nephrocalcinosis on evaluation. In the symptomatic group, 4 patients required immediate correction for acute severe hypercalcemia. Subjects in the asymptomatic group were identified on evaluation of unrelated sickness such as while evaluating for fever, or preoperative evaluation for unrelated surgery such as cholecystectomy or thyroidectomy. Some patients were detected incidentally on routine health checkups.

We compared the data on symptomatic and asymptomatic groups, and this has been summarized and presented in **Table 1**. In our analysis, 57% of asymptomatic patient were females. Mean age at presentation of asymptomatic patients was 50.58 (±14.67) compared to 47.28 (±14.78). The mean serum iPTH levels were almost double in patients who presented with symptomatic PHPT, though this difference was not statistically significant. The levels of calcium, phospho-

Table 1. Comparison of clinical profile in patients with symptomatic and asymptomatic hyperparathyroidism

Characteristic (Mean)	Symptomatic	Asymptomatic	*p*-value
Number (n=80)	47	33	
Age in years	47.28 (±14.78)	50.58 (±14.67)	0.34
Sex	Male 19 (40%) Female 28 (59%)	Male 14 (42%) Female 19 (57%)	0.85
iPTH (baseline) (pg/ml)	1212.5	678.5	0.31
Corrected calcium (mg/dl)	12.47 (±2.26)	12.27 (±1.82)	0.70
Phosphate (mg/dl)	2.59 (±0.74)	2.38 (±0.77)	0.27
ALP (IU/l)	172.5	135.5	0.245
25 OH Vitamin D (IU)	12	10.85	0.78
24 hr. urinary calcium (mg/day)	292.6	262	0.64
Duration of surgery (mins)	151	150	0.55
Gland weight in gms	4.74	2.20	0.52
Post-op hypocalcemia	Yes: 3/12 (25%) No: 9/12 (75%)	Yes: 11/25 (44%) No: 14/25 (56%)	0.34

rous, alkaline phosphatase, vitamin D and urinary calcium were very similar between the two groups. Although the duration of surgery did not differ between the two groups, the weight of the excised gland was more than double in the symptomatic group, and this was found to positively correlate with iPTH levels. The adenoma weight increased by 0.5291 mg for every unit increase in iPTH in the entire cohort of patients with PHPT. Both serum calcium and gland weight were found to have significant positive correlation with PTH, at serum iPTH values above 600 pg/ml ($p \leq 0.001$). Contrary to expectation, the incidence of post-op hypocalcemia was higher in the asymptomatic group. Although this was not statistically significant, this observation may allude to the fact that asymptomatic patients were not adequately treated pre-operatively for vitamin D deficiency that seemed equally prevalent in both groups.

DISCUSSION

Primary hyperparathyroidism is a common endocrine condition[6] with a variable presentation with a spectrum including: a) symptomatic hyperparathyroidism, b) normocalcemic hyperparathyroidism, and c) asymptomatic hyperparathyroidism. There is still a debate amongst clinicians whether to treat asymptomatic PHPT as one end of a spectrum in PHPT or to treat it as a separate entity. The effect on morbidity and mortality including effect on behavioral, renal, gastrointestinal, cardiovascular systems needs to be established, if asymptomatic PHPT is to be treated as a separate entity.[7-9] In this study, we have attempted to differentiate between the characteristics of symptomatic and asymptomatic cases at the time of detection and see if there are significant differences in their clinical profile.

We found that asymptomatic patients formed a significant portion of our PHPT cohort (41%). However, they did not differ from the symptomatic group with regards to levels of calcium, phosphorous, alkaline phosphatase, and vitamin D measured in serum; nor was there any noticeable difference in urinary calcium excretion. The asymptomatic patients tended to be older in age, had lower levels of serum iPTH, and exhibited lower weight of the excised gland. Though these differences did not achieve statistical significance, they suggest that we may have detected the disease at an earlier stage and could explain the lack of symptoms attributable to PHPT.

When serum calcium and gland weight were compared with variations in the iPTH levels, there was a significant positive correlation with iPTH>600 pg/ml irrespective of the group the patient belonged to. The adenoma weight increased by 0.5291 mg for every unit increase in iPTH in the entire cohort. These findings suggest that the degree of hypercalcemia and the size of the gland goes hand in hand with the severity of parathyroid hormone excess, even in patients who were asymptomatic at detection. The incidence of post operative hypocalcemia also did not statistically differ between the two groups, suggesting that significant physiological changes have occurred in the asymptomatic group in a similar fashion to what is seen in classic PHPT. It is noteworthy to mention that asymptomatic PHPT patients are also prone to develop post operative hypocalcemia and should receive appropriate preventive measures and close monitoring during the post-op period.

In comparison to a study by Mithal et al.[9], done in our country from 2009 to 2012, we found no statistical differences in age at presentation between symptomatic and asymptomatic patients; whereas they found that asymptomatic patients were older. The proportion of asymptomatic PHPT cases were very similar to what we found in our study (38% vs. 41%). When compared to another study by Arya et al.[10], the proportion of patients diagnosed with asymptomatic PHPT was more in our cohort (10% vs. 41%).

While Arya et al. included patients diagnosed with PHPT from 1995 onwards, our cohort only included patients from 2011 onwards. This difference seen in our study and the one by Mithal et al. probably reflects the changing spectrum of PHPT, with more and more asymptomatic cases being detected as a result of enhanced health screening packages and better access to health care. The adenoma gland weight and iPTH were lower in our findings too, similar to the above-mentioned studies, but these differences were not statistically significant in our cohort. A study by Parfitt et al.[11] from Michigan reported the clinical course of patients incidentally detected to have asymptomatic PHPT; the mean serum calcium and phosphate levels in their cohort were 11.08 mg/dL and 2.71 mg/dL, respectively. This suggested that incidentally detected asymptomatic patients are likely to have lesser degree of hypercalcemia and less pronounced hypophosphatemia. However, in our cohort of asymptomatic PHPT patients, there was a considerable degree of hypercalcemia (mean calcium – 12.27 mg/dl) and significant hypophosphatemia (mean phosphate – 2.38 mg/dl). We speculate that these differences could be related to the highly prevalent vitamin D deficiency in our cohort along with other environmental and nutritional factors. A recently published analysis of the Indian PHPT registry showed that close to 20% of patients were truly asymptomatic with no renal or skeletal manifestations.[12] This is in stark contrast with data from the western world, where more than 80% of cases of PHPT are incidentally detected and are asymptomatic.[13]

Our study does have some limitations. Firstly, it is a retrospective study that has captured available data for analysis. Extensive pre-operative evaluation and assessment may not be possible in all patients as the cost of therapy is completely borne by the patient. Bone mineral density assessment was not done in most of the patients, particularly in those from the lower economic strata. It would have been interesting to see if bone mineral density differed between the two groups; this would have added more value to our study. The relatively small sample size is another limitation, which may have contributed to the lack of statistical significance in the differences observed between the two groups. The strength of our study lies in the fact that this data has been obtained in the last decade and is more likely to be representative of the current trend.

All patients in this cohort were counseled for surgery if calcium levels warranted the same. Those with mild hypercalcemia were given the option of immediate surgery versus watchful observation. Though we have only included those who underwent surgery for this analysis, most patients in our experience opted for surgery as opposed to watchful waiting. This may be influenced by the fact that some patients come from distant and remote areas, which makes frequent follow-up visits very difficult. For many, single visit surgical curative therapy may be a better option when compared to multiple follow up visits with adding costs of investigations. This trend may be different from what is seen in the west, where the incidence of parathyroidectomy is maximum in the eighth decade of life[14], and younger patients opt for watchful waiting more often.

CONCLUSION

Our data obtained from asymptomatic PHPT patients who were incidentally detected reveal that their clinical profile is very similar to those with classical symptomatic PHPT. It is likely that the disease would have evolved in these patients and manifested as classic PHPT if they were left untreated. This leads us to believe that the asymptomatic PHPT patients identified incidentally belong to one end of the spectrum and should be evaluated for renal and skeletal manifestations and should be advised surgical intervention if and when necessary. Awareness among physicians and general practitioners about the need for further evaluation of incidentally picked up asymptomatic hypercalcemia will facilitate prompt referral to a specialist and timely intervention. There is felt need for more detailed and prospective studies comparing the outcomes in cases opting for surgery versus those opting for long-term follow-up.

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Author contributions

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Новая картина бессимптомного гиперпаратиреоза в Южной Индии – шестилетнее ретроспективное исследование

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Резюме

Введение: Первичный гиперпаратиреоз (ПГПТ) — распространённое эндокринное заболевание с вариабельной клинической картиной. В последнее время наблюдается рост числа бессимптомных случаев из-за использования многоканальных автоматических анализаторов.

Цель: Поэтому мы решили проанализировать тенденцию изменения пациентов с ПГПТ из Южной Индии.

Folia Medica

Материалы и методы: Мы собрали данные о клинической картине, биохимии, рентгенологических особенностях и оперативных результатах пациентов с ПГПТ, проходивших лечение в нашей больнице в течение шести лет, и рассмотрели различия между симптоматическим и бессимптомным ПГПТ.

Результаты: В наше исследовании было включено 80 пациентов. У значительной части (~41 %; n=33) пациентов симптомов не было. 57 % бессимптомных пациентов были женщинами. Средний возраст на момент обращения бессимптомных пациентов составил 50.58 (±14.67) по сравнению с 47.28 (±14.78) для симптоматической группы, что не было статистически значимым (p=0.34). Средние уровни сывороточного кальция, фосфора, 25(OH)D, iPTH и 24-часовая экскреция кальция с мочой у пациентов с симптомами и у бессимптомных пациентов составляли 12.47 (±2.26) mg/dl против 12.27 (±1.82) mg/dl (p=0.70) и 2.59 (±0.74) mg/dl против 2.38 (±0.77) (p=0.27) mg/dl, 12 (±1.2) ng/ml против 10.85 (±1) ng/ml (p=0.78) и 1212.5 pg/ml против 678.5 pg/ml (p=0.31), 292.6 mg/сут.против 262, статистически не значимо (p=0.64) соответственно. Ca и вес железы сравнивали с вариациями уровней PTH, наблюдалась значительная положительная корреляция с PTH>600 pg/ml (p=0.001) без различий между группами. Масса аденомы железы увеличилась на 0.5291 mg на единицу увеличения PTH во всей когорте первичного гиперпаратиреоза (без различий между группами) (p=0.52).

Заключение: Бессимптомный гиперпаратиреоз всё чаще выявляется в клинической практике и составляет значительную долю первичного гиперпаратиреоза. Хотя ожидается, что бессимптомный ПГПТ будет более лёгким, в нашем исследовании такая разница в проявлениях не была очевидной.

Ключевые слова

бессимптомный гиперпаратиреоз, масса паращитовидной железы, тенденция

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Original Article

Association of IL-4 Polymorphism with Severe Periodontitis in a Sample of Iraqi Population

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Abstract

Introduction: Specific bacterial plaque and environmental factors cannot be considered the only cause of periodontitis. Still, several genetic factors affect the host response to the bacteria, like gene polymorphisms in anti-inflammatory cytokines. Several studies have reported that clones of T-helper 2 lymphocytes (TH2) are generated in response to dental plaque in periodontitis patients, while in healthy individuals, they are regulated by T-helper 1 (TH1) lymphocytes. Accordingly, such patients consistently produce more IL-4 (TH2) in response to bacterial stimulation, whereas healthy controls with intact periodontal tissues produce a significantly higher level of TH1.

Aim: The current work aimed to investigate the association between variations in IL-4 gene polymorphisms and susceptibility to periodontitis.

Materials and methods: The current study employed a case-control observational methodology involving 120 Iraqi participants. These participants were divided into two groups: the periodontitis group, consisting of 63 subjects, and the control group, consisting of 57 subjects. Clinical periodontal parameters were assessed for all participants, and subsequent genetic analysis of IL-4 was conducted using DNA sequencing. Venous blood samples were collected from each participant enrolled in the study. SPSS was used to conduct descriptive and inferential statistical analyses, including odds ratio, Hardy-Weinberg equilibrium, and Pearson correlation.

Results: The Hardy-Weinberg equilibrium for study groups regarding the rs1801275 and rs1805016 polymorphisms of IL-4 showed a non-significant difference between the observed and expected genotypes in both groups involved in the study and the overall sample. Moreover, there was no significant association between the IL-4 gene polymorphism and the clinical periodontal parameters.

Conclusion: The research conducted revealed a lack of correlation between IL-4 gene polymorphism and the susceptibility to periodontitis in individuals from Iraq. More research with a bigger sample size is required to validate these findings.

Keywords

gene polymorphisms, interleukin-4, periodontitis.

INTRODUCTION

Periodontitis is a complex multifactorial inflammatory disease of the teeth's supporting structures, characterized by progressive destruction of the alveolar bone and periodontal ligament, the formation of periodontal pockets, loss of clinical attachment, and tooth mobility.[1] Dental plaque, with its specific microorganisms, is responsible for initiating periodontitis. Still, the breakdown of the connective tissue and the bone is affected mainly by the host response influenced by risk factors like age[2], systemic diseases like diabetes mellitus, smoking, gender, patient's oral hygiene, and genetic factors.[3,4] Although bacterial dental plaque is the key to the development of periodontitis, each individual may have a dose-dependent response to the bacterial challenge that determines their susceptibility to periodontitis.[5]

Several genes with or without their polymorphisms could affect the severity and susceptibility to periodontitis. Polymorphisms in genes can cause changes in proteins or their expression, potentially influencing innate and adaptive immune responses and, ultimately, disease outcomes. On the other hand, specific genetic variants may have a protective function in the progression of diseases.[6] However, genes are accountable for about 50% of susceptibility to periodontitis.[7,8]

Pathogenic bacteria present in periodontal tissues elicit an immune response, which can gradually lead to the destruction of the periodontium through the inflammatory process.[9] Certain microorganisms present in dental plaque can induce the production of cytokines and other biologically active substances by cells in the periodontium, thereby modulating the immune response.[10] Periodontal diseases may be triggered and maintained by excessive production of pro-inflammatory cytokines or insufficient production of anti-inflammatory cytokines.[11]

IL-4 is one of the essential anti-inflammatory cytokines[12] that has a role in down-regulating the macrophage function[13] and suppressing the secretion of prostaglandin E2 (PG-E2)[14], tumor necrosis factor (TNF-α), and many pro-inflammatory interleukins, including IL-1 and IL-6[15]. The cytokine gene cluster located on the human chromosome 5q31-33 region is responsible for the genetic mapping of interleukin-4. It encompasses several polymorphisms[16], so genetic polymorphisms in the IL-4 gene may be responsible for the aggravation of periodontal disease by altering the level of IL-4[17] since the expressions of cytokines may be affected by gene polymorphisms, and this affects the progression and susceptibility to periodontitis[18].

AIM

Accordingly, it was hypothesized that there is no relationship between periodontitis and IL-4 gene polymorphism in Iraqi individuals; thus, the current study sought to investigate the association of IL-4 gene polymorphism with susceptibility to periodontitis in the Iraqi population.

MATERIALS AND METHODS

Subjects and study design

The study employed a case-control observational design and was conducted in the College of Dentistry, University of Baghdad. The data collection commenced in September 2019 and concluded in March 2020. The researchers acquired ethical approval for the current study from the Research Ethical Committees of the College of Dentistry, the University of Baghdad, in September 2019. Additionally, all participants voluntarily participated in the study and provided informed consent regarding the study's objectives and methodology.

Around 345 subjects were examined, and only 120 Iraqi subjects between the ages of 30 and 50 met the inclusion criteria. The participants were individuals of Arab Iraqi nationality who were categorized into two groups: the periodontitis group (cases) and the control group. The subjects involved in this study exhibited systemic health, did not engage in smoking, and provided informed consent to participate. Moreover, a physician examined all participants to ensure their fitness for inclusion criteria and excluded anyone with systemic disease. Furthermore, subjects with any form of oral disease/condition, subjects using medicaments, pregnant or lactating mothers and those who could not collaborate in the study were also excluded.

The periodontitis group had 63 subjects, all of whom were diagnosed with periodontitis according to the criteria outlined in Tonetti et al.[19], in which radiographical interdental bone loss was detectable at two or more non-adjacent teeth, or the patients had buccal clinical attachment loss more than 3 mm with probing pocket depth more than 3 mm detected at more than 2 teeth. Additionally, periodontitis patients should fully fit the following characteristics:

1. Generalized periodontitis, where over 30% of teeth show attachment loss.
2. Unstable periodontitis, when bleeding pockets were evident at a depth of 4 mm or when the pocket depth reached or exceeded 5 mm.
3. The percentage of interdental bone loss is more than 35%.

The remaining 57 subjects all had a clinically healthy gingiva with intact periodontium and were considered valid to be enrolled in the study according to the criteria given in Dietrich et al.[20], - probing pocket depths ≤3 mm and bleeding on probing less than 10% with no clinical attachment loss.

Clinical periodontal parameters

Calibration sessions were conducted between the initial examiner and a qualified periodontist on 10 patients who were not part of the study. These sessions continued until a consensus level of above 75% was achieved for all clinical periodontal parameters.

The process of obtaining blood samples and doing genotyping

Venous blood from the antecubital vein was collected using 2 ml vacutainer glass blood collection tubes. The collected blood was then transported into a buffered tube containing sodium citrate 3.2% and kept at −40°C for the genotyping of IL-4. The experimental procedures encompassed the extraction of DNA, wherein the genomic DNA was obtained from the blood sample using the QIAamp DNA Mini Kit, following the QIAGEN protocol. The process of PCR amplification was initiated by preparing and optimizing the primers. The primers regarding IL-4 were selected as shown in **Table 1**.

Table 1. Selection of IL-4 primers

IL-4 primers	The Forward Primer	5-GACACCTGGAGGAAGTAGAA-3
	The Reverse Primer	5-CAAGAGGACATGCACCTAAG-3

The primers utilized in this study were subjected to lyophilization and were obtained from Macrogen Company.

The primer template's optimal annealing temperature was investigated by amplifying it using the same primer pair described in **Table 1**. The annealing temperatures tested were 55°C, 58°C, 60°C, 63°C, and 65°C. Subsequently, the polymerase chain reaction (PCR) amplifications were conducted. The PCR cycling procedure was conducted using the PCR Express instrument (Thermal Cycler, BioRad, USA) with the subsequent temperature program: the denaturation process was carried out at a temperature of 94°C for 4 minutes, followed by a series of thirty cycles. Each cycle consisted of denaturation at 94°C for thirty seconds, annealing at temperatures of 55°C, 58°C, 60°C, 63°C, or 65°C for 30 seconds, and extension at 72°C for 30 seconds. The experimental protocol involved a concluding extended incubation period lasting 7 minutes at a temperature of 72°C, which was thereafter followed by a 10-minute incubation at a temperature of 4°C to halt the ongoing processes. Agarose gel electrophoresis was utilized to validate the presence of the amplified PCR product during the loading process. The PCR product was put into the well by immediately adding 5 µl. The electrical power supply was activated at a voltage of 100 volts and a current of 50 milliamperes for 90 minutes. The movement of DNA occurs from the cathode to the anode poles. The bands stained with ethidium bromide in the gel were observed utilizing gel imaging equipment. The PCR product underwent Sanger sequencing using ABI3730XL, an automated DNA sequencer, at Macrogen Corporation, Korea (http://dna.macrogen.com/eng). The data were gathered via electronic mail and subsequently examined by an expert in software analysis. Subsequently, the sequences of all samples were aligned with the source sequence and subjected to analysis utilizing the Basic Local Alignment Search Tool Program (BLAST) (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Statistical analysis

Detailed statistical tests were performed using both descriptive and analytical statistics. For the analytical statistics, the Shapiro-Wilk test was done for normality of distribution to ensure whether the collected data followed a normal distribution. Chi-square and Fisher's exact test were employed for categorical variables. The odds ratio (OR) was used to measure the strength of the association of IL-4 SNP with health and periodontitis. The Hardy-Weinberg equation was used to compute the predictable homozygotes, heterozygotes, predictable rare homozygotes, and the frequency domain of the alleles from the detected genotypes. The statistical analysis was conducted using the SPSS software (version 21, IBM, USA).

RESULTS

The study included participants aged between 32 and 55 years. The mean age of the study group was 46.86±6.6, while the mean age of the control group was 38.86±4.4 **(Table 2)**. Moreover, the sex of patients was also illustrated in **Table 2**. In the periodontitis group, there were 57 male and 6 female participants, whereas the control group consisted of 36 male and 21 female participants. Concerning the documented clinical periodontal measures, it was observed that the group diagnosed with periodontitis exhibited significantly elevated values for plaque index (PI), gingival index (GI), bleeding on probing (BOP), and tooth loss in comparison to the control group (refer to **Table 2**).

Table 2. Demographic characteristics and clinical periodontal parameters of groups

	Periodontitis	**Control**	***P* value**
N	63	57	
Age range	32-55	32-55	
Age†	46.86±6.60	38.86±4.4	<0.001*S
Sex			
Male	57	36	0.035**
Female	6	21	
Clinical periodontal parameters†			
PI	2.66±0.38	0.52±0.04	<0.001*S
BOP	69.34±22.52	8±0.01	0.001**S
GI	1.83±0.32	0.49±0.03	<0.001*S
PPD	5.19±0.35		
CAL	6.47±0.52		
Missing teeth	6.39±2.15	0.59± .11	<0.001*S

PI: plaque index; BOP: bleeding on probing; GI: gingival index; PPD: probing pocket depth; CAL: clinical attachment loss; † Mean ± standard deviation; * The statistical significance level of $p<0.05$ was determined using the Mann-Whitney test. ** Significance at $p<0.05$ using chi-square test

The PCR loading showed the primer optimization for IL-4 according to primer design as illustrated in **Fig. 1**.

After Sanger sequencing, two polymorphisms were detected according to the primer design as demonstrated in **Figs 2, 3** at rs in rs1801275 and rs1805016.

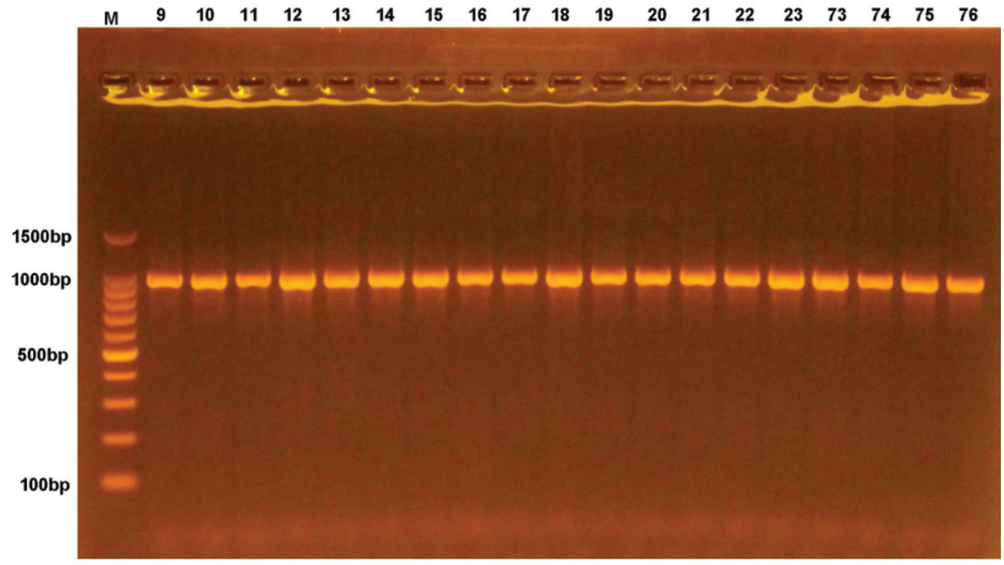

Figure 1. The amplification outcomes of rs1801275 were observed using fractionation on a 1% agarose gel electrophoresis, which was afterward stained with ethidium bromide. A 100 bp ladder marker was used as a reference for size determination.

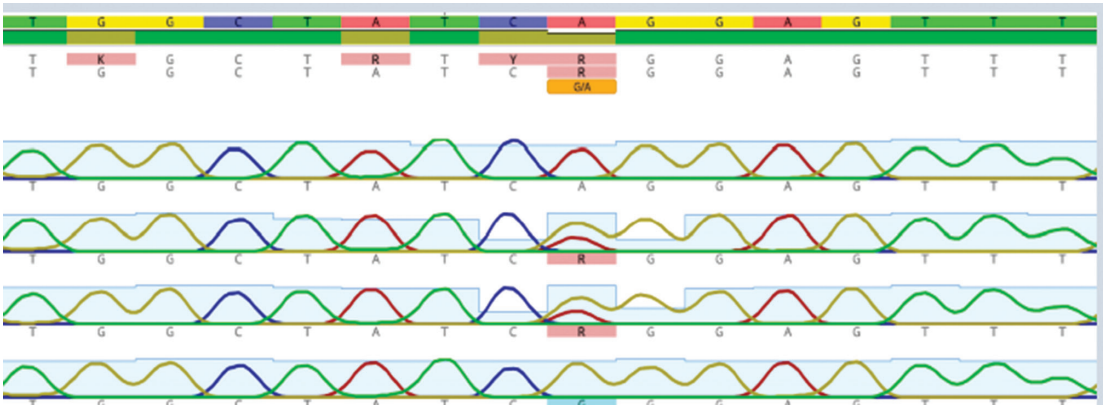

Figure 2. In this study, the rs1801275 SNP was analyzed using Sanger sequencing. The presence of a single peak denoted explicitly as 'A' is suggestive of an individual possessing a homozygous allele for the A gene. The presence of a single 'G' peak is indicative of a homozygous allele for the G variant. The presence of both the 'A' and 'G' peaks is indicative of the presence of an A/C heterozygous allele.

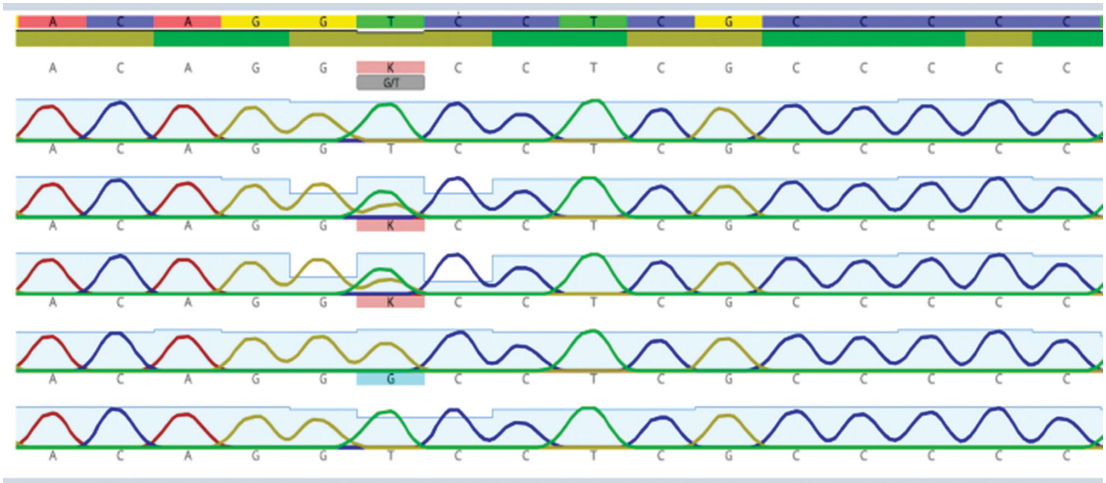

Figure 3. The rs1805016 SNP is analyzed via the Sanger sequencing method. A single peak denoted as 'A' is evidence of a homozygous allele, A solitary. The 'C' peak is suggestive of a homozygous C allele. The presence of the 'A' and 'T' peaks indicates the presence of an A/C heterozygous allele.

The two SNPs were analyzed by Hardy-Weinberg equilibrium. The results were non-significant in the periodontitis group, the control group, and in the total sample, as shown in **Table 3**.

Furthermore, concerning the rs1801275 polymorphism, twelve SNPs were observed in both the periodontitis and control groups, displaying no statistically significant variations. In contrast, the periodontitis group exhibited 6 SNPs, whereas the control group displayed 12 SNPs at rs1805016, with no statistically significant distinction observed between the two groups. The impact of IL-4 SNPs on the distribution of periodontitis was evaluated by calculating the odds ratio. The odds ratio was found to be 0.882 for rs1801275 and 0.3947 for rs1805016, as presented in **Table 4**.

Furthermore, **Table 5** described the Fisher exact test to find the genotype frequency of both SNPs in IL-4; it showed a non-significant difference in their distribution in both groups. The research demonstrated the impact of individual genotypes of IL-4 SNPs on the distribution and prevention of diseases. The study revealed that individuals with genotype A-G exhibited a more significant odds ratio (1.18) in relation to other genotypes for disease susceptibility in the rs1801275 locus, despite the lack of statistical significance in the p-value. The genotype T-G exhibits a higher susceptibility (0.53) to disease development in comparison to other genotypes present at the rs1805016 locus.

Regarding the correlation of IL-4 SNPs with clinical periodontal parameters, the current study illustrated a non-significant negative weak correlation between rs1801275 and all periodontal parameters in the periodontitis group while a non-significant positive weak correlation was found between rs1801275 and PlI, GI in the control group. For 1805016, a non-significant negative weak correlation was found between 1805016 and all periodontal parameters in the periodontitis group except for the PPD, which showed a non-significant weak positive correlation. In the control group, a non-significant positive weak correlation was found between rs1805016 and PlI, GI **(Table 6)**.

Table 3. Hardy-Weinberg equilibrium for groups in rs1801275 and rs1805016 polymorphisms

rs1801275 polymorphism	Periodontitis N=63		Control N=57		Total N=120	
	Observed in	**Expected in**	**Observed in**	**Expected in**	**Observed in**	**Expected in**
AA	51	51.6	45	42.9	96	94.5
AG	12	10.8	9	12.9	21	24
GG	0	0.6	3	0.9	3	1.5
Hardy-Weinberg equilibrium	0.232		1.815		0.611	
P value	0.629		0.17		0.43	
rs1805016 polymorphism	**Periodontitis N=63**		**Control N=57**		**Total N=120**	
	Observed in	**Expected in**	**Observed in**	**Expected in**	**Observed in**	**Expected in**
TT	57	57	45	42.9	102	99.9
TG	6	5.7	9	12.9	15	19.2
GG	0	0.0	3	0.9	3	0.9
Hardy-Weinberg equilibrium	0.0525		1.82		1.89	
P value	0.818769		0.177		0.17	

Table 4. The quantity of SNPs observed in the IL-4 gene among individuals in the periodontitis and control groups

	Periodontitis		Control		Fisher exact	*P* value	Odds ratio	CI
	n	**%**	**n**	**%**				
rs1801275	12	19.05%	12	21.05%	1	0.8743	0.882	0.1873 to 4.1577
rs1805016	6	9.52%	12	21.05%	0.39	0.3188	0.3947	0.0635 to 2.4544

CI: confidence interval.

Table 5. Genotype frequency of IL-4 SNPs

Genotype Frequency		Periodontitis		Control		Fisher exact test	*P* value	Odd ratio	CI 95%	Population penetrance
		%	**Frequency**	**%**	**Frequency**					
rs1801275	AG	12	19.05%	9	15.79%	1	0.847	1.18	0.23–6.13	0.24%
	GG	0	0%	3	5.26%	0.484	0.465	0.295	0.0112 to 7.790	0%
rs1805016	TG	6	9.52%	9	15.79%	0.64	0.51	0.530	0.08–3.56	0.12%
	GG	0	0%	3	5.26%	0.457	0.425	0.26	0.0101 to 6.967	0%

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Table 6. The rs1801275 and rs1805016 correlation with clinical periodontal parameters

Sperman correlation			PlI	GI	BOP	PPD	CAL
1801275	Periodontitis	*r*	−0.321	−0.37	−0.302	−0.360	−0.350
		p	0.155	0.100	0.183	0.108	0.119
	Control	*r*	0.120	0.190			
		p	0.624	0.434			
1805016	Periodontitis	*r*	−0.040	−0.150	−0.188	0.026	−0.121
		p	0.86	0.515	0.412	0.91	0.602
	Control	*r*	0.120	0.190			
		p	0.624	0.434			

DISCUSSION

In addition to environmental factors, genetics is a significant factor that decisively affects the host's susceptibility to periodontitis.[21,22] In 1990, the first argument emerged by Schafer et al. in that genetics had a fundamental part in the initiation and advancement of periodontal diseases as they assumed that the primary key to whether individuals are susceptible to developing periodontitis or not is mainly dependent on the way their bodies responded to the microbial attack.[23] In this regard, the association between the SNP of the inflammatory immune response and periodontitis has gained important attention in recent studies as a possible contributor to periodontal disease[24,25] affording a more detailed understanding of the development and progression of periodontal disease and contributing to the advancement of novel diagnosis, treatment, and preventive approaches. Based on the idea proposed by Seymour et al.[26] concerning the role of TH1 and TH2 lymphocytes in periodontal disease progression, they proposed that in patients with progressing periodontitis, clones of TH2 are generated upon activation with bacteria. Accordingly, such patients constantly revealed amplified secretion of IL-4 upon stimulation of T-helper 2 lymphocytes by pathogenic dental plaque bacteria, while the non-progressing disease is regulated by TH1 clones, suggesting that periodontally healthy individuals with intact periodontal tissues produced a significantly increased level of IFNγ (TH1).[10] For the above-mentioned information, the current study was conducted investigating the association of IL-4 polymorphisms with susceptibility to periodontitis. Yet, the present work demonstrated no significant association between IL-4 polymorphism and periodontitis in the Iraqi human population. This finding was supported by the non-significant results of Hardy-Weinberg equilibrium for rs1801275 and rs1805016 polymorphisms between the observed and expected genotypes in the periodontitis and control groups and the total sample. Furthermore, there were non-significant associations between the IL-4 gene polymorphism and the clinical periodontal parameters, which was in agreement with several other populations, such as Czech[27], Macedonian[28], Iranian[29], Brazilian[30,31], and Japanese[32], as their studies showed no association between polymorphisms in the IL-4 gene and periodontitis, as well as consistent with the longitudinal study done by Walther et al., as they reported IL-4 polymorphisms were unpredictable for further CAL loss[33].

Inversely, a study on the population in Germany showed a borderline association between IL-4 and periodontitis.[34] In another Brazilian study, the results showed that IL-4 gene was associated with periodontitis.[35] The small sample size, number, and sites of investigated polymorphisms, other ethnicities, and the effect of various environmental factors on susceptibility to periodontitis could explain the conflicting results reported in other literature sources. It is conceivable that a deduction reached in one population or racial group may be completely different in another population.[36]

The impact of IL-4 single nucleotide polymorphisms on the vulnerability to periodontal disease throughout the Iraqi population was shown to be negligible. On the contrary, another study reported that stimulation with dental plaque bacteria, for instance, *T. forsythia* and *P. intermedia,* would bring about meaningfully increased levels of the inflammatory response, leading to high production of different cytokines, and the IL-4 gene polymorphisms in periodontitis patients would not only affect the production of cytokines such as IL-4, IL-10, IFNγ, IL-1β, IL-6, and TNF-α, which in turn can affect periodontal disease. Polymorphisms in genes encoding some cytokines or their receptors can affect the production of not only their own but also other mediators. However, one of the major limitations of the current study is the lack of quantitative measurement of important cytokines that had a role in the development and progression of periodontal disease.[10] Furthermore, the small sample size of the people studied was due to financial considerations, which suggests the need for a future study with more patients. However, to mitigate the influence of confounding variables, this study did not include diabetes and smoking as risk factors for periodontal disease. This decision was made due to the potential for these diseases to exacerbate susceptibility to periodontitis in the presence of SNP. Another limitation of this study is the genetic heterogenicity of periodontal disease, which restricted the generalizability of the results. However, this study represents a limited number of investigations that have examined the role of IL-4 polymorphisms in periodontal disease within a sample of the Iraqi population.

CONCLUSION

Considering the limitations, it is possible to conclude that there is no evident association between polymorphisms in IL-4 and the susceptibility to periodontitis. Moreover, it was observed that there was a lack of association between clinical periodontal measures and the IL-4 single nucleotide polymorphism, indicating that the SNP has a minimal impact on the advancement and intensity of the illness. It is crucial to acknowledge that this study was constrained by its modest sample size and the fact that it was conducted at a single institution. Additional research with bigger sample size and multicenter methods is necessary to validate these findings.

Ethical Clearance

This study was approved by the Ethical Committee, College of Dentistry University of Baghdad.

Conflict of Interest

None.

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Ассоциация полиморфизма IL-4 с тяжёлым периодонтитом в выборке населения Ирака

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Резюме

Введение: Специфический бактериальный налёт и факторы окружающей среды не могут считаться единственной причиной пародонтита. Тем не менее, несколько генетических факторов влияют на реакцию организма-хозяина на бактерии, например полиморфизм генов противовоспалительных цитокинов. В нескольких исследованиях сообщалось, что клоны Т-хелперов 2 (TH2) генерируются в ответ на зубной налёт у пациентов с пародонтитом, тогда как у здоровых людей они регулируются Т-хелперами 1 (TH1) лимфоцитами. Соответственно, такие пациенты последовательно производят больше IL-4 (TH2) в ответ на бактериальную стимуляцию, тогда как здоровые люди с интактными тканями пародонта производят значительно более высокий уровень TH1.

Цель: Целью настоящей работы было изучение связи между вариациями полиморфизма гена IL-4 и предрасположенностью к пародонтиту.

Материалы и методы: В настоящем исследовании использовалась методология наблюдения „случай-контроль“ с участием 120 иракских участников. Эти участники были разделены на две группы: группа пародонтита, состоящая из 63 человек, и контрольная группа, состоящая из 57 человек. Клинические параметры пародонта были оценены у всех участников, а последующий генетический анализ IL-4 был проведён с использованием секвенирования ДНК. Образцы венозной крови были взяты у каждого участника, включенного в исследование. SPSS использовался для проведения описательного и статистического анализа, включая отношение шансов, равновесие Харди-Вайнберга и корреляцию Пирсона.

Результаты: Равновесие Харди-Вайнберга для исследуемых групп относительно полиморфизмов rs1801275 и rs1805016 IL-4 показало незначимую разницу между наблюдаемыми и ожидаемыми генотипами в обеих группах, участвовавших в исследовании, и в общей выборке. Более того, не было выявлено значимой связи между полиморфизмом гена IL-4 и клиническими параметрами пародонта.

Заключение: Проведённое исследование выявило отсутствие корреляции между полиморфизмом гена IL-4 и предрасположенностью к пародонтиту у лиц из Ирака. Для подтверждения этих выводов необходимы дополнительные исследования с большей выборкой.

Ключевые слова

полиморфизмы генов, интерлейкин– 4, пародонтит

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Original Article

Minimally Invasive Extractions with Physics Forceps – Clinical Evaluation and Comparison

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Abstract

Introduction: Tooth extraction is still one of the most common dental procedures, routinely performed for a variety of reasons. Tooth extraction forceps and elevators are well-known extraction instruments which have been the standard in tooth extraction procedures for well over a hundred years. Physics forceps are one possible alternative, aiming to perform less traumatic and more predictable extractions.

Aim: The aim of study was to compare the effectiveness of physics forceps as an alternative, less traumatic, tool to the conventional extraction forceps.

Materials and methods: All patients in the study were split into two groups: 26 patients in whom conventional extraction forceps were used (a control group) and 28 patients in whom we used physics forceps (a study group). For each group, we assessed the success of the extraction, the buccal cortical plate preservation, pain experience, and early wound healing.

Results: There was no statistically significant difference in extraction success scores between the two groups. Physics forceps extractions preserved the buccal cortical plate in 81.1% of instances, while traditional forceps extractions did so in 71.8%. The patients in the study group reported less pain on the seventh day. At 10 days, the study group had a marginally higher proportion of completely healed extraction wounds.

Conclusions: Atraumatic extractions preserve more hard and soft tissues at the extraction site. Physics forceps are a tool similar to the well-known conventional extraction forceps. They provide for somewhat better results in most extractions.

Keywords

atraumatic, minimally invasive, physics forceps, tooth extraction

INTRODUCTION

Tooth extraction remains one of the most common dental procedures, performed for a variety of reasons.[1] Dental implantology has emerged as the preferred method of replacing missing teeth, and even teeth with questionable conservative treatment outcomes are being seriously considered for extraction and replacement. Dental implants, however, are not just placed anywhere; they frequently need to be planned and executed properly in order to remove a tooth first.[2]

Tooth extraction forceps and elevators are well-known and have served as the main tools for tooth extractions for over a century. They do not make it difficult to conduct an atraumatic extraction, but they frequently result in unforeseen complications. Even the most skilled oral surgeons may have difficulty performing certain tooth extractions. Inadequate manipulation technique and approach can

result in a wide range of postoperative abnormalities in the extraction site, affecting both hard and soft tissues.[3,4] This is why specialists are still exploring different techniques and looking for the most predictable approach.

Different criteria can be used to assess the amount of trauma caused during an extraction. However, post extraction pain is undoubtedly one of the main indicators of how much damage was caused. Normally extractions are followed by mild to no discomfort at all, but their healing can be delayed and accompanied by severe pain, as well as symptoms such as swelling, trismus, infection. All of these indicate postoperative complications, oftentimes a result of excessive trauma.[5] This, of course, leads to an expectation that less bone tissue will remain after the healing process and the soft tissue contour may be harmed.[6]

Physics forceps are an alternative tooth extraction tool, aiming to perform less traumatic and more predictable extractions. They were first introduced by Dr. Richard Golden in 2004. Unlike the conventional forceps, these tools do not rely on the luxation of the tooth and expansion of the socket. These forceps rely on the phenomenon of solid materials known as 'creep'. This is the process of a material undergoing slow deformation while subjected to persistent stress. In this way, they tear the periodontal ligament fibers and free the tooth from its retention to the socket, all the while protecting the buccal cortical plate with a silicon covered bumper.[7,8]

AIM

The aim of study was to compare the effectiveness of physics forceps as an alternative, less traumatic, tool to the conventional extraction forceps.

MATERIALS AND METHODS

Study design

This study was a crossover randomized control trial.

Subjects and sample

The study was conducted between January 2022 and December 2022. It included 54 patients who were recruited from the Department of Oral Surgery at the Faculty of Dental Medicine, in the Medical University of Plovdiv. The study was approved by the Ethics Committee of Medical University of Plovdiv with protocol P-3499/21.12.2021.

A convenience sampling method was used to select patients. The participants were split into two equal groups using block randomization assigning every next patient to the group with the least participants. Group I (control group) consisted of 26 patients who had an extraction with conventional extraction forceps and was considered the control group. Group II consisted of 28 patients who were treated with physics forceps and were considered the study group. Two patients from the study group did not turn up for the complete follow-up period and were thus excluded.

Inclusion criteria:

- Patients with teeth indicated for extraction
- Patients without contraindications for surgical intervention (ASA 1 or 2)
- Patients with good oral hygiene

Exclusion criteria:

- Patients with severe systemic conditions or immunosuppression
- Patients with acute odontogenic infections
- Drug or alcohol abuse
- Patients with psychiatric conditions
- Patients on anticoagulant or antiaggregant drugs refusing to complete prior tests and preparation for tooth extraction
- Patients on chemotherapy, radiotherapy, or oral bisphosphonate intake

Clinical procedure

Patients in the control group were treated with the conventional extraction forceps, which are taught to all dental students and are well-known among dental practitioners. Patients in the study group were treated with physics forceps, which are suggested to provide a more predictable, less traumatic extraction of teeth.

In both groups, infiltration anesthesia was applied using 4% articaine hydrochloride with adrenaline (dilution, 1:200000; Septodont, Saint-Maur-des-Fossés, France). To prevent soft tissue tearing, the tooth was freed from the gingival margin using a scalpel blade #15C. The multi-rooted teeth in both groups were not separated before the extraction began.

For the extractions with physics forceps the instrument was positioned so that the bumper would lie on the buccal side at the level of the mucogingival junction. The beak was positioned over sound hard tissues on the oral side of the tooth. The forceps were activated with a slight buccal rotation and held until the tooth came loose. Then the tooth was picked out with either a hemostat or conventional extraction forceps with no additional luxation or rotation.

Evaluation method

Extraction success assessment

Extraction success was graded from 1 to 5 based on the scale of Choi et al.[9] and its later modification by Patel et al.[10]

- Complete success (score 5): extraction without crown and root fracture.
- Limited success with root tip fracture (score 4): extraction involving root tip fracture.
- Limited success with root fracture (score 3): extraction involving root one or more root fracture or

crown fracture.
- Limited success with osteotomy (score 2): fracture-free extraction and partial osteotomy in case divergent roots and thick cortical bone was present.
- Failure (score 1): Failure to extract.

Buccal cortical plate preservation assessment

The level of the buccal cortical plate was ranked as preserved (no difference), partially preserved (<4 mm) and missing (>4 mm). The difference was measured based on the preoperative and postoperative probing distance on the buccal side of the socket. This is part of the newly suggested single-rooted extraction wound classification by Hamoun et al.[11] where they evaluate the missing buccal cortical plate in percentages.

Pain intensity assessment

Pain was scored on a linear VAS scale and measured in centimeters (10 cm total length) on the day of extraction (day 0), and days 1, 3, and 7 after the extraction. Participants were asked to place a mark on the linear scale where pain grows from left to right, based on their pain experience on the given day.

Wound healing assessment

Wound healing was assessed at 3, 7, and 10 days after the extraction. Scoring was based on Landry's index (LWHI – Landry Wound Healing Index, also known as Landry, Turnbull, and Howley index).[12] The index evaluates the extraction socket based on wound size, tissue color, bleeding on palpation, presence of granulation tissue, presence of pus, and gingival margin status.

Statistical analysis

The statistical package for Social Sciences (SPSS) v. 27 (2020) was used to analyze the data. Non-normally distributed variables were presented with median values and interquartile ranges (IQRs) and between-group comparisons were performed using the Mann-Whitney U test. The chi-square test and Fisher's exact test were utilized to determine the relationships between categorical data presented as numbers and percentages. All statistical tests were two-tailed and performed at a type I error α=0.05. We performed Z-tests to compare column proportions in cross-tabulations involving variables with more than two levels. All statistical tests were two-tailed and performed at a type I error (α) of 0.05.

RESULTS

Fig. 1 shows the extraction success rate in the control group and the study group, graded on a scale from 1 (unsuccessful) to 5 (full success). The median success rate in the study group was 5 (IQR=0.00), and it was the same in the control group (median=5, IQR=0.00). The Mann-Whitney U test showed a lack of significant differences in the distribution of the success scores in the two groups (p=0.657).

Absolute success (score=5) was achieved in 81.1% of the extractions with physics forceps and in 84.60% of the extractions with conventional extraction forceps. Limited success with root fracture (score=3) was observed in 10.80% of the extractions with physics forceps and in 10.40% of those with conventional forceps. The extractions with a score of 2 (limited success with osteotomy) amounted to 2.7% in the physics forceps group and to 2.6% in the conventional forceps group. Failure (score=1) was observed in 5.40% of the physics forceps extractions and in 2.6% of the conventional

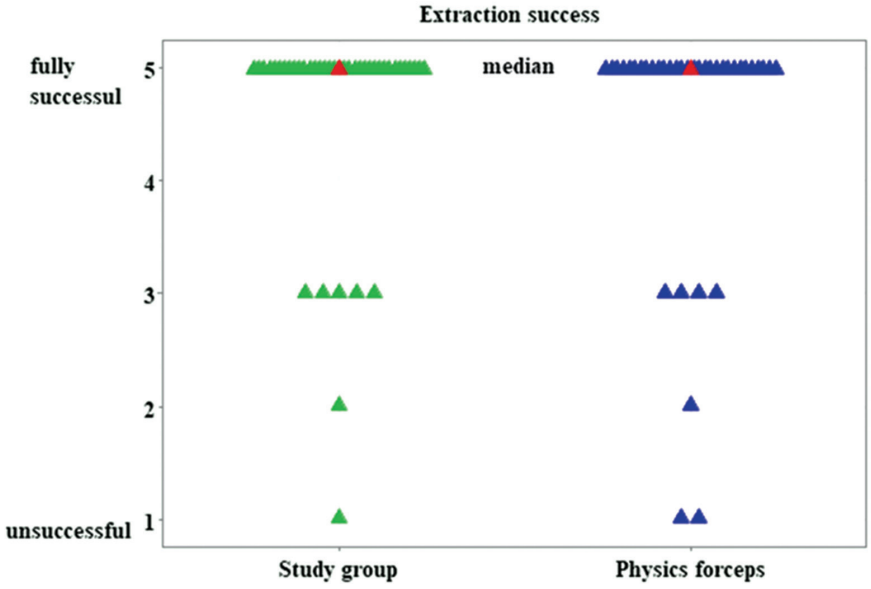

Figure 1. Distribution of the extraction success rate scores in the control and study group.

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forceps extractions. Cases with limited success with root tip fracture (score=4) were not recorded.

The buccal cortical plate was preserved in 81.1% of the extractions in the study group and 71.8% in the control group, with no statistically significant difference between the two groups (p=0.729). Detailed results are presented in **Fig. 2**. The preservation of buccal cortical plate for the extractions with physics forceps by group of teeth is presented in **Fig. 3**. The least preserved buccal bone was in the molars group, most probably due to the greater force needed for their extraction.

The results of the subjective pain intensity score measured in centimeters on the visual analogue scale (VAS) are presented in **Fig. 4**. During the healing period, both groups showed almost equal spikes and reductions in pain, with no statistically significant difference.

The healing score based on the early wound healing index (LWHI) can be seen in **Table 1**. The study group showed marginally better healing scores, yet with no statistically significant difference between the two groups.

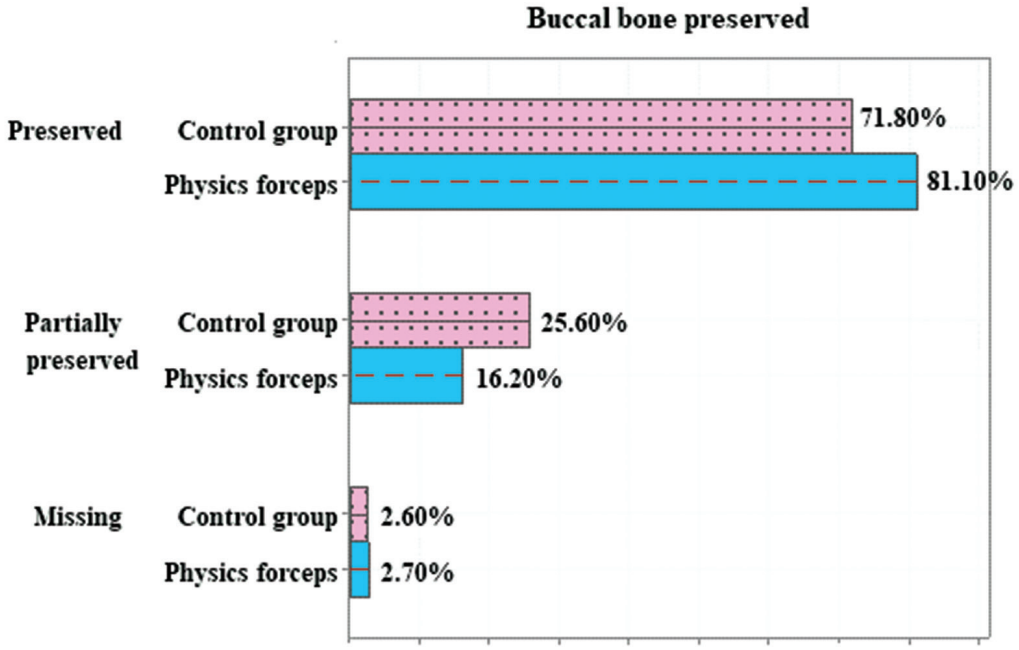

Figure 2. Buccal cortical plate preservation.

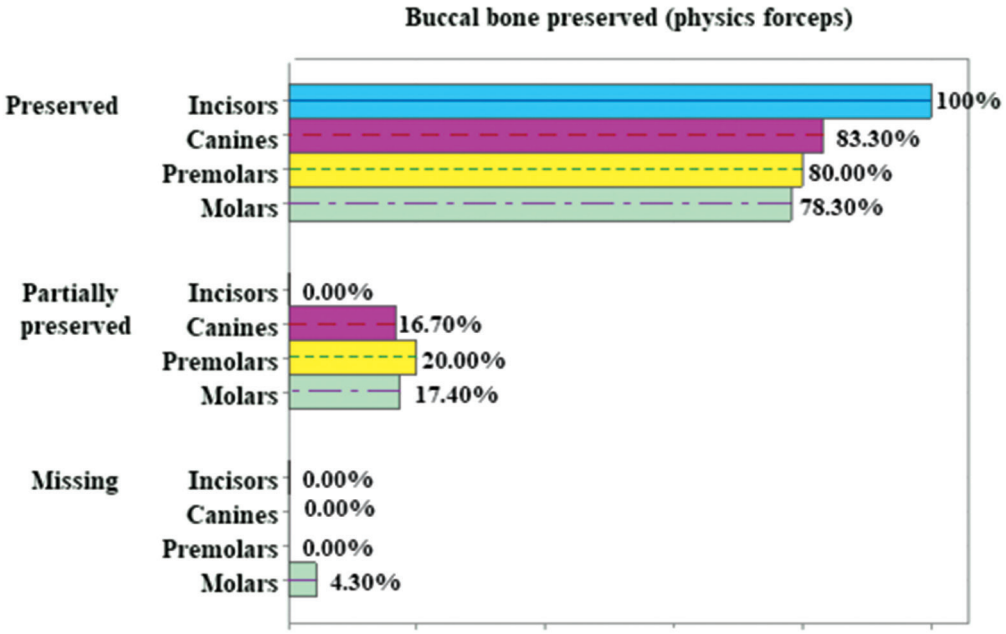

Figure 3. Buccal cortical plate preservation by tooth.

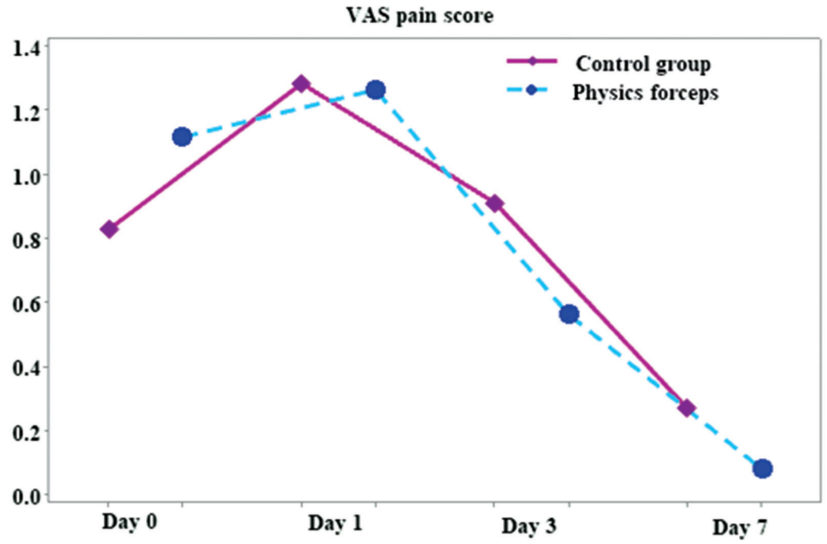

Figure 4. VAS pain intensity score.

Table 1. Early wound healing score

Early wound healing score	Physics forceps n = 26	Conventional instruments n = 28	*p*-value
	Day 3		
Very poor	0.00% (0)	0.00% (0)	N/A
Poor	84.60% (22)	78.60% (22)	0.786
Good	11.50% (3)	17.90% (5)	0.508
Very good	3.80% (1)	3.60% (1)	0.879
Excellent	0.00% (0)	0.00% (0)	N/A.
	Day 7		
Very poor	0.00% (0)	0.00% (0)	N/A
Poor	23.10% (6)	28.60% (8)	0.645
Good	26.90% (7)	35.70% (10)	0.486
Very good	38.50% (10)	21.40% (6)	0.169
Excellent	11.50% (3)	10.70% (3)	0.925
	Day 10		
Very poor	0.00% (0)	0.00% (0)	N/A
Poor	3.80% (1)	14.30% (4)	0.183
Good	7.70% (2)	7.10% (2)	0.932
Very good	30.80% (8)	28.60% (8)	0.859
Excellent	57.70% (15)	50.00% (14)	0.602

DISCUSSION

Extraction techniques, which predictably improve the success of the procedure, while causing less trauma are something that dental practitioners, and especially dental implantologists, are constantly striving for. The trauma is in direct correlation with how much hard and soft tissues will be affected and lost during the healing period.[13] Since extraction trauma is hard to avoid, a variety of socket and ridge preservation techniques have been developed, aimed at preserving the volume of the tissues present at the time of extraction. However, techniques are not entirely predictable, and may require a long waiting period for complete recovery. Therefore, aiming to be as less traumatic as possible is a must.[14,15]

In our study, we included all teeth with indications for extraction. Several similar studies exist. El-Kenawy and Ahmed[16] extract an overall of 200 teeth on patients divided in two groups – one with physics forceps and one with conventional instruments. Patole and Chidambar's[17] study is very similar, where they also perform 200 extractions in a similar manner. The study of Raghu et al.[7] includes 241

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extractions with physics forceps alone.

The studies of El-Kenawy and Ahmed[16] and Patole and Chidambar's[17] record the time it takes to perform the extractions in both groups. They both conclude that it takes considerably less time to perform the extraction with physics forceps than it takes to do so with the conventional extraction forceps. The studies of Sonune Avinash et al.[18], Patel et al.[10] and Panchal et al.[19] compare the physics forceps with the conventional extraction forceps in orthodontic extractions. They also find that it takes less time for the extractions with physics forceps. However, all these studies record the time of the extraction differently in that they consider the beginning of the extraction to be the placement of anesthesia, or from the time the patient is numb, or once the instrument is in position. This means that the results are not directly comparable.

El-Kenawy and Ahmed[16] report that a total of 83 out of 100 (83%) extractions were successful. In our study, we achieved successful extractions in 81.1% of the cases, which is on par with the results of the other study. Neither result is of statistical significance when compared with the control group. Raghu et al. report that 226 out of 241 extractions were successful, which is 93.77% and is a little bit higher than our study and that of El-Kenawy and Ahmed.

The study of Choi and Bae[9] performs planned replantation of 96 teeth. They mobilize the teeth with braces and then extract them with physics forceps. Their results show that about 93% of the extractions occur successfully. This matches the results reported by El-Kenawy and Ahmed, but given the fact that teeth were already mobile, the overall success would otherwise be closer to what the studies of El-Kenawy and Ahmed and our study achieved.

It is a well-established understanding that after a tooth extraction, the hard and soft tissues undergo remodeling and are ultimately reduced.[20] The loss of a tooth leads to the initiation of resorption processes, which mainly affect the bone on the buccal side of the extraction site.[21] Even with the use of ridge preservation techniques, the soft tissues, too, do not remain unaffected with a predominant change in their buccal contour.[22] The bumper of the physics forceps aims to support the buccal tissues and counteract the extraction forces, preserving the buccal cortical plate and the soft tissues over it intact.

In our study, of all the physics forceps extractions, in 81.10% of the cases the buccal cortical plate remained intact (preserved). There was no statistically significant difference in comparison with the control group. In the studies of both El-Kenawy and Ahmed and Patole and Chidambar, there were only 3 cases (3%) of buccal cortical plate fracture. The results of Raghu et al. show that 35 of 241 cases had a buccal cortical plate fracture, which means that the cortical plate was intact in 85.48% of the cases, which somewhat coincides with our findings.

The studies of El-Kenawy and Ahmed and Patole and Chidambar and our study all show that, even though with no statistically significant difference, there were less buccal cortical plate fractures in the study group in comparison to the control group. However, the results of the orthodontic extractions in the study of Sonune Avinash et al.[18] had more buccal cortical plate fractures with the physics forceps.

In our study, we followed up the extractions for up to 10 days and evaluated the extraction wound healing using the LWHI. While the physics forceps extractions showed marginally better results, there was no statistically significant difference between the two groups. Patole and Chidambar[17] report that 89% of the extraction wounds in the study group had healed. This is in contrast with our study where we found that only about 50% of the extractions with physics forceps had healed. However, on the 10th day 88.5% of the wounds had healed, which matches the results of the other study.

Limitations

We acknowledge that the lack of appropriately calculated patient sample size for the study is a major limitation, which might have an impact on the ultimate credibility of the results presented.

CONCLUSIONS

Atraumatic extractions allow for more hard and soft tissues to be preserved in the extraction site. Physics forceps are a tool that many practitioners are familiar with, but they are used differently than traditional extraction forceps. However, this might allow for a quicker getting used to. Although the clinical reports show mixed results with marginally better outcomes for the physics forceps, the fact that the extractions are quicker and the instrument is not much different than the well-known conventional counterpart, it might be considered an appropriate upgrade to atraumatic extraction armamentarium in modern dentistry.

Ethical statement

The study was approved by the Ethics Committee of the Medical University of Plovdiv.

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Competing Interests

The authors have declared that no competing interests exist.

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Минимально инвазивное удаление с помощью физических щипцов – клиническая оценка и сравнение

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Резюме

Введение: Удаление зубов по-прежнему остаётся одной из наиболее распространённых стоматологических процедур, которые регулярно выполняются по разным причинам. Щипцы и элеваторы для удаления зубов — хорошо известные инструменты для удаления зубов, которые уже более ста лет являются стандартом в процедурах удаления зубов. Физические щипцы — одна из возможных альтернатив, позволяющая выполнить менее травматичное и более предсказуемое удаление.

Материалы и методы: Все пациенты в исследовании были разделены на две группы: 26 пациентов, для которых использовались обычные щипцы для экстракции (контрольная группа), и 28 пациентов, для которых мы использовали физические щипцы (основная группа). Для каждой группы мы оценивали успешность удаления, сохранение кортикальной пластинки щеки, ощущение боли и раннее заживление ран.

Результаты: Статистически значимой разницы в показателях успешности экстракции между двумя группами не наблюдалось. При экстракции физическими щипцами буккальная кортикальная пластинка сохранилась в 81.1% случаев, а при экстракции традиционными щипцами – в 71.8%. Пациенты основной группы сообщили об уменьшении боли на седьмой день. На десятый день в исследовательской группе доля полностью зажывших экстракционных ран была немного выше.

Заключение: При атравматическом удалении сохраняется больше твёрдых и мягких тканей в месте удаления. Физические щипцы – это инструмент, аналогичный широко известным обычным щипцам для удаления зубов. Они обеспечивают несколько лучшие результаты в большинстве экстракций.

Ключевые слова

атравматичный, малоинвазивный, физические щипцы, удаление зубов

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Original Article

Presence of Single Nucleotide Polymorphisms in Transforming Growth Factor β and Insulin-Like Growth Factor 1 in Class II Malocclusions due to Retrognathic Mandible

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Abstract

Aim: The aim of this study was to evaluate specific single nucleotide polymorphisms (SNP) of transforming growth factor-beta (TGF-β) (rs1800469) and insulin-like growth factor-1 (IGF-1) (rs17032362) genes in Class II individuals with a normal maxilla and retrognathic (short) mandible.

Materials and methods: The study had 25 participants: 5 were assigned to the control group, which had a normal maxilla and mandible, and 20 to the experimental group, which had a structurally retrognathic mandible and a normal maxilla. The polymerase chain reaction was used with preselected primers after which Sanger sequencing was used to identify individual mutations.

Results: SNP at rs1800469 (TGF-β) in the study and control groups showed significant difference (p=0.009). The Odds Ratio of 5.28 signified that the individuals with SNP at rs1800469 were at 5.28 times higher risk of developing mandibular retrognathism. The IGF SNP showed its presence in experimental group but was not statistically significant.

Conclusion: Our study reports for the first time on the association between TGF-β SNP and mandibular retrognathism. Other SNP also showed its presence in the study group and its complete absence from control group directs us for further research.

Keywords

insulin-like growth factor-1, retrognathic mandible, single nucleotide polymorphisms, transforming growth factor-β

INTRODUCTION

Advanced molecular techniques have assisted us in determining DNA alterations by providing an infinite variety of genetic markers for the creation of genetic maps, allowing us to study a disease's genetic predilection. DNA sequencing can be used for detecting genetic variations in the nucleic acid sequence, known as single nucleotide polymorphisms. It can help orthodontists to determine the genetic component in a specific malocclusion so as to better predict the end result of further growth or treatment.

Mandibular condyle performs a crucial function in the development of orofacial structures by providing endochondral ossification. Therefore, any disturbance in the development of condyle can lead to mandibular asymmetries or retrognathism.

Mandibular condylar cartilage is designated as secondary cartilage as it shows differences in histological organiza-

tion when compared to primary skeletal cartilage. However, it is considered to be a part of the primary cartilaginous skeleton as it develops into permanent cartilage unlike other secondary cartilages which are mostly transient in nature.[1] The growth of cartilage is influenced by both inherent genetic factors and the broader category of epigenetic factors, which encompasses genetic elements susceptible to external influences or other modifying factors.[1-6]

The role of TGF-β and IGF in enhancing the growth of mandibular condylar cartilage (MCC) and their downstream events have been well documented by our team. They both have a synergistic role in the mandibular cartilage synthesis and remodeling.[2,3,5,6] Studies on the skeletal Class III malocclusion have identified SNPs and various genetic makers contributing to a prognathic mandible[7-10], whereas the studies concerning a retrognathic mandible or a skeletal Class II are scarce.

AIM

Our aim was to assess the genetic markers, specifically the SNPs of TGF-β (rs1800469) and IGF-1 (rs17032362), in relation to a structurally retrognathic mandible associated with a skeletal Class II malocclusion. By focusing on key genetic factors, including SNPs of TGF-β (rs1800469) and IGF-1 (rs17032362), our objective was to elucidate the genetic underpinnings of mandibular growth and condylar cartilage development in individuals with a Class II malocclusion. This exploration is crucial for enhancing our understanding of the complex interplay between genetic variations and craniofacial development, ultimately providing valuable insights that may contribute to improved predictions and treatment strategies for individuals with such malocclusions.

MATERIALS AND METHODS

Adult subjects of both sexes with a mean age of 26±3 years were included in the study. The experimental group consisted of 20 subjects with orthognathic maxilla and retrognathic mandible i.e., SNA=82°±2°, SNB≤78°, effective mandibular length (Co-Gn: Co condylium is the most posterior point of the condyle, and Gn is the lowest point of the lower jaw) ≤118 mm, mandibular length (Go-Me: Go is the most posterior point of the lower jaw, Me is the contact point of the corticalis of the mandibula and the symphysis) ≤76 mm. The control group consisted of 5 subjects with orthognathic maxilla and mandible i.e., SNA=82°±2°, SNB=80°±2° (Skeletal Class I), effective mandibular length =122±4 mm (Co-Gn: Co condylium is the most posterior point of the condyle, and Gn is the lowest point of the lower jaw), mandibular length =79±2 mm (Go-Me: Go is the most posterior point of the lower jaw, Me is the contact point of the corticalis of the mandibula and the symphysis).

The patient selection criteria for the experimental group of 20 individuals included specification regarding their feeding history. It is imperative to note that, as part of this criterion, individuals in the experimental group were exclusively those who were fed naturally during infancy. This consideration is essential due to the inherent compensatory nature of embryonic mandibular retrognathism up to 6 months, a process facilitated through natural nutrition.

Participants with harmful habits, such as mouth breathing and finger sucking, were deliberately excluded from the study. This exclusion was implemented to mitigate potential external factors known to contribute to a distal bite, with finger sucking additionally linked to the risk of a shortened lower jaw due to obstruction.

The study was approved by the Institutional Ethics Committee. A written informed consent, in adherence to their diagnostic and involvement in the results for scientific review without revealing their identity, was obtained from all the participants. Tracings of the lateral cephalograms, taken for every participant were done manually on acetate matte tracing paper.

Angular and linear parameters, including SNA (Sella-Nasion-A point) and SNB (Sella-Nasion-B point) within angular measurement, as well as mandibular length and effective mandibular length within linear measurement, were assessed in the tracings of both subjects and controls.

For detecting the presence of selected SNPs, 2 ml of blood sample was withdrawn from the cubital fossa of each subject and stored in an EDTA vacutainer at 4°C in a refrigerator. Testing of DNA amplicons on 0.8% agarose gel, found the quality of DNA samples to be satisfactory **(Fig. 1)** for further procedures described below. Further tests were conducted, where the DNA was isolated with the help of Xcelgen Blood DNA isolation kit.

The protocol was as follows:

1. 200 µl of human blood sample was taken and transferred to a sterile 1.5 ml Eppendorf tube.
2. One volume of red blood cell lysis buffer was added to the tube and inverted back and forth 5 times for proper mixing. Centrifugation was done at 10,000 rpm for 1 minute.
3. One volume of phosphate buffered saline was added to the tube and inverted back and forth 5 times to mix well. Centrifugation was done at 10,000 rpm for 1 minute.
4. 10 µl of proteinase K was added to the Eppendorf tube.
5. One volume of buffer lysate, e.g., 200 µl of buffer to 200 µl of blood sample, was added.
6. Incubation was done at 55°C for 20-30 minutes on thermomixer.
7. After incubation, 200 µl of ethanol (96%-100%) and 200 µl of DNA wash buffer was added to the lysate. Mixing of the entire lysate was done by pipetting.
8. Entire lysate was transferred to the DNA spin column and centrifuged at 10,000 rpm for 1 minute.
9. The column was placed into the same collection tube. 500 µl of DNA wash buffer II was added and centrifuged at 10000 rpm for 1 minute.

Primers for each restriction site are listed in **Table 1**. Polymerase chain reaction (PCR) was performed using

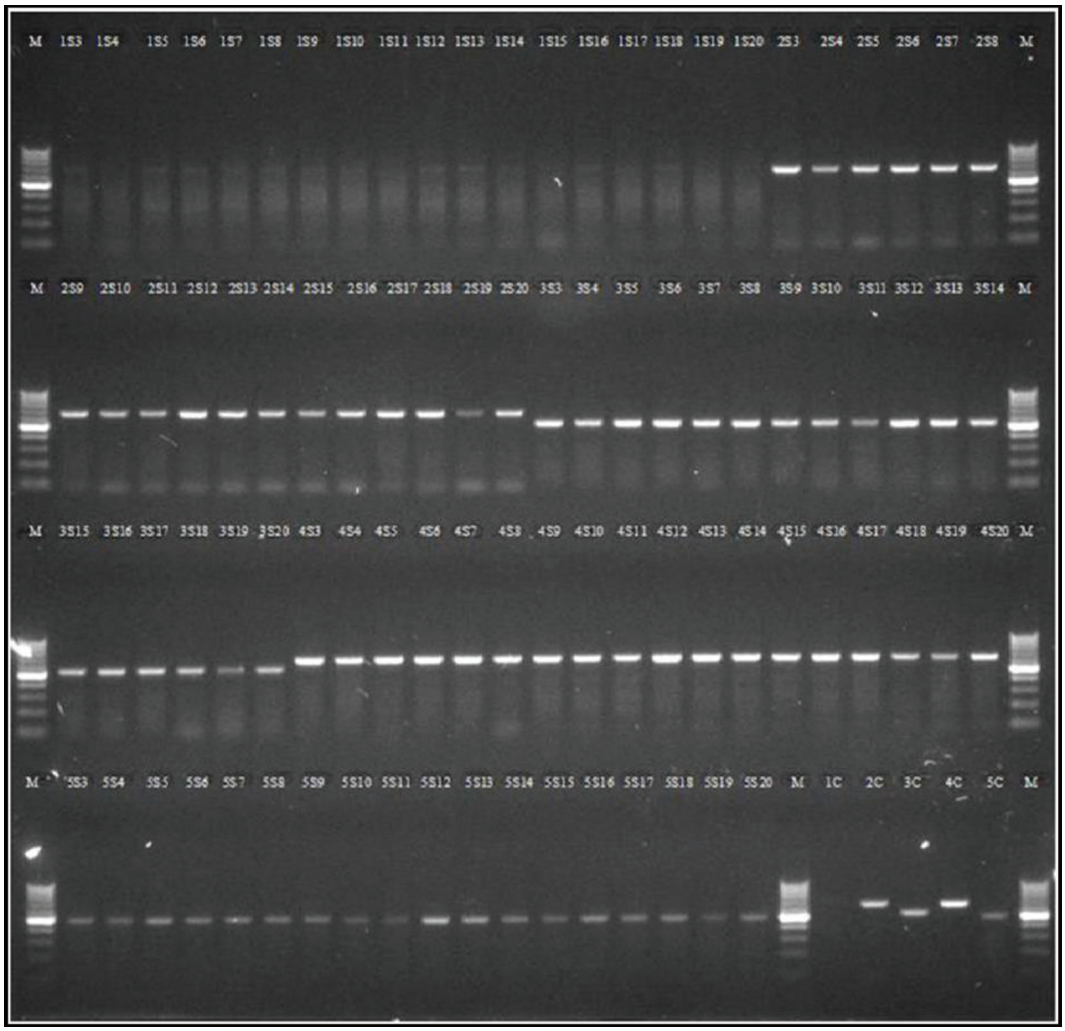

Figure 1. Quality check of the DNA on 0.8% agarose gel.

synthesized primer sets using isolated DNA as template. Briefly, 20 ng of DNA was used to carry out PCR amplification in final reaction volume of 25 µl. Composition of reaction mixture is given in **Table 2** and the PCR conditions used are provided in **Table 3**.

All PCR products were sequenced by the ABI sequencer, 3730xl (Sanger Sequencing). The amplicons were then purified and automated DNA sequencing was carried out on ABI 3730xl Genetic Analyzer (Applied Biosystems, USA). The BigDye Terminator v. 3.1. Cycle Sequencing Kit was used for sequencing as per manufacturer's protocol, where sequencing cycle was set with the thermal ramp rate of 1°C per second for 30 cycles.

Statistical analysis

Statistical analyses were performed using Student's *t*-test and *p*-values were calculated to evaluate the distribution

Table 1. Restriction sites selected with respect to the corresponding gene

Sr. No	Gene	Restriction site
1.	Transforming growth factor β	rs1800469
2.	Insulin-like growth factor 1	rs17032362
3.	Myosin 1H	rs11611277

Table 2. Components of reaction mixture for PCR

	Components	Quantity
1.	Nuclease free water	10.5-X µl
2.	Template DNA	X* µl
3.	Forward primer (10 pmole/µl)	1.0 µl
4.	Reverse primer (10 pmole/µl)	1.0 µl
5.	2XPCR master mix	12.5 µl
	Total volume	25 µl

*X represents variable volume of genomic DNA.

Table 3. PCR conditions

Initial denaturation	Denaturation extension	Annealing (X35)		Final extension	Hold
95°C	94°C	66°C	72°C	72°C	4°C
5 min	30 sec	45 sec	45 sec	10 min	15 min

*X represents the number of cycles

of each SNP across all samples. Additionally, odds ratios were computed to determine the precise risk. These analyses were conducted utilizing Sigmaplot (v. 13) software package.

RESULTS

Table 4 proves the homogeneity of study and control groups. The mean cephalometric parameters like ∠SNA 81.0, ∠SNB 73.65, effective mandibular length 102.6 mm and mandibular length 65.1 mm in the study group confirms that it included cases with true mandibular retrognathism, whereas cephalometric parameters like ∠SNA of 81.4, ∠SNB of 80.2, effective mandibular length 119 mm and mandibular length 79.2 mm confirms that the control group included skeletal class I individuals.

The distribution of SNP at rs1800469 in cases and controls showed significant difference as shown in **Table 5** with *p*-value of 0.009. The percentage of samples showing mutations in the study group was 65%, as compared to 0% in the control group. The Odds Ratio of 5.28 signifies that the individuals with SNPs at rs1800469 were at 5.28 times higher risk of developing mandibular retrognathism than those without mutation.

The distribution of SNP at rs17032362 in cases and controls showed a non-significant difference indicated by a *p*-value of 0.356 as shown in **Table 6**. The percentage of

Table 4. Comparison of measurements between study group and control group

	Groups	n	Mean	Standard deviation	t-test value	*p* value
SNA	Study	20	81.0	0.91	−0.925	p=0.365
	Control	5	81.4	0.54		
SNB	Study	20	73.65	1.30	−10.87	p<0.001
	Control	5	80.2	0.44		
Effective mandibular length	Study	20	102.6	5.67	−6.324	p<0.001
	Control	5	119.0	1.22		
Mandibular length	Study	20	65.1	6.41	−4.834	p<0.001
	Control	5	79.2	0.44		

*p<0.05: significant difference; **p<0.001: highly significant difference

Table 5. Association of presence of SNP at rs1800469 between study and control group

rs1800469	Study n (%)	Control n (%)	Odds Ratio (95% CI)	t test	*p* value
Present	13 (65%)	0 (0%)	5.28 (3.46-7.81)	6.771	p = 0.009 highly significant
Absent	7 (35%)	5 (100%)			

p<0.05: significant difference; p<0.001: highly significant difference

Table 6. Association of presence of SNP at rs17032362 between study and control group

rs170323662	Study n (%)	Control n (%)	Odds Ratio (95% CI)	t test	*p* value
Present	3 (15%)	0 (0%)	3.17 (1.97-4.86)	0.852	p=0.356, no significant difference
Absent	17 (85%)	5 (100%)			

p<0.05: significant difference; p<0.001: highly significant difference

samples showing mutation in the study group was 15% as compared to 0% in the control group. The Odds Ratio of 0.852 signifies that the individuals with SNP at rs17032362 were at a 0.852 times higher risk of developing mandibular retrognathism than those without mutation.

In restriction site 1800469, C allele was over-presented in the control group subjects (p=0.006). In restriction site 17032362, G allele was over-presented in the mandibular retrognathism subjects (p=0.47) **(Table 7)**.

DISCUSSION

Human gene mapping studies of maxilla and mandible (normal or retrognathic position and size) are scarce; they have focused majorly on the skeletal Class III malocclusion.[18,19,21] Previous studies have found correlation between mandibular prognathism and genes EPB-41, GHR, LTBP-2, MATRILIN-1, TGF-β3, and MYO-1H indicating that molecular mechanisms involved in bone (TGF-β3, LTBP) and cartilage (MATRILIN-1, GHR) growth influence mandibular size discrepancies.[7-9,11-13] Skeletal Class II malocclusion is always considered to be of a multifactorial etiology with various permutations and combinations related to length and position of both the maxilla and the mandible.[20,22-26] Thus, we attempted to focus on skeletal Class II malocclusion with a normal maxilla and retrognathic mandible.

In our previous studies[2,3,5,6], TGF-β and IGF-1 have proven to be important and synergistic factors in development and growth of the mandible. The role undertaken by TGF-β in repair of cartilage is well explained. Administration of growth factors like TGF-β and IGF-1 in our animal study also proved to have a positive correlation with the growth of MCC.[6] As the growth of MCC with administration of these factors increased, it urged us to investigate its role in a Class II individual with normal maxilla and a retrognathic mandible.

TGF-β has proven to be an important factor in MCC growth and differentiation.[2,5,14] It has a role in the differentiation of chondrocytes and also prevents the growth of any other cells. TGF-β also maintains the surrounding microenvironment by increasing the deposition of extra cellular matrix and maintaining vascularity by promoting angiogenesis. In our present study, we found a significant role of TGF-β SNP (rs1800469) in producing a phenotype with a retrognathic mandible leading to a skeletal Class II malocclusion. The Odds Ratio of 5.28 signifies the higher risk of a skeletal Class II with the respective SNP.

IGF-1 plays a significant role in cartilage growth and differentiation by controlling cartilage homeostasis. It blocks the cytokine stimulated cartilage degradation.[5] In this study, presence of IGF-1 SNP (rs17032362) in the study group was about 15% and hence its complete absence in the control group validates some of its role in not producing a normal skeletal Class I phenotype.

The factors that influenced the development of a malocclusion may not be the only ones that will influence how the patient will respond to a given treatment.[15] Different patients respond differently to the same treatment as described by Carlson DS[16] and Hartsfield et al.[17] The normal patients have SNPs which respond to usual treatment whereas some patients may have SNPs (abnormal) which do not respond to normal treatment. The predictability of treatment outcome for a particular patient will thus be dependent on the identification of such SNPs.

Genomics and epigenomics are a duality i.e., no malocclusion can only be genetic or environmental in origin completely. They may be opposing but are interdependent on each other. This study for the first time has identified the role of TGF-β SNP (rs1800469) in a retrognathic mandible.

CONCLUSION

This study established an association between TGF-β SNP rs1800469 and mandibular retrognathism. The presence of IGF-1 SNP rs17032362 in the study group and its absence in the control group directs us for further research.

The strengths and limitations of the study should be considered when interpreting the results. The major strength is the standardization of participants with an average maxilla and exclusion of participants with a protruded maxilla. A relatively small sample size of the study conducted can be considered as a limitation, which may have influenced the relation between a retrognathic mandible and TGF-β gene polymorphisms. Even though previous studies have confirmed an association between TGF-β and incidence

Table 7. Comparison of each allele marker

Marker	Genotypes	Study	Control	Chi-square	*p* value
rs11611277	C	35 (87.5%)	10 (100%)	1.389	p=0.239
	A	5 (12.5%)	0 (0%)		
rs17032362	G	37 (92.5%)	10 (100%)	0.519	p=0.471
	A	3 (7.5%)	0 (0%)		
rs1800469	C	21 (52.5%)	10 (100%)	7.661	p=0.006
	T	19 (47.5%)	0 (0%)		

p<0.05: significant difference; p<0.001: highly significant difference

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of retruded mandible, the limited power of this study necessitates repetition with larger sample size. This study is a proof-of-concept study depicting the association of TGF-β with a retrognathic mandible or a skeletal Class II malocclusion due to retrognathic mandible.

Declarations of interest

None

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Conflict of interest

The authors report there are no competing interests to declare.

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Наличие однонуклеотидных полиморфизмов в трансформирующем факторе роста β и инсулиноподобном факторе роста 1 при аномалиях прикуса II класса, обусловленных ретрогнатической нижней челюстью

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Резюме

Цель: Целью данного исследования было оценить специфические однонуклеотидные полиморфизмы (SNP) генов трансформирующего фактора роста-бета (TGF-β) (rs1800469) и инсулиноподобного фактора роста-1 (IGF-1) (rs17032362) в классе II особей с нормальной верхней челюстью и ретрогнатической (короткой) нижней челюстью.

Материалы и методы: В исследовании приняли участие 25 участников: 5 были отнесены к контрольной группе с нормальной верхней и нижней челюстью, и 20 – к экспериментальной группе со структурно ретрогнатической нижней челюстью и нормальной верхней челюстью. Полимеразную цепную реакцию использовали с предварительно выбранными праймерами, после чего использовали секвенирование по Sanger для идентификации отдельных мутаций.

Результаты: SNP при rs1800469 (TGF-β) в основной и контрольной группах показал значительную разницу (p=0.009). Отношение коэффициентов от 5.28 означало, что у людей с SNP при rs1800469 риск развития нижнечелюстного ретрогнатизма был в 5.28 раза выше. SNP IGF показал своё присутствие в экспериментальной группе, но не был статистически значимым.

Заключение: В нашем исследовании впервые сообщается о связи между SNP TGF-β и нижнечелюстным ретрогнатизмом. Тот факт, что другой SNP также показал своё присутствие в основной группе, а его полное отсутствие в контрольной группе побуждает нас к дальнейшим исследованиям.

Ключевые слова

инсулиноподобный фактор роста-1, ретрогнатическая нижняя челюсть, однонуклеотидные полиморфизмы, трансформирующий фактор роста-β

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Original Article

Evaluation of Calcium Hydroxide Root Canal Filling Materials by Cone Beam Computed Tomography and Three-Dimensional Modeling

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Abstract

Aim: The aim of this study was to compare the effectiveness of filling pastes in resin prototype primary molars by cone beam computed tomography (CBCT) and 3D modeling.

Materials and methods: The canals of 27 primary second molar teeth models were shaped with a rotary endodontic motor to file No. 30/04. The samples were randomly divided into three groups and filled with calcium hydroxide-distilled water, Viopex, and calcium hydroxide-propylene glycol, respectively (n=9). Pre-filling and post-filling CBCT images of the shaped canals were obtained, 3D models were reconstructed with MiMiCS® software and volume measurements were made in cubic millimeters (mm^3). Differences between the volume of the shaped canals and filling materials were calculated as the miss-filling areas of various filling materials were also recorded. Dependent 2-group I-test, Bonferroni test, and one-way analysis were used for statistical analysis. Data were statistically analyzed at $p<0.05$ significance level by IBM SPSS 25 software.

Results: Among the three root canal sealers, the filling capacity of the calcium hydroxide-propylene glycol group was found to be the highest ($p=0.001$).

Conclusion: When calcium hydroxide mixed with propylene glycol was compared to calcium hydroxide alone, calcium hydroxide demonstrated a greater potential for root canals in primary maxillary second molar models. It is clear that clinical studies with follow-ups of the subjects will contribute to literature and clinical success.

Keywords

miss filling, primary molar teeth, root canal filling

INTRODUCTION

Endodontic treatment in primary teeth aims to preserve the teeth's integrity and health, as well as the periodontal and periapical tissues, until they are replaced by permanent teeth. Comprehensive cleaning and filling of the root canal system is essential to prevent bacterial infection and toxins. However, it is claimed that a lack of understanding of pulp anatomy is the cause of root canal failure.[1-3] The filling material must reach and fill the entire root canal in three dimensions, providing a biocompatible seal.

Furthermore, the root canal system in primary teeth has unique characteristics that make complete sealing difficult. Gomes et al. found that anatomical changes such as collateral or lateral canals and apical delta within the root canal significantly affect the success of root canal treatment. If an

existing excess canal is overlooked, the treatment prognosis will be adversely affected.[4]

Various filler materials and techniques have been aimed at increasing the chances of successful root canal treatment in primary teeth. Zinc oxide eugenol, calcium hydroxide, and iodoform fillers are commonly used and recommended, but no material has yet been accepted as a gold standard.[5,6] Hence, the canal filling potency of root canal pastes continues to be of interest to researchers. Apex reachability, filled root canal volumes, and the presence of miss-fillings are some of the evaluation criteria to assess the success of root canal fillings.[7,8]

AIM

Thus, the present study aimed to compare the filling capacities of calcium hydroxide-based root canal sealers by using cone beam computed tomography (CBCT) images and three-dimensional modeling of root canals of primary maxillary second molar models.

MATERIALS AND METHODS

The study was conducted using commercially available prototypes of primary molar teeth containing root canals. The use of these prototyped resin replicas has the potential to be used for educational purposes, endodontic training, and research due to the sample standardization.

Twenty-seven polymer-based prototype upper primary second molar teeth were fixed on wax and shaped by a rotary endodontic motor and endoart Pedo blue nickel titanium files. 18-mm 15/06, 25/04, and 30/04 files were used according to the recommendation of the manufacturer's protocol. At each file change, irrigation was provided with the saline solution using a perforated needle. After the irrigation process, the canals were dried with paper points. All samples were divided into three equal groups according to the filling materials: G1 - calcium hydroxide-distilled water, G2 - injectable calcium hydroxide-iodoform mixture (Viopex), and G3 - calcium hydroxide-propylene glycol.

Equal amounts of calcium hydroxide paste with three different carriers were placed in the root canals. The calcium hydroxide pastes were prepared as follows: one scoop of calcium hydroxide powder and one drop of distilled water (G1), one scoop of calcium hydroxide powder, and one drop of propylene glycol (G3) were mixed in a 1:1 ratio of powder and liquid on a mixing paper. These mixtures were prepared fresh and sent to the canals by a Lentulo spiral. Finally, Viopex (G2) was applied to its own syringe until it overflowed from the canal orifices. The cavity was cleaned with cotton pellets and restored with glass ionomer cement and CBCT images were re-obtained.

CBCT images were obtained from shaped root canals before and after filling both in the same position and protocol. In order to standardize the positions of the teeth, groups were identified with a marker on the anterior side of the block and a number was given for each block. All CBCT images were obtained by the same operator with Moritta® 3D Accuitomo 170, J. Morita MFG. CORP, Tokyo, Japan (Moritta®) CBCT device in 0.8 mm^3 isotropic voxels and 0.480 mm slice thickness. The operating parameters were 90 kVp, 2.5 mA, and 30.8 s, the field of view (FOV) was 4×4 cm^2, and images were obtained in the high-resolution imaging mode. The device delivers each irradiation with a single 360° rotation around the model. The images obtained were saved in Digital Imaging and Communications in Medicine (DICOM) format. 3D reconstructions of the shaped canals and filling materials, from the isthmus to the apex of the roots, were done with the MiMiCS® software, and their volumes were calculated in mm^3 **(Fig. 1)**.

A single researcher performed all evaluations, and 15 randomly selected CBCT images were re-evaluated four weeks later for intra-observer reliability assessment. The intraclass correlation coefficient (ICC) was used to investigate the consistency between measurements in quantitative data. In terms of the study's objectivity, the researcher who made the measurements differed from the researcher who worked on the models.

The data were analyzed statistically, and the success of intracanal filling of three different calcium hydroxide pastes were evaluated. SPPS 25 (IBM Corp. Publication. 2017 IBM SPSS Statistics, Version 25.0. Armonk, NY: IBM Corp.) statistical package was used to analyze the data. Mean ± standard deviation, percentages, and frequency values are used. Variables were evaluated for normality and homogeneity of variances after prevaccination control

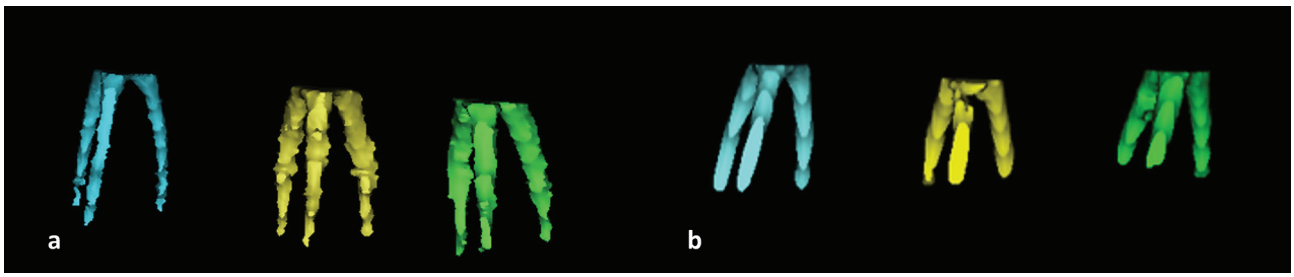

Figure 1. 3D reconstruction of a group of polymer-based prototype upper primary second molar teeth root canals, **a)** shaped root canals, **b)** filled root canals.

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(Shapiro-Wilk and Levene tests). While performing data analysis, Bond 2 group *t*-test (pairing surplus test) was used for the comparison of two groups, and one-way analysis of variance and comparison test were evaluated by Bonferroni test for further group comparison. Intra-class correlation (ICC) for inter-measure agreement. The $p<0.05$ and $p<0.01$ values were accepted for the significance level of the tests.

RESULTS

The aim of this study was to compare the filling effectiveness and internal voids of different obturation materials in polymer prototyped primary molars. Intraclass correlation coefficient (intrakappa value) was high, with a value of 99.8%, indicating an agreement between the first and second measurements of the same root canal volume **(Table 1)**. There was a statistically significant difference between the empty and filled canal volumes in the calcium hydroxide-distilled water, Viopex, and calcium hydroxide-propylene glycol groups. The calcium hydroxide-propylene glycol group showed the highest filling success ($p=0.001$), followed by Viopex group ($p=0.011$). Calcium hydroxide-distilled demonstrated the least filling capacity of root canals ($p=0.014$) **(Table 2)**.

DISCUSSION

Researchers have focused on developing new root canal pastes to overcome the filling difficulties due to miss-filling or overfilling.[7-9] In dental literature, there are limited data on the filling effectiveness of calcium hydroxide in primary teeth when used by different carriers.[10] In this study, CBCT imaging and 3D modeling were used to evaluate the filling capacity of calcium hydroxide based filling materials with different carriers namely distilled water, propylene glycol, and iodoform.

The present study used polymer-based prototype primary molars that ensured standardization by eliminating different results due to different root canal anatomies and physiological root resorptions. However, since the interaction between dentin and canal sealer cannot be evaluated in polymer teeth, results may differ in vivo.

Some studies have used radiography as an evaluation method, resulting only in two-dimensional interpretation.[11,12] In the present study, CBCT was used to assess the filling capacity of root canal pastes. CBCT uses cone-shaped X-ray to acquire data by revealing root canal morphology in a single 360° rotation. Compared to computed tomography (CT), CBCT provides improved accuracy, low scanning time, low radiation doses, and high resolution.[13]

For standard enlargement, pediatric files were used with rotary endodontic motor movement. In addition, 15/06, 25/04, and 30/04 taper files were used by a single operator. Samples with the same canal anatomy made the technique reproducible and the results consistent.

In this study, calcium hydroxide-distilled water and calcium hydroxide-propylene glycol mixture were applied with lentulo, while Viopex was applied with its syringe in compliance with the manufacturer's recommendations.

In another study, zinc oxide eugenol paste was applied to the canals with lentulo and injector. Lentulo showed the best results in reaching the apex, while the injector filled the intracanal volume the best.[11]

In dental literature, there are a limited number of studies with different evaluation criteria, which makes the results incomparable. For example, some assess the reaching apex, while some evaluate the void presence.[12-15] Furthermore,

Table 1. Intra class correlation coefficient test

	Intraclass correlation	95% Confidence interval		*p*
		Lower bound	Upper bound	
Average measures	0.998	0.992	0.999	0.001

99.8% correspondence between first and second shaped canals measurements.

Table 2. Filling success of different materials

Group		Mean	SD	*P*
KALSIYUM HIDROKSIT-DISTILE SU	Shaped canal volume	23.34	3.26	0.014*
	Filling material volume	19.77	1.12	
VIOPEX	Shaped canal volume	23.06	1.92	0.011*
	Filling material volume	21.06	3.46	
KALSIYUM HIDROKSIT-PROPILEN GLIKOL	Shaped canal volume	25.48	1.27	0.001*
	Filling material volume	23.03	2.19	

Paired t test, $p<0.05$

since different filling techniques and different canal sealants are used, the designs of the studies also vary.

Walia et al. designed their study on 45 extracted teeth separated into three groups calcium hydroxide + iodoform with a syringe, zinc oxide eugenol paste using rotary lentulo, and handheld lentulo.[15] Apex reachability of pastes were assessed by radiographic examination. The syringe method demonstrated overfilling, whereas both lentulo techniques showed better-filling quality.

Miss-filling areas in the canal can cause microleakage and lead to reinfection. Hence, selecting the appropriate root canal paste in clinical practice assists in the avoidance of this formation.[16,17]

CONCLUSION

The results of this study suggest that when calcium hydroxide is mixed with propylene glycol it shows a higher canal filling success compared to distilled water and iodoform mixtures.

Future studies should focus on studying extracted teeth with great sample numbers to reveal differences between filling materials. In addition, further clinical studies should be conducted for both primary and permanent teeth to confirm the results.

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Conflict of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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Оценка материалов для пломбирования корневых каналов на основе гидроксида кальция с помощью конусно-лучевой компьютерной томографии и трёхмерного моделирования

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Резюме

Цель: Целью данного исследования было сравнение эффективности пломбировочных паст в прототипах временных моляров из смолы с помощью конусно-лучевой компьютерной томографии (КЛКТ) и 3D-моделирования.

Материалы и методы: Каналы 27 моделей молочных вторых моляров были сформированы с помощью роторного эндодонтического двигателя с напильником № 30/04. Образцы были случайным образом разделены на три группы и заполнены дистиллированной водой гидроксида кальция, Viopex и гидроксидом кальция-пропиленгликолем соответственно (n=9). Были получены КЛКТ-изображения сформированных каналов до и после пломбирования, 3D-модели были реконструированы с помощью программного обеспечения MiMiCS®, а измерения объёма были выполнены в мм^3. Различия между объёмом сформированных каналов и пломбировочными материалами рассчитывались по мере того, как фиксировались площади незаполнения различными пломбировочными материаламн. Для статистического анализа использовались зависимый 2-групповой I-тест, тест Bonferroni и односторонний анализ. Данные статистически анализировали при уровне значимости $p<0.05$ с помощью программного обеспечения IBM SPSS 25.

Результаты: Среди трёх герметиков для корневых каналов наполняющая способность группы гидроксида кальция и пропиленгликоля оказалась самой высокой ($p=0.001$).

Заключение: При сравнении гидроксида кальция, смешанного с пропиленгликолем с чистым гидроксидом кальция, гидроксид кальция продемонстрировал больший потенциал при заполнении корневых каналов на моделях первичных вторых моляров верхней челюсти. Очевидно, что клинические исследования с последующим наблюдением за субъектами будут способствовать увеличению числа публикаций и клиническому успеху.

Ключевые слова

неправильное пломбирование, молочные коренные зубы, пломбирование корневых каналов

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Original Article

Development and Validation of a High-Performance Thin-Layer Chromatography Method for Detection of Sibutramine in Dietary Supplements

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Abstract

Introduction: In the period between 1997 and 2010, sibutramine-containing drugs were widely prescribed for obesity and over-weight management. Due to safety concerns, in 2010 all medicines containing sibutramine were urgently withdrawn from the USA and European pharmaceutical market. Although sibutramine is no longer available in pharmaceutical products, there have been numerous reports of mislabeled weight-loss dietary supplements containing sibutramine.

Aim: This work aimed to develop and validate an accurate and sensitive high-performance thin-layer chromatographic method for the detection of sibutramine.

Materials and methods: The method was developed using a CAMAG HPTLC system. Silica gel 60 F254 aluminum HPTLC plates were used as stationary phase and toluene:ethyl acetate:methanol (7:2:1 v/v/v) as mobile phase.

Results: The calibration curve was built in the range of 0.250–1.250 μg/band. The method provided satisfactory linearity, specificity, precision, and accuracy. The LOD and the LOQ were 0.0765 μg/band and 0.2318 μg/band, respectively.

Conclusions: The method allows for the simultaneous analysis of multiple samples as well as the rapid and sensitive monitoring of sibutramine levels in dietary supplements.

Keywords

dietary supplements, food supplements, high-performance thin-layer chromatography, HPTLC, sibutramine

INTRODUCTION

Obesity and overweight are today regarded as some of the most pressing global health issues. Furthermore, over the last 30 years, the prevalence of obese and overweight people has reached pandemic proportions. Pharmaceutical interventions have played a significant role in the pursuit of effective treatments.[1,2] One such medication that gained considerable attention was sibutramine. Between 1997 and 2010, sibutramine was widely prescribed to treat obesity. However, due to safety concerns, it was withdrawn in 2010.[3,4]

Sibutramine is a centrally-acting serotonin-norepinephrine reuptake inhibitor (SNRI). Its primary mode of action involves altering neurotransmitter levels in the brain, resulting in appetite suppression and increased thermogenesis. By targeting the central nervous system, sibutramine aims to aid individuals in achieving weight loss and maintaining long-term weight management.[5]

Clinical studies demonstrated that sibutramine, combined with a low-calorie diet and exercise, can lead to significant weight loss in obese individuals. Its efficacy was particularly notable in patients with a body mass index (BMI) exceeding 30 kg/m² or a BMI above 27 kg/m² with comorbidities such as dyslipidemia, hypertension, or type 2 diabetes. The medication exhibited promising results by promoting sustained weight loss and improving metabolic parameters.[6]

However, despite its initial success, concerns regarding the safety profile of sibutramine emerged over time. Later, it was announced that the intake of sibutramine increases the risk of cardiovascular events such as heart attacks, strokes, and arrhythmias.[5,7-9]

In 2010, all medicines containing sibutramine were urgently withdrawn from the USA and European pharmaceutical market. Although sibutramine is no longer available in pharmaceutical products, there have been numerous reports of adulterated weight-loss dietary supplements (DSs) containing this compound without proper labeling.[10-12]

One of the main reasons for sibutramine's presence in DSs is the lack of appropriate and obligatory quality control for DSs.[13] As a result, consumers may unknowingly consume high doses of sibutramine, which can increase the risk of adverse effects, especially when combined with other medications.[14] Something more, cases of sibutramine overdose from adulterated DS were reported as well.[15]

Dietary supplements aimed at weight loss, according to Wrobel et al., are among the most commonly purchased supplements on the Internet.[16] However, the missing quality control and the high demand for these products expose consumers at high risk. The quality control of the active compounds in DSs and the screening for adulterants are critical for ensuring the safety of consumers. There are only a few published analytical methods for detecting sibutramine[10-12,17], the majority of which rely on high-performance liquid chromatography and gas chromatography.

AIM

The aim of the current study was to develop a simple and rapid high-performance thin-layer chromatography (HPTLC) method for the identification and quantification of sibutramine.

HPTLC is an advanced and more sophisticated version of the classical thin-layer chromatography (TLC), designed to provide higher separation efficiency, faster and more precise analysis, improved resolution, and enhanced sensitivity.[18,19]

HPTLC offers several advantages over other chromatographic techniques, such as HPLC and GC: it is more cost-effective and time-saving. HPTLC allows multiple samples to be analyzed simultaneously on a single plate, reducing the overall cost per analysis (up to 75 samples simultaneously).[20] It is a versatile technique that can be used to analyze a variety of compounds, including organic and inorganic substances, natural products, pharmaceuticals, DS, and others. It can be adapted to various sample matrices and compound classes, making it suitable for diverse applications. HPTLC techniques seem to be much more appropriate for the detection of sibutramine in DSs than other techniques because of the possibility for simultaneous screening of multiple samples.

MATERIALS AND METHODS

Standards, reagents, and samples

Standard of sibutramine hydrochloride monohydrate was purchased from Sigma-Aldrich, Steinheim, Germany. The toluene, ethyl acetate, and methanol were of analytical grade purchased from Sigma-Aldrich, Steinheim, Germany. Bismuth subnitrate, potassium iodide, glacial acetic acid were purchased from Sigma-Aldrich, Steinheim, Germany. Forty DSs were purchased in pharmacies, herbal shops, and via the Internet.

Sample preparation

The stock solution of sibutramine was prepared by diluting with methanol in concentration of 1 mg/mL. Better dilution was accomplished using ultrasonic bath (BANDELIN, Berlin, Germany).

Dragendorff's reagent was prepared by dissolving 0.85 g of bismuth subnitrate in a mixture of 40 mL of water and 10 mL of glacial acetic acid (Solution A) and dissolving 8 mg of potassium iodide in 20 mL of water (Solution B). Five milliliters of both solutions were diluted with 20 mL of glacial acetic acid and 100 mL of water.[21]

The samples of DSs were prepared by diluting 250 mg of each sample with 10 mL methanol. After that they were homogenized by vortex. The next step involved the use of an ultrasonic bath for 30 minutes and then the samples were filtered through 0.45 µl PTFE Syringe filters (GVS North America Sanford, USA).

Apparatuses

The method was developed using a CAMAG HPTLC system (CAMAG, Muttenz, Switzerland). The configuration of the HPTLC system consisted of Limomat 5; Automatic Developing Chamber 2, and TLC Visualizer 2. The system was controlled by VisionCATS version 3. An ultrasonic bath (BANDELIN, Berlin, Germany) and a dipping chamber (Biostep-Desaga, Burkhardtsdorf Germany) were also used.

Chromatographic conditions

The analyses were carried out using silica gel 60 F254 aluminum HPTLC plates, 10×20 cm, 200 μm layer thickness (E. Merck KGaA, Darmstadt, Germany). The mobile phase comprised toluene, ethyl acetate, and methanol at a ratio of 7:2:1 v/v/v. The volume of the mobile phase was 10 mL. Application type: band. Front: 70 mm. Time for development: 25 minutes. Drying: 5 minutes. After development, derivatization was performed with Dragendorff's reagent in a dipping chamber for 20 seconds. The plate was observed with the CAMAG TLC visualizer at 254 nm.

RESULTS AND DISCUSSION

Method development

To achieve good linearity, accuracy, precision, robustness, limit of detection, and limit of quantification different chromatographic conditions were tested, including a reversed-type chromatography, using RP-18 modified silica gel-coated aluminum plates, with F254 fluorescent indicator.

However, the best results were achieved using silica gel 60 F254 aluminum HPTLC plates, 10×20 cm, 200 μm layer, and mobile phase – toluene : ethyl acetate : methanol at a ratio of 7:2:1 v/v/v.

Compared to the classical TLC, this technique requires a smaller volume of the mobile phase (10 mL). The standard solutions and the samples were applied as bands. All steps of the progress development were carefully monitored using specialized software. The time for the development was 25 minutes. After reaching a front of 70 mm, the plates were dried for 5 minutes. In the HPTLC techniques, the drying process is independent of human interaction, it is much safer for the researcher who performs the analysis, and it is performed automatically in a specific place of the development chamber. The next step involved derivatization with Dragendorff's reagent in a dipping chamber for 20 seconds. The plate was observed with the CAMAG TLC visualizer after 5 minutes at 254 nm.

Method validation

The optimized HPTLC method was validated for linearity, accuracy, precision, limit of detection and quantification, and robustness according to the guidelines of the International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use (ICH).[22]

Linearity

According to the ICH guidelines, the linearity must be evaluated using a minimum of five concentrations. The calibration curve was built using concentrations of sibutramine standard solutions in range from 0.250 to 1.250 μg/band.

A stock solution of sibutramine 100 μg/ml was prepared in methanol. Different volumes of the stock solution were applied by automatic sampler on HPTLC plate to obtain concentrations of 0.250 μg/band, 0.500 μg/band, 0.750 μg/band, 1.000 μg/band, and 1.250 μg/band.

After the development and derivatization, the plate was observed with the CAMAG TLC visualizer at 254 nm **(Fig. 1)**.

The peak areas of the standard solutions were measured with CAMAG HPTLC system. Isometric profile of sibutramine in different concentrations is represented in **Fig. 2**. The linear regression line was y=0.0055933+0.000995. R^2=0.9977. The concentration range 0.250–1.250 μg/band showed a good linearity.

Accuracy

Accuracy was assessed as a percentage of recovery. Recovery was evaluated using 3 known concentrations and 6 replicates for each concentration level. For accuracy tests from each examined substance were used 3 different quality con-

Figure 1. HPTLC chromatogram of sibutramine after derivatization with Dragendorff's reagent under 254 nm. Concentrations 250 ng/band, 500 ng/band, 750 ng/band, 1000 ng/band, and 1250 ng/band.

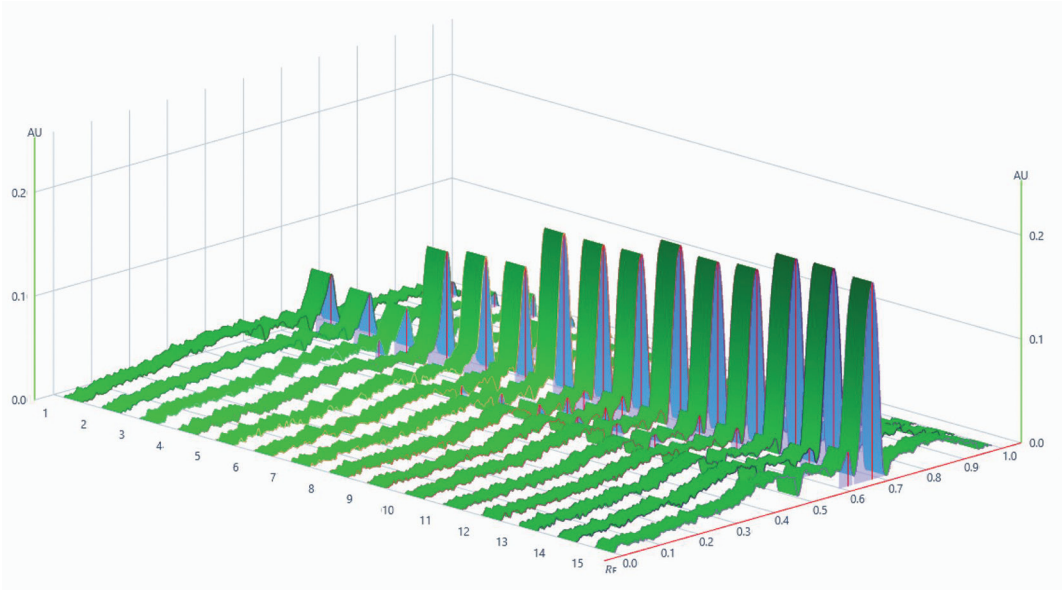

Figure 2. Isometric profile of sibutramine in different concentration levels.

trol (QC) levels: lower QC (LQC; 0.500 µg/band), middle QC (MQC; 0.750 µg/band), and high QC (HQC; 1.000 µg/band) with six replicates. The results from the accuracy evaluation are presented in **Table 1**.

Precision

The interday and intraday precision of the method were determined by evaluating the coefficient of variation for the 3 known level concentrations, each concentration with 6 replicates **(Table 2)**.

Limit of detection and limit of quantification

The detection limit and quantification limit were expressed by the standard deviation of the slope (s) and the slope of the calibration curve (S). We used the following formulas: LOD=3.3 s/S and LO-Q=10 s/S. The limit of detection was 0.0765 µg/band, and the limit of quantification was 0.2318 µg/band.

Comparison of the proposed method with other HPLC/TLC methods

The study by Phattanawasin et al. was the first validated TLC method for quantification of sibutramine in DSs. The analysis was carried out using silica gel 60 F254 TLC plates, where the mobile phase comprised toluene-n-hexane-diethylamine, and for visualizing the spots Dragendorff's reagent was used. The calculated LOD and LOQ were 190 ng/spot and 634 ng/spot respectively.[10]

Later on, Hayun et al. developed a TLC-densitometry method using TLC silica gel 60 F254 aluminum plate, tol-

Table 1. Results on the accuracy of the developed HPTLC method for detection of sibutramine in dietary supplements

Concentration (µg/band)	Mean (µg/band)±SD	Recovery, %	CV%
0.500	0.5164±0.0135	103.278	2.606
0.750	0.7697±0.01599	102.622	2.078
1.000	0.9991±0.0209	99.911	2.092

Table 2. Results on the precision of the developed HPTLC method for detection of sibutramine in dietary supplements

Concentration (µg/band)	Intraday precision			Interday precision		
	Mean (µg/band)±SD	Standard error	CV%	Mean (µg/band)±SD	Standard error	CV%
0.500	0.5076±0.0105	0.0043	2.073	0.5241±0.0152	0.0062	2.892
0.750	0.7619±0.0151	0.0062	1.984	0.7589±0.0220	0.0090	2.900
1.000	1.0140±0.0192	0.0078	1.891	1.0015±0.0315	0.0128	3.140

uene-diethylamine (10:0.3) as mobile phase, and densitometric scanning at 227 nm. Lower amounts of LOD/LOQ were reported, 217.5 ng and 724.9 ng/spot, respectively.[23]

With the advent of new improved techniques based on the principles of thin-layer chromatography, HPTLC instruments were developed. HPTLC represents a significant advancement in the TLC methodology, owing to the incorporation of several enhancements: automatic and improved sample application, automatic development and drying, automatic monitoring of the analytical procedure, increased resolution, etc. All these essential improvements lead to more precise results and better safety for the researchers.[18,19]

Ariburnu et al. developed and reported much sensitivity HPTLC technique compared to previous TLC methods, where the LOD and the LOQ were 77.34 and 257.79 ng respectively. HPTLC was performed with glass plates silica gel 60 F254, n-hexane-acetone-ammonia as a mobile phase, where the densitometric observation was set at 225 nm.[24]

The discussed studies reported not only novel methods for sibutramine detection but also, positive samples adulterated with sibutramine in various concentrations, reaching up to 35 mg per capsule.[10,23,25-27] A comparison between previous HPTLC/TLC techniques for the detection of sibutramine in DSs is presented in **Table 3**.

The method established and validated in this study demonstrated excellent linearity, accuracy, and precision. The accuracy of sibutramine was between 99.911% and 103.278%. The limit of detection was 0.0765 μg/band, and the limit of quantification was 0.2318 μg/band which shows good sensitivity of the proposed method. The HPTLC assay for the determination and quantification of sibutramine could be used for the analysis of DSs.

In contrast to prior approaches used for analysis, derivatization was performed in a dipping chamber. Dipping allows for a more controlled and even application of the sample onto the HPTLC plate. Dipping provides better resolution of the separated spots on the HPTLC plate compared to spraying. The controlled application of the sample by dipping ensures that the spots remain well-defined and do not merge, leading to more accurate identification and quantification of the analytes.

Analysis of dietary supplements and confirmation of the method

The research carried out by Hachem et al. investigated 164 dietary supplements, of which 43 samples had sibutramine adulteration, with concentrations varying from 0.1 mg to 22 mg.[28] In 2016, a study conducted by Adela Krivohlavek and her team revealed that sibutramine was present in 20% of the 123 analyzed supplements, with the highest recorded level reaching 26.41 mg/g.[29] A comparable investigation was conducted by Zeng et al., who analyzed 447 weight loss dietary supplements, among which 55 out of 119 contaminated samples were found to contain sibutramine.[30] In 2014, Kim et al. discovered that 29 out of 188 DSs contained sibutramine, with quantities ranging from 0.03 mg/g to 132.40 mg/g.[11]

The results indicated that a large number of DSs contain undeclared sibutramine.

For determining sibutramine in DSs, the validated HPTLC method was used. The samples and standard solutions were spotted on the same plate and analyzed. Bands of dietary supplements were identified by comparing the Rf value, after dipping in Dragendorff's reagent, with those from standard of sibutramine. Forty DSs were tested and the presence of sibutramine in 5 dietary supplements was confirmed. The chromatogram and isometric profile of sibutramine standards and 5 dietary supplements are presented in **Figs 3, 4**, respectively.

Table 3. Comparison between previous HPTLC/TLC techniques for analyzing sibutramine in dietary supplements

Technique	Mobile phase	Wavelength	Reagent	Sibutramine concentration detected	LOD/LOQ	Ref.
HPTLC	N-hexane-acetone-ammonia (10:1:0.1)	225 nm	-	-	77.34/257.79 ng/spot	(24)
HPTLC	Toluene-methanol (9:1)	225 nm	-	Amounts reach 35 mg per capsule	-	(25)
TLC	Toluene-diethylamine (10:0.3)	227 nm	-	Range from 2.45 to 26.24 mg in a single dosage	217.5/724.9 ng/spot	(23)
HPTLC	MTBE-toluene-methanol (9:1:1)	225 nm	-	-	-	(17)
HPTLC	Toluene-ethyl formate-formic acid (5:4:1)	254 nm 366 nm	Dragendorff's reagent	-	-	(27)
TLC	Methanol: ammonia (100 : 1.5)	245 nm	Dragendorff's reagent	4.38 mg/capsule and 26.37 mg/capsule		(26)
TLC	Toluene-n-hexane-diethylamine (9:1:0.3)		Dragendorff's reagent	Range from 6 mg to 24 mg per single dosage	190/634 ng/spot	(10)

Figure 3. Chromatogram of sibutramine standard in concentrations from 0.250 µg/band to 1.250 µg/band and 5 dietary supplements.

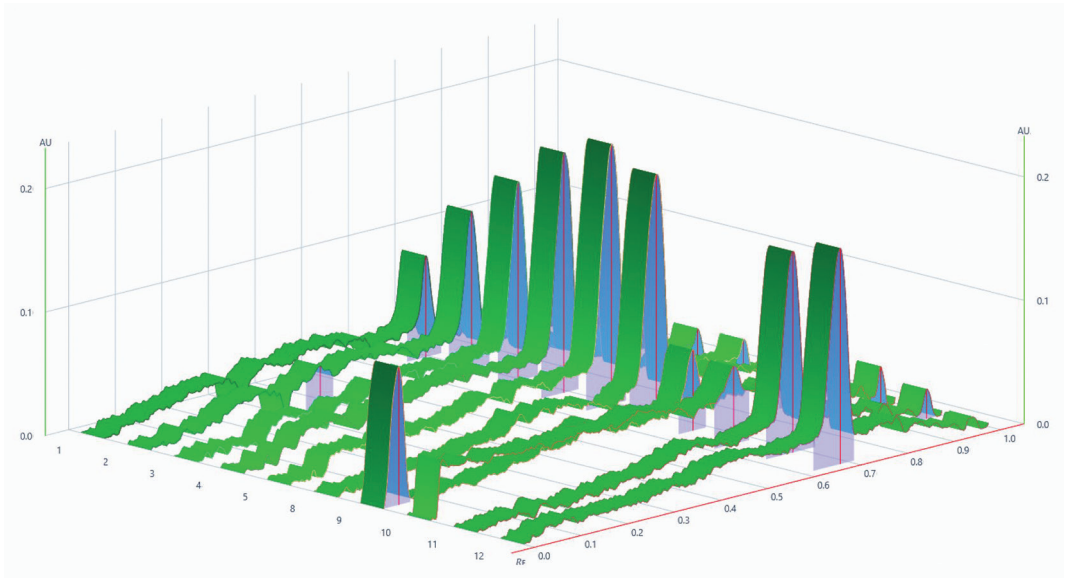

Figure 4. Isometric profile of sibutramine standards and 5 dietary supplements. 1. Sibutramine 0.250 µg/band. 2. Sibutramine 0.500 µg/band. 3. Sibutramine 0.750 µg/band. 4. Sibutramine 1.000 µg/band. 5. Sibutramine 1250 µg/band. 8–12 DSs.

Three DSs showed a higher amount of sibutramine. The results from HPTLC were confirmed through GC-MS analysis by a previously validated method.[12]

Despite the high level of consumption of DSs, the lack of regulations is currently a critical issue.[31] The majority of DS users believe these products are safe and represent a healthy lifestyle. In general, consumers are unaware of the risks associated with the intake of DSs.[32] Unintentional intake of sibutramine from DSs can affect levels of norepinephrine and serotonin in the body, resulting in an increased heart rate and tachycardia. These effects raise the risk of cardiovascular events such as heart attacks, strokes, and arrhythmias.[3,5,7,9] Moreover, sibutramine intake can cause sleep disorders, anxiety, dizziness, and restlessness.[33] People with cardiovascular disease, including those with a history of heart attack, stroke, arrhythmias, congestive heart failure, or uncontrolled hypertension, are at a higher risk of adverse cardiovascular events when using sibutramine. Sibutramine intake can potentially increase the likelihood of cardiovascular events, as mentioned above.[3]

Furthermore, DSs are essential components of professional athletes' diets and may offer advantages like quick recovery from strenuous exercise regimens, improved exercise performance, and dietary enrichment.[34] However, unintentional doping can occur after intake of DSs, if an athlete unknowingly consumes substances that are prohibited by the World Anti-Doping Agency (WADA). Sibutramine falls into this category because it is classified as a banned substance by WADA.[35] The reason for its prohibition is primarily due to appetite suppression and weight loss.

The five contaminated DSs, from our study, could expose consumers not only to serious health side effects but also expose many professional athletes to a significant risk of unintentional doping.

CONCLUSIONS

The presence of sibutramine in weight-loss DSs is a serious concern. DSs, unlike prescription medications, are not

subject to the same testing procedures and regulations, putting consumers' safety at risk. The presence of sibutramine in DSs without labeling could result in the consumption of high doses of this compound, increasing the risk of adverse effects, especially when combined with other medications. The current study reported on the development of a simple and rapid HPTLC method for the identification and quantification of sibutramine. The method was subsequently validated and showed good linearity, accuracy, and precision over a concentration range of 0.250–1.250 μg/band. The LOD and LOQ were determined as 0.0765 μg/band and 0.2318 μg/band, respectively. Furthermore, the developed method is sensitive, rapid, dependable, and precise, making it useful for detecting sibutramine in dietary supplements. The method was applied for the analysis of 40 DSs. Five of the samples were contaminated with sibutramine in different concentrations. The method contributes to the ongoing efforts in monitoring and controlling the undeclared presence of sibutramine in DSs.

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Author contributions

Conceptualization: V.K., S.I., and K.I.; methodology: V.K., K.I., and S.I.; validation: V.K. and S.I.; investigation: V.K., K.I., D.C., and S.I.; resources: V.K.; data curation: V.K. and S.I.; writing original draft: V.K., K.I., and S.I.; review and editing: K.I., D.C., and S.I.; supervision: K.I. and S.I. All authors have read and agreed to the published version of the manuscript.

Competing Interests

The authors have declared that no competing interests exist.

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Разработка и валидация метода высокоэффективной тонкослойной хроматографии для обнаружения сибутрамина в пищевых добавках

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Резюме

Введение: В период 1997-2010 гг. сибутраминсодержащие препараты широко назначались для лечения ожирения и избыточной массы тела. Из соображений безопасности в 2010 году все препараты, содержащие сибутрамин, были срочно изъяты с фармацевтического рынка США и Европы. Хотя сибутрамин больше не доступен в фармацевтических продуктах, были многочисленные сообщения о фальсифицированных пищевых добавках для снижения веса, содержащих сибутрамин, которые не были маркированы.

Цель: Целью данной работы было разработать и апробировать точный и чувствительный высокоэффективный тонкослойный хроматографический метод обнаружения сибутрамина.

Материалы и методы: Метод разработан с использованием системы CAMAG HPTLC. Алюминиевые пластины для HPTLC с силикагелем 60 F254 использовали в качестве неподвижной фазы, а толуол:этилацетат:метанол (7:2:1 объём/ объём/ объём) в качестве подвижной фазы.

Результаты: Построена калибровочная кривая в диапазоне 0.250–1.250 μg/лента. Метод обеспечил удовлетворительную линейность, специфичность, скрупулёзность и достоверность. LOD и LOQ составляли 0.0765 μg/лента и 0.2318 μg/лента соответственно.

Заключение: Метод позволяет проводить одновременный анализ нескольких образцов, а также быстрый и чувствительный мониторинг уровня сибутрамина в пищевых добавках.

Ключевые слова

пищевые добавки, высокоэффективная тонкослойная хроматография, HPTLC, сибутрамин

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Original Article

An Osteoporosis Knowledge Assessment Instrument – Development and Validation

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Abstract

Introduction: The consequences of osteoporotic fractures are extremely detrimental to the individual as well as to society. Adopting effective preventative measures is a top public health priority.

Aim: This paper deals with the development and validation of an osteoporosis knowledge measurement tool.

Materials and methods: The study sample included 335 healthy women aged between 25 and 51 years. The osteoporosis knowledge measurement tool is an adapted version of the osteoporosis knowledge assessment tool (OKAT). To determine the validity and reliability of the tool, we examined the psychometric properties. Nonparametric methods were used for the statistical analysis.

Results: Flesch reading ease index was 55.14. The Cronbach's α value was 0.884. The corrected item-total correlations varied between 0.340 and 0.611. The items' mean difficulty was 0.46. The mean discrimination index was 0.61. The mean score of the sample was M=12.64±5.164, a little bit higher than 50% of the success rate.

Conclusion: The tool can be used in different settings to assess educational needs and plan interventions. The results indicate a need for educational and preventive initiatives.

Keywords

evaluation, awareness, osteoporotic fractures, prevention

INTRODUCTION

Osteoporosis is a disease with significant social implications that initially presents as an inconspicuous condition but can cause serious complications in later stages. Osteoporotic fractures are a serious public health problem. The consequences of these fractures have major negative economic effects on society as a whole as well as on individuals. They are associated with long-term difficulties in carrying out daily activities, long-term treatment, permanent disability, and job loss. Impaired quality of life and dependence on care provided by relatives or medical personnel leads to social isolation and low self-esteem.[1]

An important public health priority is the implementation of effective prevention strategies. Bone density before menopause is as important as bone loss after menopause in predicting the risk of future fractures. Aging people can increase their bone density through healthy behaviors that include proper diet, physical activity, and a healthy lifestyle. Women of active age from 25 to 51 are a very important target group for prevention. Results of a study[2] showed that more than half of the surveyed women over 45 were not informed about the disease, 85.7% did not take enough calcium, and 30.5% had a family history, which is a risk factor for osteoporosis.

There are studies in the scientific literature that assess

the risk of developing osteoporosis among women of active age, but the implementation of prevention methods aimed at lifestyle changes requires active work in the group and at the individual level. Calcium-rich foods and exercise are recommended to increase bone density among premenopausal women. Endicott[3] believes that health education about risk factors and preventive measures for osteoporosis should begin well before menopause. Education campaigns aimed at raising awareness of the condition, its risk factors, and ways to reduce them should target both women active reproductive age and young people in school. Increasing health knowledge is a predictor of motivated engagement for long-term preventive behavior. Planned health education aims at changing attitudes, beliefs, and a greater self-efficacy[4] and is built upon a valid and reliable diagnostic tool to assess the knowledge of osteoporosis of the targeted population. For this purpose, we developed an adapted version of OKAT[5] that takes into account the cultural peculiarities and lifestyle of women in Bulgaria.

AIM

This paper deals with the development and validation of an osteoporosis knowledge measurement tool.

MATERIALS AND METHODS

Sample selection and description

The participants, 335 healthy women aged between 25 and 51 years, were selected randomly from the city of Plovdiv and the Plovdiv region. After being informed about the purpose of the study, the women agreed to participate voluntarily and anonymously. A paper-based questionnaire was administered to the participants. The study was approved by the Institutional Ethics Committee of the Medical University of Plovdiv (protocol No. 1/19.01.2023). The opinion of the Committee was that the research meets the standards of ethics and complies with the requirements of the Helsinki Declaration, the principles of good clinical practice, Bulgarian laws, and regulations for conducting clinical and scientific research with the participation of people.

Development of the instrument

Our osteoporosis knowledge measurement tool is an adapted version of the OKAT.[5] OKAT was translated from English to Bulgarian by a professional licensed translator with experience in translating medical literature. We examined the statements in detail, paraphrased some of them, and left others without change. We added new statements and produced an assessment tool that contained 27 statements. Then we submitted the questionnaire to three experts - a rheumatologist, an endocrinologist, and a specialist in general medicine. On the experts' advice, we modified some of the questions to avoid ambiguity and improve validity. Each of the items had three answer options: *yes*, *no*, and *I do not know*. Indication of the correct answer was considered a correct response, and incorrect responses are considered those that indicated an incorrect answer and the "I do not know" answer. We assigned 1 point for each correct response and 0 points for each wrong one.

Validation procedure

To determine the validity and reliability of the questionnaire, we examined the following psychometric properties:

- Flesch reading ease. We did not find a Flesch reading ease formula validated for the Bulgarian language, so we calculated this index according to the formula proposed by Ivanov et al.[6] for text readability in academic texts in Russian as a Slavonic language close to Bulgarian. The scores ranged between 0 and 100 - a greater value meant easier reading and better understanding by people.
- Internal consistency measurement Cronbach's α. We excluded the items that would increase the scale's homogeneity of omitted. We considered acceptable Cronbach's α >0.70.
- Corrected item-total correlations. Items with negative or low correlation should not be included in the assessment tool, because they do not correlate enough with the scale. A correlation lower than 0.3 was considered negligible.[7]
- Items discrimination index (DI). We calculated it as the difference between the mean score on an item of the students in the first 27th percentile of scores on all items ranging from the highest to the lowest and the mean score on the same item of the students in the last 27th percentile. DI of 0.40 and up indicates a good item's distinguishing ability.[8]

Items' difficulty level was defined as the ratio between correct responses and all answers. Higher values mean easier questions. The optimal range was 20-80%.[8] We accepted items with a difficulty level lower than 0.75.[5,9]

Statistical analysis

We did not use any software for the determination of the Flesch reading ease index, due to the lack of the corresponding functionality for the Bulgarian language in MS Word. The index was calculated independently by two of the authors. We accepted that the index was accurate when the results of the two separate calculations were identical. We calculated the difficulty and discrimination index of the statements in MS Excel. The rest of the statistical analysis was done in SPSS v. 23. The nonparametric methods of Kruskal-Wallis and Mann-Whitney tests, and Spearman correlation were used for the analysis of ordinal variables and variables that were not normally distributed. Central tendencies were reported with a mean value and a standard

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deviation (M±SD). We assumed a level of statistical significance α=0.05.

RESULTS

The Flesch reading ease index was 55.14. The Cronbach's α value was estimated at 0.790. Item-total statistics indicated that removing items 2, 3, 17, and 19 would increase Cronbach's α value. These items also had negative corrected item-total correlations. We removed items 2, 3, 17, and 19, performed the analysis again, and received Cronbach's α=0.884 for a scale with 23 items that do not necessitate any omissions. The corrected item-total correlations varied between 0.340 and 0.611. The items' difficulty varied between 0.23 and 0.66. The mean difficulty of all items was 0.46. The DI was negative for items 2, 3, 17, and 19. For the rest of the statements the DI varied between 0.43 and 0.81, mean D-value 0.61. We removed items 2, 3, 17, and 19 from the scale. The psychometric properties by items of the osteoporosis knowledge measurement tool are presented in **Table 1**.

The mean score of the sample in the developed assessment scale was M=12.64±5.164.

DISCUSSION

We aimed to create an instrument assessing knowledge about osteoporosis validated for the Bulgarian population. We have carefully selected statistical methods to investigate the reliability of a psychometric test for measuring knowledge. In validating a similar instrument, the test-retest method was used.[9,10] We did not use the test-retest method on purpose because there was a chance that the first filling out of the questionnaire would pique the respondents'

Table 1. Psychometric characteristics of the scale with items 2, 3, 17, and 19 removed

Items	Difficulty	Discrimination index	Item-total correlation
1. Osteoporosis leads to an increased risk of bone fractures.	0.66	0.57	0.496
4. Higher bone density in childhood protects against the development of osteoporosis later in life.	0.35	0.43	0.370
5. Women suffer more from osteoporosis.	0.55	0.67	0.512
6. People with lighter skin color are at a higher risk of developing osteoporosis.	0.23	0.46	0.369
7. Low bone density can be the cause of bone fracture in minor traumas.	0.55	0.81	0.611
8. Most people develop osteoporosis by the age of 80.	0.39	0.67	0.486
9. After the onset of menopause (the cessation of menstruation), most women can expect at least one fracture.	0.34	0.64	0.438
10. A family history of osteoporosis is an important prerequisite for the development of the disease.	0.50	0.81	0.605
11. Smoking can contribute to the development of osteoporosis.	0.37	0.59	0.422
12. Moderate physical activity outdoors protects against osteoporosis.	0.47	0.78	0.569
13. Playing sports in childhood prevents the development of osteoporosis in adulthood.	0.47	0.67	0.487
14. Exposure to direct sunlight for at least 30 minutes a day prevents the development of osteoporosis.	0.55	0.61	0.496
15. The daily intake of milk and milk products supplies the body with enough calcium.	0.58	0.62	0.474
16. Fish is a good source of calcium.	0.63	0.52	0.401
18. Eggs are a good source of calcium.	0.59	0.58	0.473
20. Raw nuts are a good source of calcium.	0.59	0.66	0.556
21. Daily alcohol use suppresses the formation of new bone density.	0.37	0.60	0.444
22. Calcium supplements alone can prevent bone loss.	0.36	0.49	0.406
23. I can determine my risk of developing osteoporosis based on my lifestyle.	0.36	0.57	0.453
24. Hormone therapy contributes to bone loss at any age.	0.30	0.53	0.425
25. It is important to prevent osteoporosis before the age of 40.	0.66	0.60	0.489
26. There is an effective therapy for osteoporosis.	0.42	0.59	0.419
27. Osteoporosis is a treatable disease.	0.37	0.50	0.340

interest, and they would seek more information about the disease and its causes. This would skew the results of a second fill and the statistical results would be biased. In the development of OKAT as a valid and reliable tool to measure knowledge in osteoporosis, the principal component factor analysis was used.[5,9] We did not perform a factor analysis because of the dichotomous type of the variables. There has been considerable controversy surrounding the appropriateness of using factor analytic techniques for dichotomous variables.[11] The factor analysis in the case of dichotomous variables will often lead to artificial factors.[12]

We achieved the set validity and reliability requirements. The questionnaire we created based on OKAT with added statements had a good Flesch reading ease index, 55.14, higher than those reported by Winzenberg et al.[5] (45) and Tardi et al.[9] (44). The calculated Cronbach's α=0.884, after removing four of the statements, showed a good internal consistency of the test, also supported by the corrected item-total correlations, all within the preset limits. Items' difficulty range was satisfactory, there were neither very easy nor very difficult questions. The differences between mean, median, and mode indicated a normal to slightly easy assessment, which was also confirmed by the average difficulty being slightly less than 0.50. The four reliability-compromising statements were found to have a negative discrimination index. They were removed from the pool. All questions in the final version had a discrimination index within the desired range. This measure shows the item's ability to differentiate between "good" and "poor" participants. The DI indicates the extent to which the answers to the question are a result of knowledge rather than guesswork. The mean D-value for the tool was 0.61%, which is higher than those reported by Tardi et al.[9] (50.4%), and Winzenberg et al.[5] (44%).

The mean test score was 55% of the maximum of 23 points and was higher than the scores reported by Winzenberg et al.[5] (44%) and Sayed-Hassan et al.[10] (less than 50%).

CONCLUSION

The results show that we succeeded in developing a valid and reliable osteoporosis knowledge measurement tool for the Bulgarian population. It can be used in different settings to assess educational needs and plan interventions. The mean score of the sample was a little bit higher than 50% of the success rate for this assessment, which indicates a need for educational initiatives and preventive activities.

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Author contributions

All authors have contributed equally to the development, research and writing of the manuscript.

Competing Interests

The authors have declared that no competing interests exist.

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Инструмент оценки знаний об остеопорозе – разработка и валидизация

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Резюме

Введение: Последствия остеопоротических переломов чрезвычайно вредны как для человека, так и для общества. Принятие эффективных профилактических мер является главным приоритетом общественного здравоохранения.

Цель: В данной статье речь идёт о разработке и проверке инструмента измерения знаний об остеопорозе.

Материалы и методы: В выборку исследования вошли 335 здоровых женщин в возрасте от 25 до 51 года. Инструмент измерения знаний об остеопорозе представляет собой адаптированную версию инструмента оценки знаний об остеопорозе (OKAT). Чтобы определить валидность и надёжность инструмента, мы исследовали психометрические свойства. Для статистического анализа использовались непараметрические методы.

Результаты: Индекс Flesch (Flesch reading ease index) составил 55.14. Коэффициент α Cronbach составил 0.884. Скорректированные корреляции общего количества пунктов варьировались от 0.340 до 0.611. Средняя сложность пунктов составила 0.46. Средний индекс дискриминации составил 0.61. Средний балл выборки составил M=12.64±5.164, что немного превышает 50% показателя успеха.

Заключение: Этот инструмент можно использовать в различных условиях для оценки образовательных потребностей и планирования хирургических вмешательств. Результаты указывают на необходимость образовательных и профилактических инициатив.

Ключевые слова

оценка, осведомлённость, остеопоротические переломы, профилактика

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Case Report

Spontaneous Thrombosis of Type II Vein of Galen Aneurysmal Malformation: a Case Report

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Abstract

Vein of Galen malformations (VGAMs) are rare and complex congenital brain vascular anomalies that pose significant diagnostic and treatment challenges. The natural history of this type of vascular anomaly is very poor, with many patients succumbing to complications such as congestive heart failure, hydrocephalus, and brain parenchymal injury. Although the clinical course of most VGAMs was considered unfortunate, with meticulous imaging, a group of lesions with a more placid presentation and course can be identified.

We present a case of spontaneous thrombosis of VGAM where no embolization or surgical repair was attempted, with excellent clinical outcomes.

This case also highlights the possibility of spontaneous thrombosis in VGAM, even in the absence of clinical symptoms, and emphasizes the importance of a regular imaging follow-up in patients with known vascular malformations.

Keywords

pediatric, thrombosis, vascular, vein of Galen malformation

INTRODUCTION

Vein of Galen aneurysmal malformations (VGAMs) are a rare type of congenital vascular pathologies that represent around 30% of pediatric vascular malformations and around 1% of all cerebral vascular malformations.[1,2] The first reported pathology case is widely thought to have been published by Jaeger et al. in 1937.[3,4] Since then, many more cases have been reported, and interest in the disorder, as well as our understanding of its underlying mechanism, has expanded.[5-7] Two classifications are commonly used in VGAM evaluation: one proposed by Lasjaunias et al.[8] and the other proposed by Yasargil.[9,10] The former categorizes VGAMs into choroidal and mural types based on the origin and insertion of the feeding arteries and the place of fistula communication (direct or intramural). They observe the difference in the clinical presentation and perspective based on their proposed subtypes. The classification proposed by Yasargil makes a more precise division into four types based on the feeding arteries: type I is an arteriovenous fistula between the posterior cerebral arteries or the pericallosal arteries and the vein of Galen, type II is an arteriovenous fistula between the thalamoperforating arteries and the vein of Galen, type III is a mix between types I and II. Type IV is reserved for arteriovenous malformations that drain into the vein of Galen and directly dilate it. Although the clinical course of most VGAMs was considered unfortunate, with better imaging, a group of lesions with a more benign presentation and course has been identified. We present a case of spontaneous thrombosis of VGAM

where no embolization or surgical repair were attempted, with excellent clinical outcomes.

CASE PRESENTATION

A 1-month-old child was referred to our hospital for cranial magnetic resonance imaging (MRI) after the routine post-birth ultrasound suspected a VGAM. Brain MRI and 3D time of flight (TOF) magnetic resonance angiography confirmed the diagnosis ***(Fig. 1)****. The child underwent further neurological and cardiological examinations, which appeared normal without any deviations. The laboratory studies were also normal, and the patient's Bicêtre score was calculated to be 21* ***(Table 1)****. Following a discussion with a multidisciplinary team of neurologists, interventional radiologists, pediatricians, and neurosurgeons, a decision for observation, conservative treatment, and close follow-up was made. Parents were carefully instructed, and monthly control medical examinations were evaluated at six monthly intervals for radiology scans.*

At one year of age, the patient came to the hospital for a routine follow-up examination. The neurological examination was normal, with a slightly higher cranium diameter. A non-contrast computed tomography (CT) confirmed the macrocephaly with a more significant biparietal index than the ages. CT angiography showed an enlargement of the vein of Galen. Due to the mass effect of the malformation, we observed dilatation of the two lateral and third ventricles. There were no changes in the brain parenchyma. On the CT angiography, we could evaluate the feeders coming from the posterior and middle choroidal arteries draining into the large aneurysmal dilatated vein of Galen, dilatated torcula and significantly narrowed proximal part of the straight sinus distal to Galen. We classified the malformation as type II by Yasargil ***(Fig. 2)****.*

The patient was scheduled for digital subtraction angiography, but the parents refused to perform the test. The cardiological consultation of the child was unremarkable. After a neurological and neurosurgical examination, we assessed that the hydrocephalus was asymptomatic and placement of a ventricular-peritoneal shunt was not needed. The patient was discharged and followed closely for the next two years with periodic head circumference measurements. During this period, his neurological examination and progress were excellent.

An MRI at three years of age revealed significant but also positive changes. There was a total occlusion of the malformation due to thrombosis of the aneurysmal dilatated vein of Galen ***(Fig. 3)****. We observed an almost double shrinkage of the varix compared to the CTA made two years ago. There were no radiological signs of hydrocephalus, and the sella media index was normal. The MRI, too, showed no changes in the brain parenchyma. The patient was neurologically intact.*

Another control MRI at eight years was performed. The thrombosed aneurysmal dilatated vein of Galen continues to shrink ***(Fig. 4)****. The venous structure appeared normal, with no radiology signs of hydrocephalus. The neurological examination remained normal. The child exhibited advanced developmental milestones compared to his peers.*

DISCUSSION

The principal pathoanatomic substrate of VGAM is represented by a formed fistula between different portions of the involuting primitive median prosencephalic vein of Markowski and adjacent brain arteries of the fetus around weeks 6-11 of gestation.[10] This, in turn, halts the regression of the venous vessel due to the high blood flow that passes through it, with the malformation not rarely being associated with dural sinus stenosis due to atresia or thrombosis, further increasing the risk of complications.[8,11] An interesting observation can be made that VGAMS can be associated with a different primitive vessel noted on angiograms of patients suffering from the condition - the falcine sinus, which most likely acts as a draining shunt and is connected to the distal 1/3 of the superior sagittal sinus.[12,13] Another noteworthy point is the disruption of the function of the deep venous brain system, which drains through mesencephalic collaterals.[14]

Pathological changes associated with VGAMs can lead to several dreadful patient outcomes. The most common clinical presentation is widely reported to be congestive heart failure due to high output.[15] Another clinical manifestation is development of hydrocephalus due to either the increased intracranial blood volume or cerebral hemorrhages and direct obstruction of cerebrospinal spaces.[16] Due to the condition primarily affecting children, arrest in brain development and focal neurological symptoms can also be observed.[17]

Large strides in the treatment of VGAMs have been made. To this day, they present a clinical conundrum that requires careful consideration of the patient's characteristics and the vascular pathology. The pretreatment era of VGAMs historically cites exceptionally high mortality rates in patients. With the progression of surgical and endovascular methods, a decline in mortality can be observed[18], even with their inherent risks.

An approach for assessing newborns with VGAM involves the application of the Bicêtre score, which aids in determining potential courses of treatment.[19] This 21-point scale assigns points based on the severity of indications and symptoms related to cardiac, pulmonary, neurological, hepatic, and renal systems. The Bicêtre score is computed for neonates with VGAM by employing clinical and laboratory data. A score of less than 8 out of 21 indicates a highly critical prognosis, rendering the infant unsuitable for immediate embolization. For neonates scoring between 8 and 12, emergent embolization is likely to be beneficial. A score exceeding 12 identifies infants eligible for the administration of medical treatment to address their cardiopulmonary insufficiency. Such medical intervention is continued until the infants reach approximately 5 months of age, at which point their larger size diminishes the risks associated with

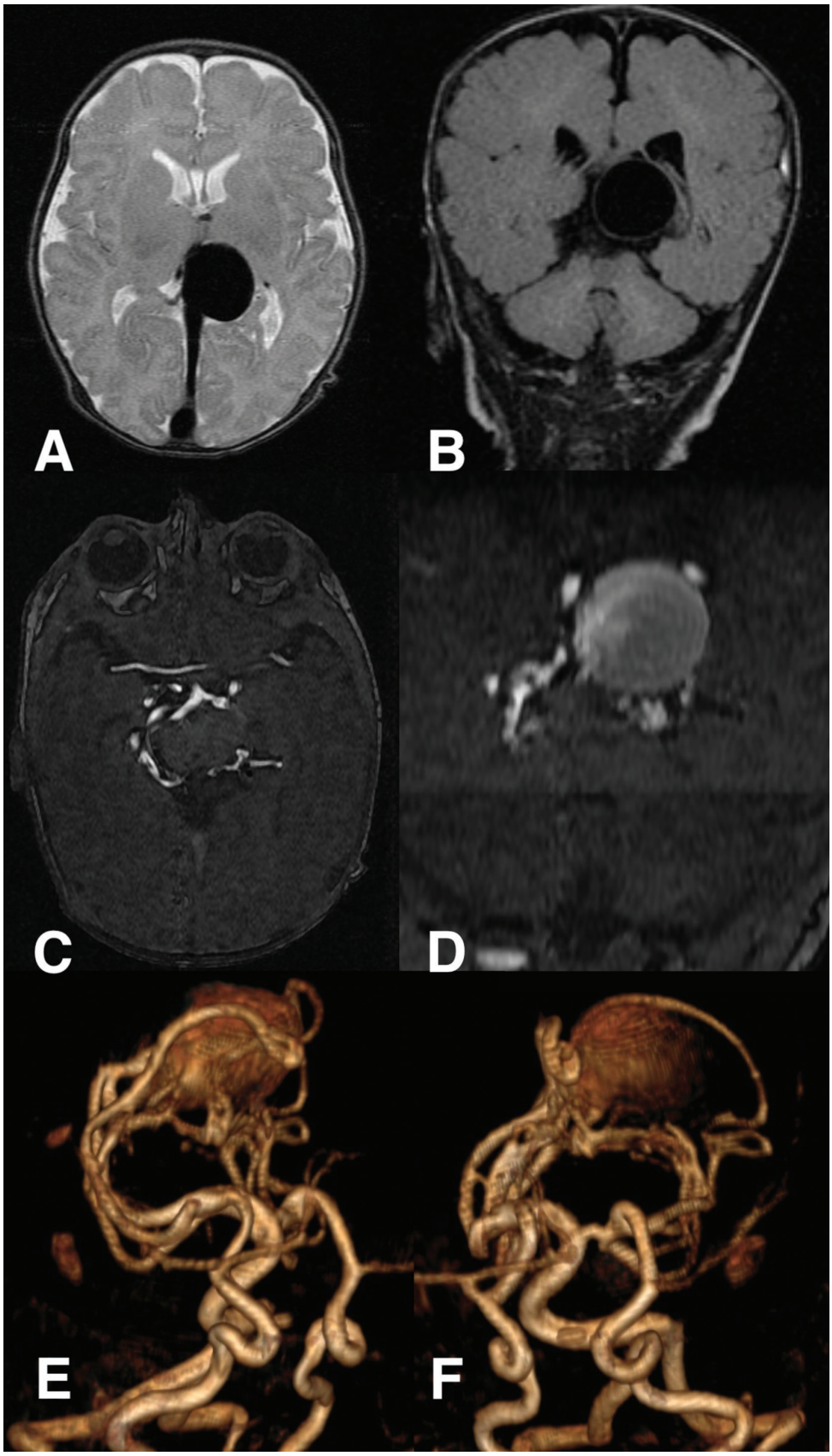

Figure 1. MRI at the age of 1 month. Axial T2 weighted and coronal FLAIR images of the brain show dilatation of the vein of the Galen up to 3 cm in diameter without any pathological findings in the brain parenchyma and ventricular system (**A, B**). 3D TOF images with VR reconstructions demonstrate the feeders coming from the posterior and middle choroidal arteries draining into the large aneurysmal dilatated vein of Galen (**C**, **D**, **E**, **F**).

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Table 1. Bicêtre score

Points	Cardiac functions	Cerebral function	Respiratory function	Hepatic function	Renal function
5	Normal	Normal	Normal	-	-
4	Overload, no medical treatment	Subclinical, isolated EEG abnormalities	Tachypnea finishes bottle	-	-
3	Failure, stable with medical treatment	Nonconvulsive intermittent neurologic signs	Tachypnea does not finish bottle	No hepatomegaly, normal hepatic function	Normal
2	Failure, not stable with medical treatment	Isolated convulsion	Assisted ventilation, normal saturation FiO_2 <25%	Hepatomegaly, normal hepatic function	Transient anuria
1	Ventilation necessary	Seizures	Assisted ventilation, normal saturation FiO_2 >25%	Moderate or transient hepatic insufficiency	Unstable diuresis with treatment
0	Resistant to medical therapy	Permanent neurological signs	Assisted ventilation, desaturation	Abnormal coagulation, elevated enzymes	Anuria

Maximal score: 5 (cardiac) + 5 (cerebral) + 5 (respiratory) + 3 (hepatic) + 3 (renal) = 21

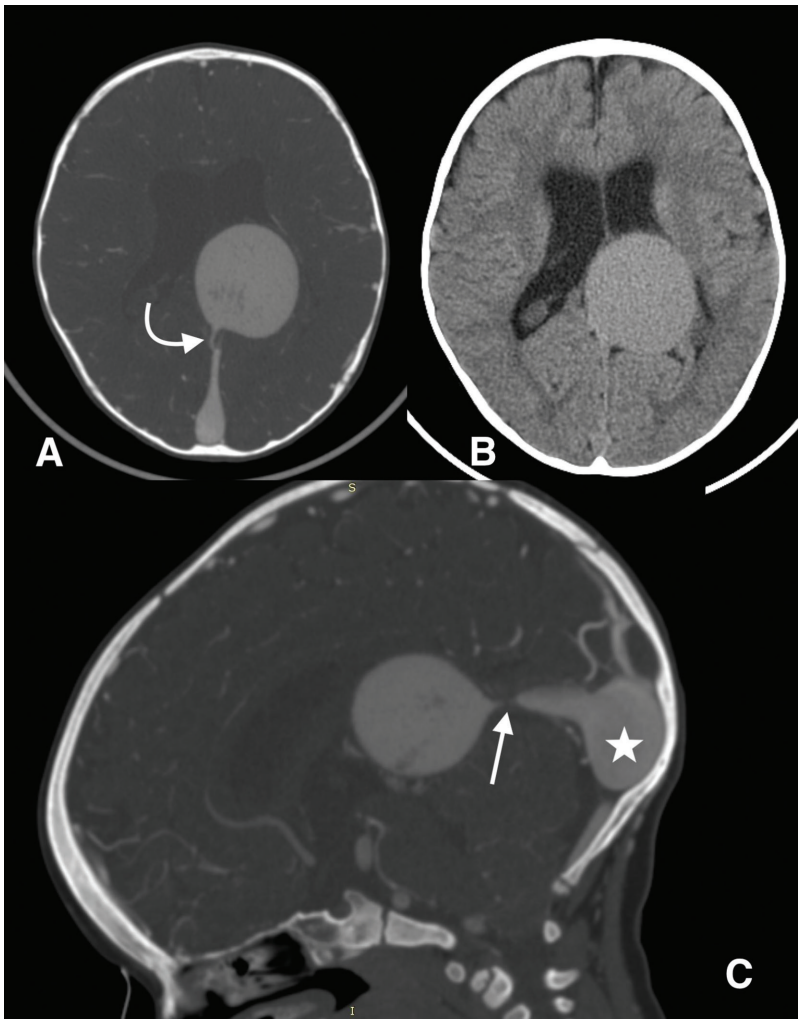

Figure 2. CT image at 1 year. Non-contrast CT demonstrated macrocephaly with elevated biparietal index and dilatated lateral ventricles (**B**). CT angiography shows significant enlargement of the aneurysmal dilatated vein of Galen compared to previous MRI, feeders coming from the posterior and middle choroidal arteries draining into it, dilatated torcula (white star) and significantly narrowed proximal part of the straight sinus (white arrows) distal to Galen (**A**, **C**).

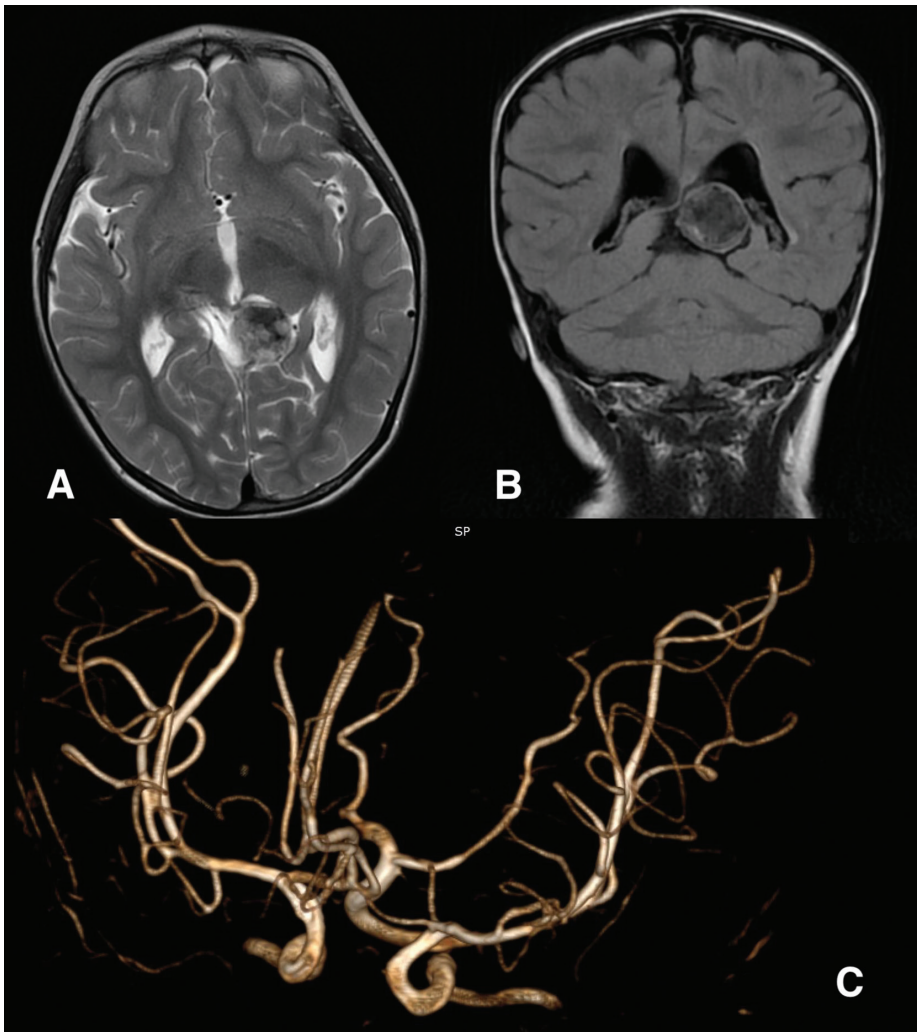

Figure 3. MRI at 3 years. Axial T2 weighted and coronal FLAIR images demonstrate total thrombosis of the malformation, double shrinkage of the varix, no signs of hydrocephalus and damage of the brain parenchyma (**A**, **B**). VR reconstruction of the 3D TOF MRI reveals a complete regression and disappearance of the pathological arterio-venous connections (**C**).

prolonged embolization. **Table 1** provides a comprehensive overview of this treatment protocol.

In one of the largest studies (317 patients) reported thus far, Lasjaunias et al. reported that approximately 19% (57 of 300; 17 lost to follow-up) were considered to be in too bad a clinical condition to be treated. Of the 216 treated patients, 73 (34%) had a bad outcome, ranging from permanent neurological symptoms and mental retardation to death. According to these numbers, the overall poor outcome is approximately 46%. Spontaneous thrombosis of the shunt occurred in only eight of 317 patients (2.5%).[8] In the study of Geibprasert et al., the poor outcome rate was 52%, and three of their reported cases (12%) spontaneously thrombosed during the follow-up period.[20]

In a recent study, Brinjikji et al. performed a meta-analysis of endovascularly treated VGAM. They reported all-cause mortality of 14% and an overall good neurological outcome rate of 62%. Their study's overall poor neurological outcome rates were 21%, in which neonates were significantly less likely to have good neurological outcomes than infants (48% versus 77%).[5]

On rare occasions, though, a spontaneous regression of the malformation can be observed. This is most likely the result of low-flow fistulas, which cause a slow filling of the dilated vascular channel and a more benign natural history, with several such cases being described in the literature.[21,22] In these cases, the commonest presenting sign was macrocephaly, but symptoms such as hemiparesis, seizures, and pyramidal and cerebellar signs were also observed. Patients presenting with spontaneously thrombosed VGAM could be classified in a discrete clinical group, as proposed by Nikas et al.[23]

Spontaneous thrombosis of intracranial vascular lesions is a well-documented phenomenon observed in conditions such as arteriovenous malformations, arteriovenous fistulas, and aneurysms. The presence of flow-related aneurysms, which can both form and regress, underscores the influence of hemodynamic alterations on thrombosis and

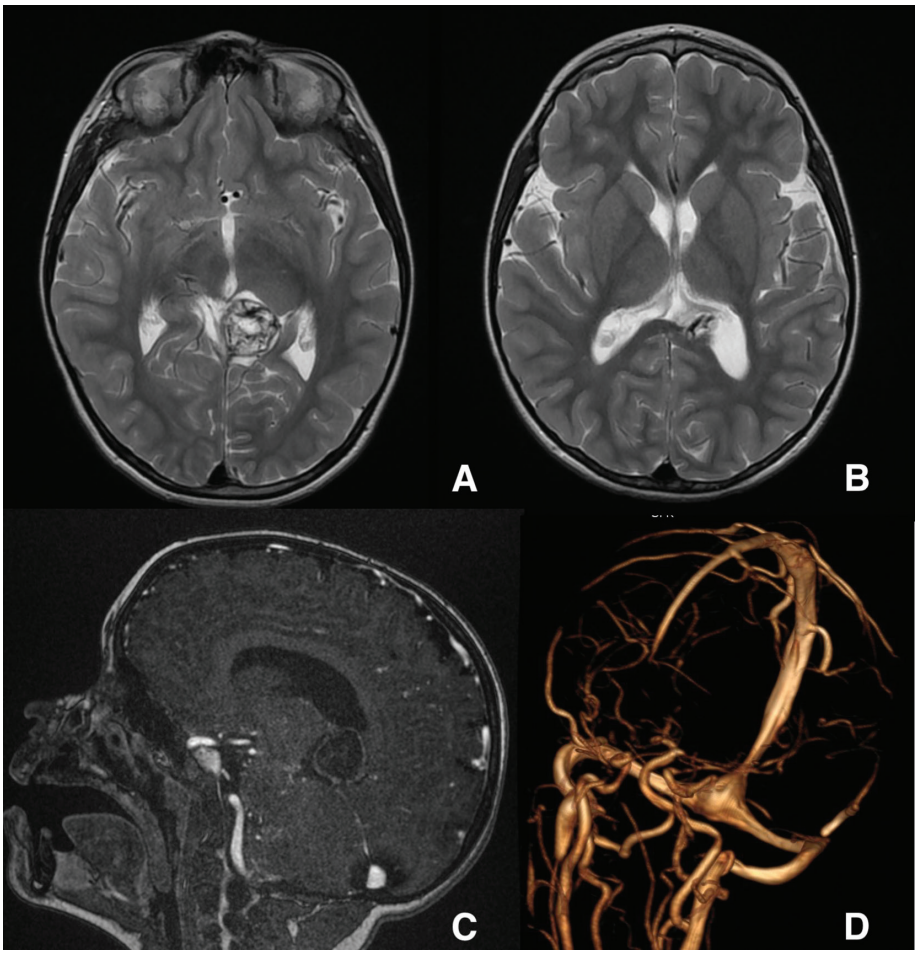

Figure 4. MRI at 8 years. Again, axial T2 and contrast MR angiography demonstrate complete occlusion of the VGAM without any abnormal finding in the brain parenchyma (**A**, **B**, **C**, **D**).

the regression of these vascular abnormalities. While the precise mechanism and clinical factors leading to thrombosis in the vein of Galen malformation are not fully understood, it can be postulated that similar to other vascular lesions, changes in hemodynamic conditions may contribute to thrombus formation. Several potential mechanisms could disrupt the hemodynamic balance in VGAM, including compression or mass effect from adjacent hematoma or intra-aneurysmal clot, posthemorrhagic edema, regressive arteriosclerosis affecting the vessel walls, vascular spasm, and gliosis resulting from fragmentary micro-bleeding.[24]

CONCLUSIONS

The case presented here highlights the possibility of spontaneous thrombosis and subsequent resolution of vein of Galen malformation in a pediatric patient. This case adds to the growing evidence suggesting that certain VGAMs may exhibit a more benign course and favorable clinical outcomes without invasive interventions such as embolization or surgical repair. The utilization of advanced imaging techniques played a crucial role in identifying this subgroup of lesions with spontaneous thrombosis. Further research and long-term follow-up studies are warranted to understand better the underlying mechanisms and factors contributing to spontaneous thrombosis in VGAMs. Nonetheless, our findings emphasize the importance of considering conservative management strategies in selected cases, which may spare patients the potential risks associated with invasive interventions.

Author contributions

S.B.: writing the manuscript; K.S.: methodology, data curation, imaging; S.S.: final and critical review

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Спонтанный тромбоз аневризматической мальформации вены Галена II типа: описание случая

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Резюме

Мальформации вены Галена (VGAMs) представляют собой редкие и сложные врожденные аномалии сосудов головного мозга, которые создают серьёзные проблемы в диагностике и лечении. Естественное течение этого типа сосудистых аномалий очень плохое, многие пациенты страдают от таких осложнений, как застойная сердечная недостаточность, гидроцефалия и повреждение паренхимы головного мозга. Хотя клиническое течение большинства VGAMs считалось неудачным, при тщательной визуализации можно идентифицировать группу поражений с более спокойной картиной и течением.

Мы представляем случай спонтанного тромбоза VGAM, при котором не было предпринято никаких попыток эмболизации или хирургического лечения, с отличными клиническими результатами.

Этот случай также подчёркивает возможность спонтанного тромбоза при VGAM, даже при отсутствии клинических симптомов, и подчёркивает важность регулярного наблюдения за пациентами с установленными сосудистыми мальформациями.

Ключевые слова

педиатрический, тромбоз, сосудистый, мальформация вены Галена

folia medica

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Case Report

A Giant Synovial Sarcoma of the Left Lung

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Abstract

Primary pulmonary synovial sarcoma is an extremely rare and aggressive neoplasm that primarily affects young people and has a poor prognosis. Establishing this diagnosis requires the exclusion of a wide number of other neoplasms with multimodal clinical, imaging, histological, immunohistochemical, and cytogenetic assessment. We present a case of synovial sarcoma of the left lung in a 44-year-old man, diagnosed immunohistochemically after left lower lobectomy with atypical resection of the 5th segment. Imaging, diagnostic workup, histological and immunohistochemical characteristics, surgical treatment, and prognosis are discussed.

Keywords

diagnostic imaging, lung, immunohistochemistry, synovial sarcoma, surgical treatment

INTRODUCTION

The majority of malignant lung tumors are of epithelial origin. Soft tissue tumors are far less common. Primary lung sarcoma is a rare and aggressive malignancy and lung metastases from extrapulmonary sarcomas are significantly more common than primary pulmonary synovial sarcoma (PPSS).

Because most mesenchymal malignancies have a benign analogue and certain epithelial tumors show sarcomatoid differentiation (e.g., renal cell carcinoma, melanoma), accurate histological diagnosis, including assessment of the size of the lesion, is critical.[1]

We present a case of synovial sarcoma of the left lung in a 44-year-old man, diagnosed immunohistochemically after left lower lobectomy with atypical resection of the 5th segment.

CASE REPORT

A 44-year-old male was admitted to the Department of Thoracic Surgery with pain in the back and the lateral part of the chest wall on the left, fatigue, and cough with mild hemoptysis for a month. He was a smoker and heavy drinker (100 ml daily). Physical examination was remarkable for decreased breath sounds on the left. The laboratory tests, pulmonary function tests, and arterial blood gas analysis showed no abnormalities.

On chest X-ray, a large heterogeneous mass in the left lower hemithorax was visualized. The computed tomography (CT) shows an ovoid lesion of 69×105×90 mm with heterodense structure up to 56 HE in the left lower lung with numerous gas deposits in the matrix, which was widely adhered to the chest wall without infiltrating it but with radicular and reticular interstitial changes around the lesion ***(Fig. 1)****.*

Fibrobronchoscopy (FBS) showed a deformed B8 segment with submucosal proliferation but was not diagnostic.

The surgical approach was through a left lateral thoracotomy in the 5th intercostal space ***(Fig. 2)****. A left lower lobectomy with atypical resection of the 5th segment, due to the proximity of the tumor to the major fissure, was performed. In the left lower lobe, a moderately dense tumor lesion of 100×70 mm was found. There was a presence of visibly*

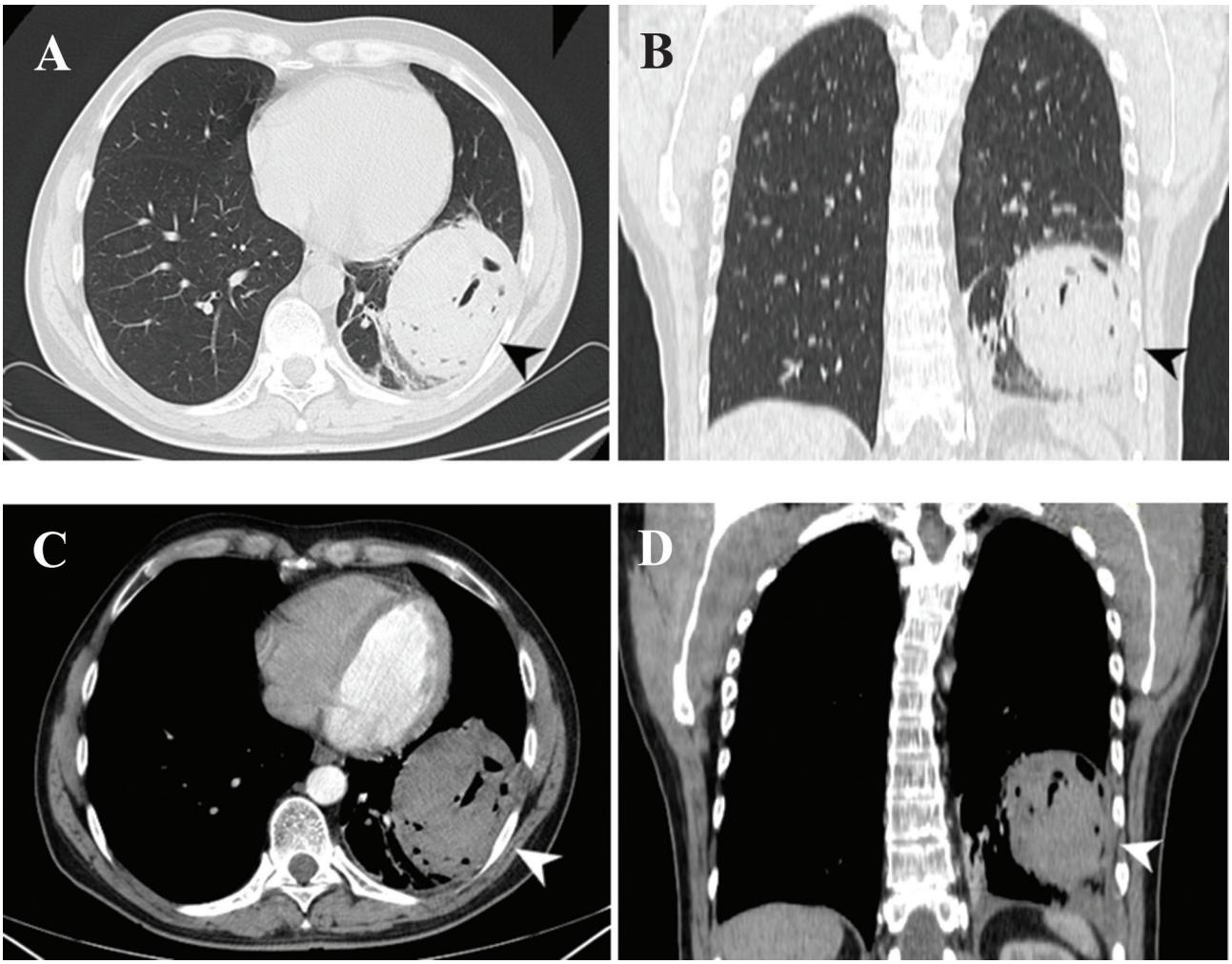

Figure 1. Axial and coronal CT images, visualizing left lower lobe synovial sarcoma.

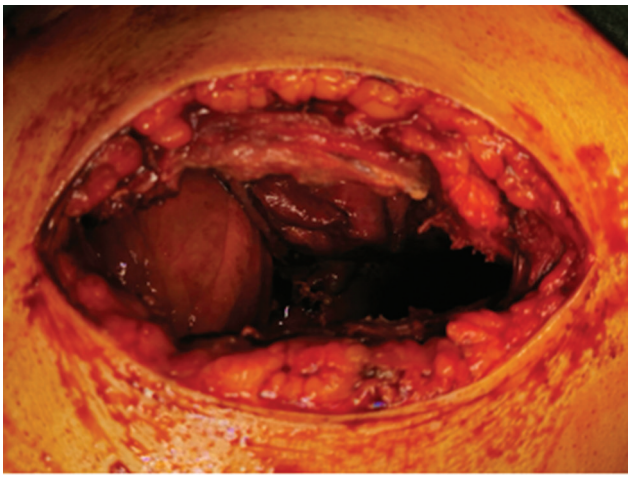

Figure 2. Intraoperative image of lateral muscle-sparing thoracotomy in the 5th intercostal space after left lower lobectomy and an atypical resection of the 5th segment.

pathological lymph nodes in the hilum and the pulmonary ligament, the first being located as a cuff around the hilum of the lower lobe. Thorough lymph dissection was performed at levels 5, 7, 9, and 10.

The specimen dissection showed cavitary tumor lesion which was filled with blood, and a large coagulum ***(Fig. 3)****. A pathohistological study demonstrated destruction of the lung parenchyma by tumor tissue (over 100 mm), represented by bundles of monotonous atypical spindle cells, focal myxoid stromal changes, areas of hemorrhages, extensive areas of necrosis, areas of cystic degeneration, and mitoses. The morphological finding refers to malignant intrapulmonary mesenchymal neoplasm. The bronchial stump margin was free of tumor infiltration. Seventeen lymph nodes were examined and all of them were without metastases.*

The immunohistochemical analysis showed positive expression in neoplastic cells for TLE-1 (1F5), CD99 (O13), and Bcl2 (bcl-2/100/D5); negative expression in tumor cells for AE1/AE3 (PANCK), desmin (DE-R-11), SOX-10; negative expression in neoplastic cells but positive in the vascular network in the tumor mass for CD34 (QBEnd/10), Actin (SMA) (αSMA-1) ***(Fig. 4)****.*

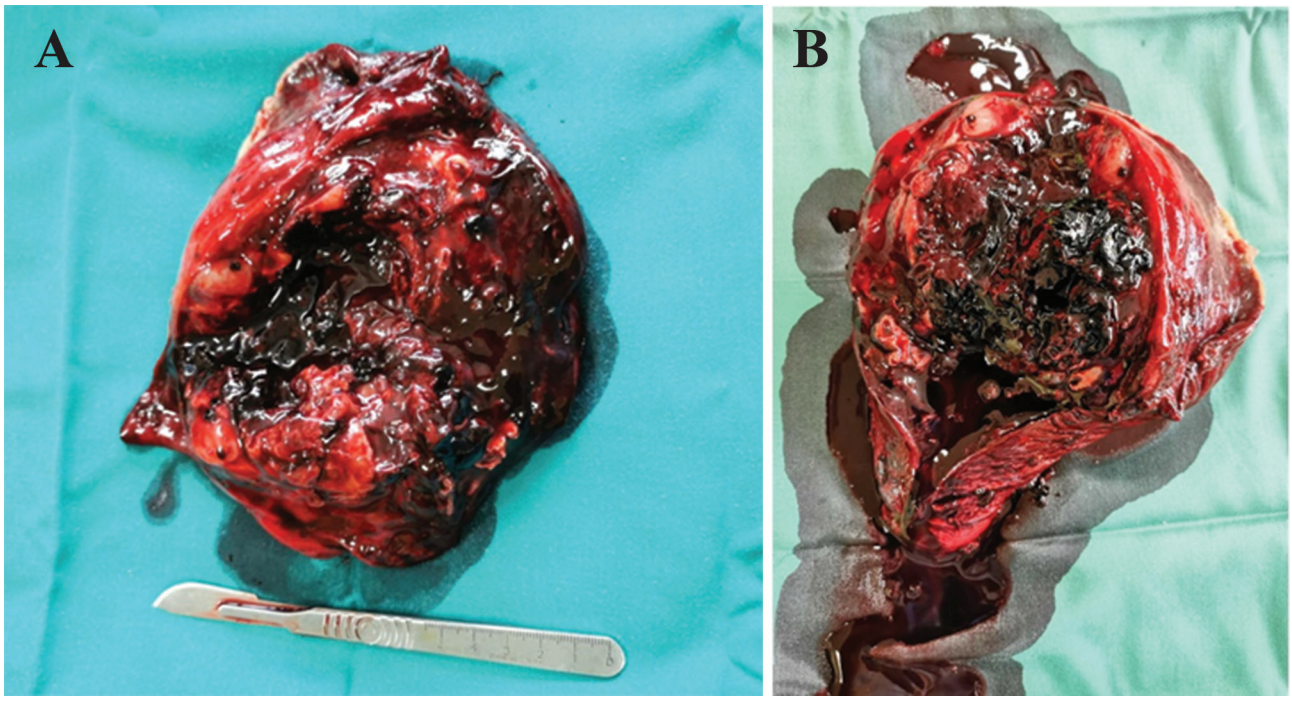

Figure 3. Macroscopic view of the tumor mass.

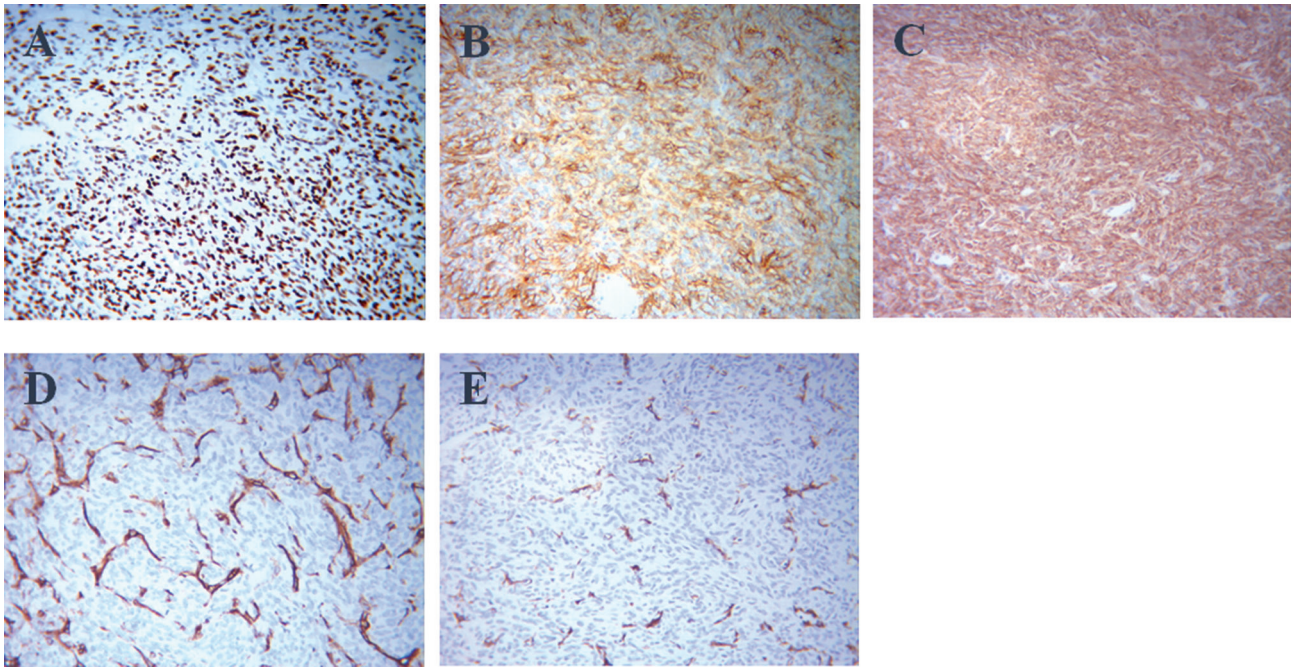

Figure 4. Photomicrograph of immunohistochemical staining, showing positive expression of neoplastic cells for TLE (**A**), CD99 (**B**), Bcl-2 (**C**), positive expression in the vascular network for CD34 (**D**), and Actin (**E**).

The immunoprofile of the neoplastic process in the lung favors synovial sarcoma (SS). Further fluorescent in situ hybridization (FISH) revealed monophasic fibrous (spindle cell) synovial sarcoma. According to the French Federation of Cancer Centers Sarcoma Group (FNCLCC) grading criteria, the tumor was graded as G3 (differentiation – score: 3; mitoses – score: 3; necrosis – score 2) and staged as T4N0M0R0.

The postoperative period was uneventful. The patient was discharged from the hospital on postoperative day 7 and was referred to an oncology clinic. One year after surgery, the patient is in excellent condition and without PET/CT data for recurrence.

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DISCUSSION

Primary lung sarcomas are uncommon and aggressive malignancies with similar treatment and prognosis compared to other soft tissue sarcomas.[2] Lung sarcomas account for only 0.1%–0.5% of all primary pulmonary malignancies[3] with a 5-year overall survival rate of 50%[4]. The most common variants of lung sarcoma are malignant fibrous histiocytoma and synovial sarcoma.[4]

Synovial sarcoma is a mesenchymal spindle cell tumor that accounts for 5%–10% of all soft tissue sarcomas and is defined by a pathognomonic chromosomal translocation t(X;18)(p11.2;q11.2).[3] It arises from immature mesenchymal elements rather than from the synovium.[4] SS is most common in the extremities (more than 90%), especially near large joints, but the head and neck, lungs, heart, mediastinum, and the abdominal wall could also be primary localizations.

PPSS usually affects young patients (median age between 31 and 50 years).[3] Patients with pulmonary synovial sarcoma usually report pleuritic chest pain (50.0%), cough (28.6%), palpable mass (7.1%), and dyspnea (7.1%).[5] The differential diagnosis is very wide, but primary lung cancer should be excluded. The absence of significant lymphadenopathy in a young adult with a relatively large ovoid tumor favors PPSS rather than primary lung cancer.[5]

PPSS presents on chest X-ray as a homogenous parenchymal formation with a well-circumscribed rounded or lobulated border, a pleural mass, and partial or complete opacification of the hemithorax, with dimensions often more than 7 cm^3. On CT scan, these tumors show commonly heterogeneous enhancement with necrotic or cystic areas, vessels in the lesion, calcifications, tumor rupture, pleural/chest wall extension, and pleural effusion.[5]

PPSS is aggressive, possibly due to its anatomic location and large tumor size generally resulting in insufficient resection and high proliferative activity.[6] Treatment of PPSS includes surgical resection followed by chemotherapy or radiation therapy. Surgical treatment is the main option as there are no other standardized approaches. Adjuvant chemotherapy improves the time to local recurrence and recurrence-free survival and tends to improve overall survival.[4] Due to the lack of preoperative morphological diagnosis and the giant size of the mass, we undertook open surgery through lateral muscle-sparing thoracotomy. A left lower lobectomy with atypical 5th segment resection was performed because of the lesion's size and proximity to the large interlobar fissure.

Primary pleuropulmonary SS seems to be with higher local aggressiveness than its soft-tissue counterpart, and this could be due to the difficulties in achieving wide surgical margins combined with a late presentation.[7] Patients with PPSS have a poor prognosis, with a 5-year overall survival rate of 50%.[4] Male gender, large tumor size, extensive tumor necrosis, neurovascular invasion, high histological grade, and mitotic rate are poor prognostic factors.[8]

CONCLUSIONS

PPSS is a rare and aggressive neoplasm that primarily affects young people and has a poor prognosis. Establishing this diagnosis requires the exclusion of a wide number of other neoplasms and a wide work-up plan. The method of choice for treatment is mainly surgical with subsequent adjuvant chemo- or radiation therapy. Due to the risk of recurrence, these patients should be carefully monitored.

Ethical Approval

Ethical approval was not necessary as this is not a clinical study. The research meets all applicable standards concerning the ethics of experimentation and research integrity, and the following is being certified/declared true. No identifiable images or information were used.

Informed Consent

Informed consent was obtained for all the procedures as a part of the patient's hospital stay.

Consent for publication

All authors read and agreed with the manuscript.

Availability of data and material

All figures and data are readily available.

Conflict of Interest

None of the authors of this paper has a financial or personal relationship with other people or organizations that could inappropriately influence or bias the content of the paper.

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Гигантская синовиальная саркома левого лёгкого

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Резюме

Первичная синовиальная саркома лёгких — крайне редкое и агрессивное новообразование, поражающее преимущественно молодых людей и имеющее плохой прогноз. Установление этого диагноза требует исключения широкого числа других новообразований с помощью мультимодальной клинической, визуализационной, гистологической, иммуногистохимической и цитогенетической оценки. Представлен случай синовиальной саркомы левого лёгкого у мужчины 44 лет, диагностированный иммуногистохимически после нижней лобэктомии слева с атипичной резекцией 5-го сегмента. Обсуждаются методы визуализации, диагностическое обследование, гистологические и иммуногистохимические характеристики, хирургическое лечение и прогноз.

Ключевые слова

диагностическая визуализация, лёгкие, иммуногистохимия, синовиальная саркома, хирургическое лечение

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Case Report

Intrathoracic Non-Tuberculous Mycobacteriosis with Endobronchial Lesion in a Child Aged 11 with HIV Infection Diagnosed by Bronchoscopic Biopsy, EBUS-TBNA, and Confocal Laser Endomicroscopy

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Abstract

The diagnosis of intrathoracic non-tuberculous mycobacteriosis (NTM) is challenging. We report a case of a pediatric pulmonary NTM with endobronchial lesion and lymphadenitis in a child with HIV infection diagnosed by bronchoscopic biopsy, EBUS-TBNA and probe-based confocal laser endomicroscopy (pCLE). The pCLE showed a large number of highly fluorescent cells and zones of density and disorganized elastin fibers at alveolar areas. A combination of diagnostic endoscopic procedures is required to establish the diagnosis of NTM.

Keywords

biopsy, bronchoscopy, child, confocal laser endomicroscopy, EBUS-TBNA, endobronchial, HIV-infection, non-tuberculous mycobacteriosis, optical biopsy

INTRODUCTION

Non-tuberculous mycobacteria (NTM) are ubiquitous in the environment, both in soil, fresh and salt-water sources.[1] Pulmonary NTM infection is probably caused by inhalation of aerosol from municipal or private water systems.[2] Furthermore, there is the *Mycobacterium avium* complex (MAC) detected in the shower biofilms that is also known to cause NTM infection.[3]

NTM infections in children usually present with cervical lymphadenitis. The annual incidence of NTM has been estimated to be 0.84 NTM infections per 100 000 children.[2] Pediatric pulmonary NTM infection is a rare condition which is usually seen in immunocompromised patients, such as those with cystic fibrosis, hematologic malignancy, congenital immunodeficiency, or acquired immunodeficiency syndrome caused by HIV infection.[4,5] The diagnosis of intrathoracic NTM is challenging and often needs a variety of bronchoscopic methods to obtain respiratory samples for microbiological analysis.[2,3] We report here a case of pediatric thoracic non-tuberculous mycobacteriosis with endobronchial lesion and lymphadenitis diagnosed

by bronchoscopic biopsy, EBUS-TBNA and confocal laser endomicroscopy.

CASE REPORT

An 11-year-old girl was admitted to our clinic with a history of dry cough, fever (up to 38.9°C), weakness, loss of appetite, and night sweats. A diagnosis of HIV infection with severe immunosuppression (CD4 count of 6 cells, viral load 2,700,000 copies/ml) was established in June 2022, when the child was hospitalized due to severe bronchitis and started on antiretroviral therapy. A year ago, when HIV infection was detected in the mother, the child was not examined then. From July 2022 to October 2022, she was treated in several hospitals with a diagnosis of an approved new coronavirus infection (COVID-19). The MSCT scans made in October 2022 revealed a small infiltrate with a cavity in six segments of the right lung and severe intrathoracic lymphadenopathy. Tuberculosis was suspected, but the T-SPOT and multiple sputum tests for mycobacteria were negative. After negative nasopharyngeal swabs in November 2022, the child was transferred to the differential diagnosis department in our clinic. The control MSCT scans showed increase of lymphadenopathy and infiltrates in both lungs, predominantly in the right upper lobe ***(Fig. 1)****.*

We decided to do a bronchoscopy with transbronchial lung biopsy and endobronchial ultrasound transbronchial needle aspiration of the lymph nodes. We used also the bronchoscopic probe-based confocal laser endomicroscopy (pCLE) method to examine in vivo the airways and alveoli at microscopic level.[6] *The bronchoscopy was performed under general anesthesia using a laryngeal mask. It showed many grey contact bleeding granulations in the area of the intermediate bronchus and the carina of the upper lobe bronchus, which narrowed the lumen by half to two thirds of its diameter* ***(Fig. 2)****. The mucosa of the upper lobe and the 1, 2, and 3 segmental bronchi was edematous and thickened, and the lumen of the bronchi was narrowed by three-fourth of the diameter with the flow of viscous secret from the lumen. The probe-based confocal laser endomicroscopy was performed using the Cellvizio system and 1.4-mm Alveoflex probe (Mauna Kea Technologies). The probe was inserted through the instrumental channel of the bronchoscope to 7, 4, 5, 1, 2, and 3 segmental bronchi. At all alveolar areas, a large number of highly fluorescent cells 20–30 μm in diameter were noted, and in the upper segments, zones of density and disorganized elastin fibers were revealed* ***(Figs 3A, 3B, 3C)****.*

We performed an endobronchial biopsy of the granulations in the upper lobe bronchus and a transbronchial biopsy of segments 1, 2, and 3. We also obtained bronchial washings. Then EBUS-TBNA of the lymph nodes in the region of interlobar carina was performed. Large lymphatic nodules with signs of necrosis were found in an ultrasound study. Samples for histological, PCR examination and culture were taken.

The bronchial washings yielded no useful results. Histological examination of endobronchial biopsy samples revealed fragments of the bronchial wall with granulation tissue and areas of necrosis, mild lymphoid infiltration, and two epithelioid-cell granulomas with giant Langhans cells and one acid-fast bacillus ***(Fig. 4A)****. Pathomorphological examination of the transbronchial biopsy samples showed fragments of lung tissue with focal mild fibrosis, granulation tissue, hemorrhage, the presence of fibrin, and areas of necrosis in a part of the alveoli, and no acid-fast bacilli* ***(Fig. 4B)****. A histological examination of the TBNA samples of lymphatic nodules revealed fragments of lymph node tissue and coagulation necrosis with focal leukocyte infiltration with no acid-fast bacilli* ***(Fig. 4C)****.*

But the PCR examination and culture (BACTEC) from lymph node samples detected NTM of Mycobacterium avium complex. The patient was started on a treatment for the MAC infection.

DISCUSSION

Thoracic non-tuberculous mycobacteriosis is a rare disease in children, usually seen in immunodeficient pa-

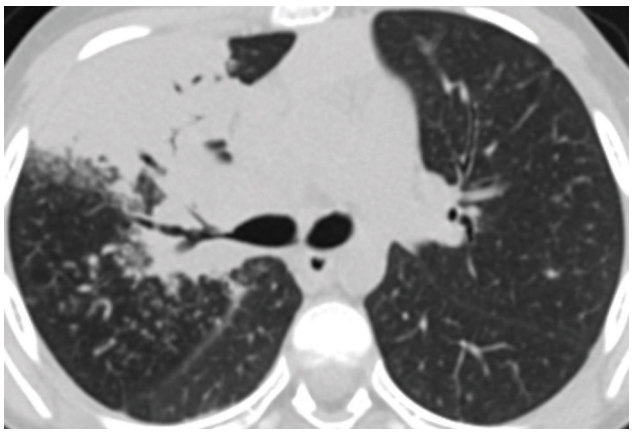

Figure 1. Infiltrative lesion of the upper lobe of the right lung and increase of paratracheal (2R up to 18×20 mm), and bifurcation (7 to 13×12 mm) lymph nodes.

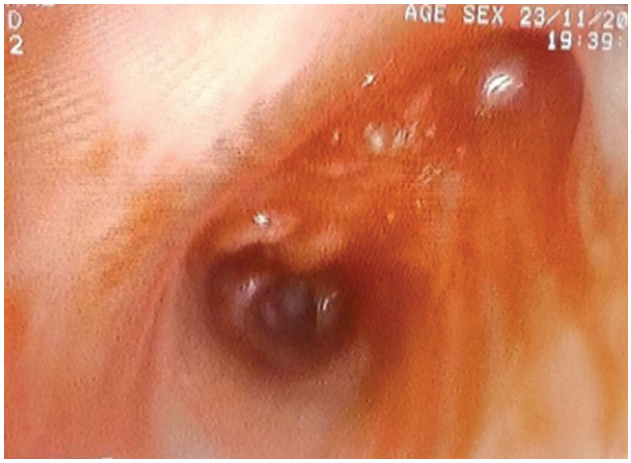

Figure 2. A large number of actively bleeding granulations in the region of interlobar carina at bronchoscopy.

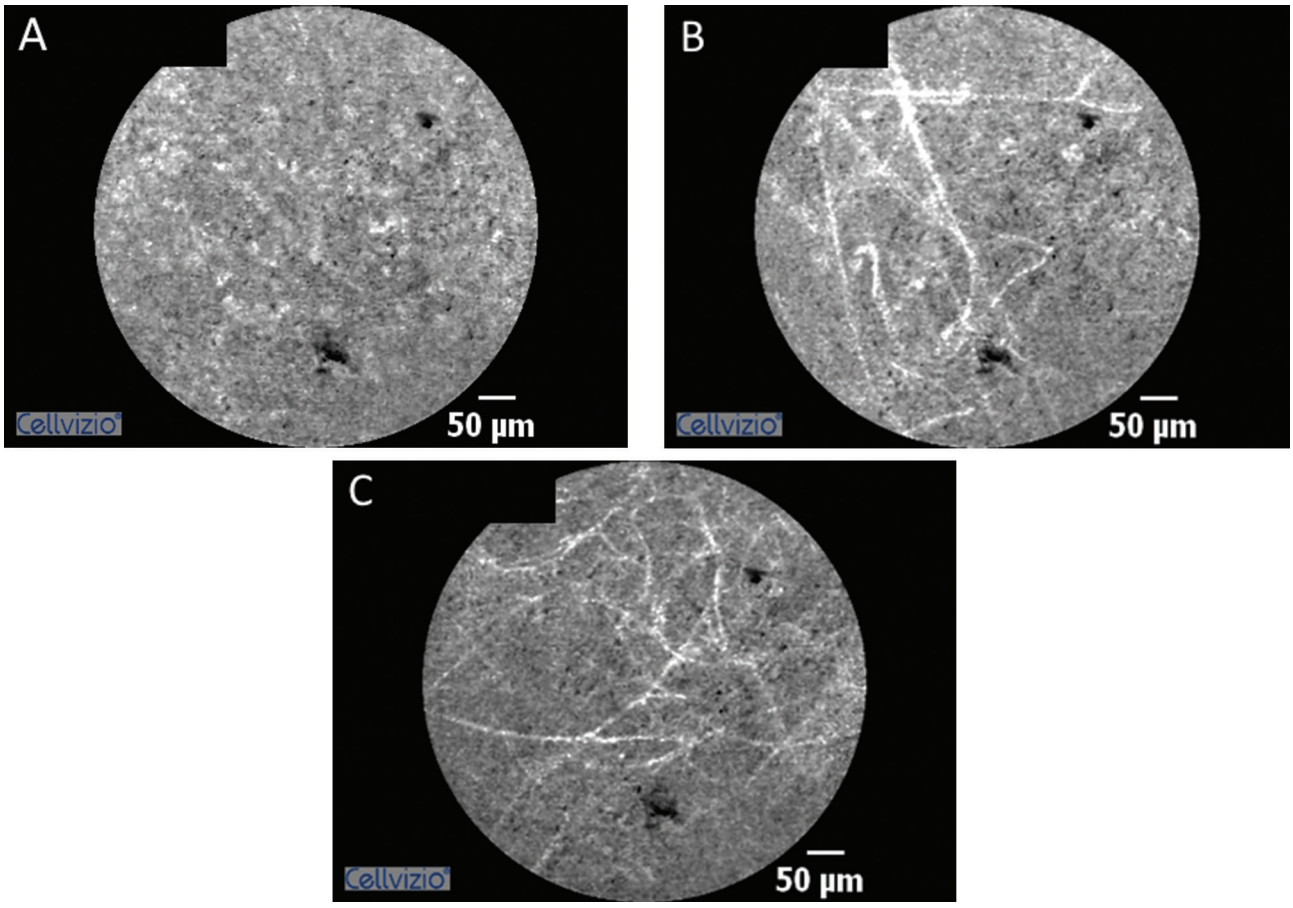

Figure 3. pCLE images in the alveolar areas of right upper lobe segments. **A**. A large number of highly fluorescent cells; **B**. A zone of disorganized alveolar fibers and fluorescent cells; **C**. A zone of density of alveolar elastin fibers.

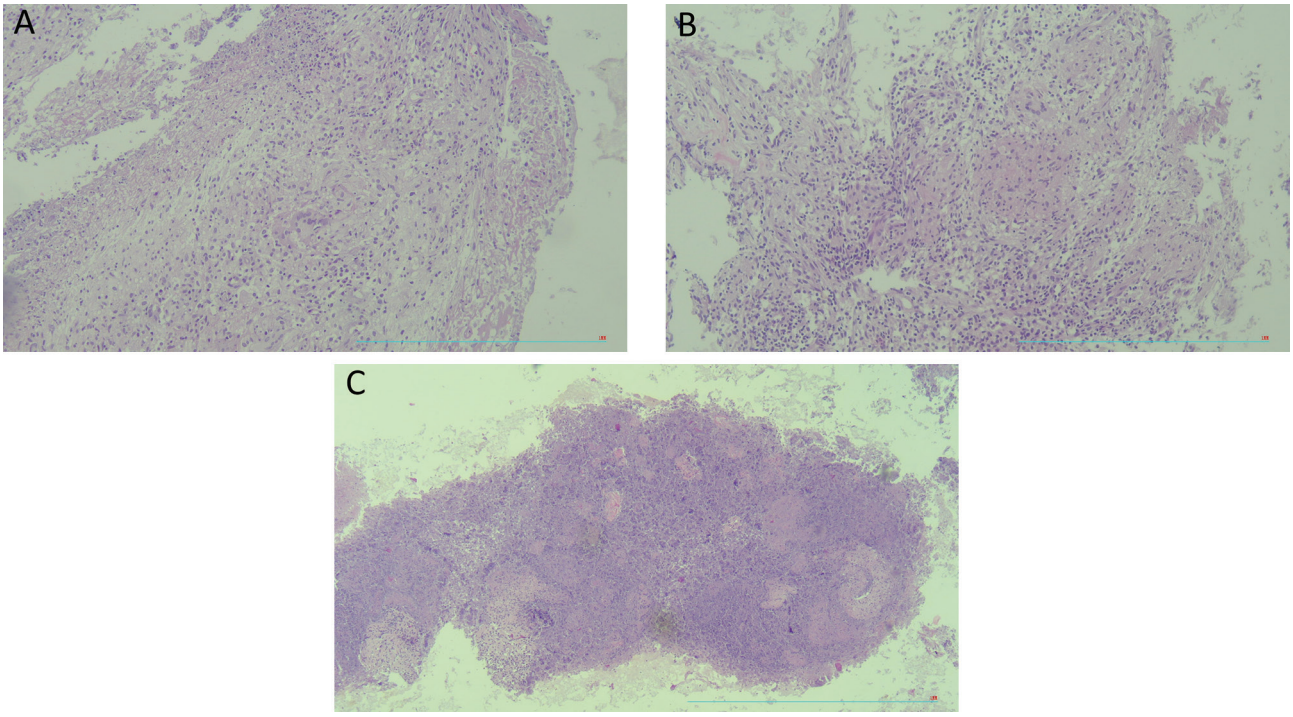

Figure 4. Histological examination of biopsy samples. **A**. Granulation tissue, epithelioid cell granuloma with Langhans cells on the bronchial wall (hematoxylin and eosin, ×100); **B**. Epithelioid cell granulomas in the area of granulation tissue in the lung (hematoxylin and eosin, ×100); **C**. Fragments of coagulative necrosis with focal leukocyte infiltration, nuclear detritus in lymphoid tissue (hematoxylin and eosin, ×40).

tients with HIV infection but could occur in healthy subjects as well.[2,4,5]

An endobronchial lesion of NTM infection is even rarer, requiring a differential diagnosis with tuberculosis and malignancy.[1,3,4] It is believed that the endobronchial granuloma is the result of the reaction to the penetration of the bronchial wall by material from adjacent lymphadenitis.[1] The key to the diagnosis of intrathoracic non-tuberculous mycobacteriosis is identification of NTM. Positive cultures of sputum are difficult to obtain in the pediatric patient. Therefore, endoscopic techniques for obtaining material for culture are important. We found no data on the sensitivity and specificity of the bronchoscopic techniques in NTM as non-tuberculous mycobacteriosis of the lungs in children is a rather rare pathology. EBUS-TBNA is considered a safe and highly effective (diagnostic yield is about 98%) tool for diagnosing lymphadenopathy in children.[7] Since NTM in culture grows for a long time, the PCR analysis is also important for faster identification of the infection.[2]

In this case, we used three types of sampling - endobronchial, transbronchial lung, and transbronchial needle biopsy of lymph nodes. And only the EBUS-TBNA allowed us to identify the NTM by PCR and culture examination. This demonstrates that we should use all available tools to diagnose such a complex disease as intrathoracic NTM infection.

We also used in this case the so-called 'optical biopsy' – the probe-based confocal laser endomicroscopy. Using pCLE to diagnose lung diseases in children is reported only in a few publications.[8,9] To the best of our knowledge, we present here the first pCLE pictures of a child with pulmonary NTM infection in the scientific literature. Earlier, our group published an article about the comparison of pCLE pictures between adult patients with tuberculosis and NTM infection. The key differences were the existence of fluorescent alveolar cells in tuberculosis patients and their absence in NTM.[10] But in the present case, we can see in a child with NTM a large number of fluorescent cells in all investigated segments. This feature requires additional investigation; however, we believe that the presence of fluorescent cells in alveoli may be connected with severe cellular and necrotic inflammation in lung tissue or/and the patient's positive HIV status.

CONCLUSIONS

Children's intrathoracic non-tuberculous mycobacteriosis is a challenging condition to diagnose, necessitating the use of a range of tools such as endobronchial, transbronchial, and EBUS-TBNA biopsies. Confocal laser endomicroscopy is a promising diagnostic tool that needs more research in children with varied clinical disorders.

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Competing Interests

The authors have declared that no competing interests exist.

Author contributions

I.V.: study conception, data analysis and interpretation, writing of the manuscript; I.M.: study conception and design, data collection, analysis and interpretation, critical revision with the introduction of valuable intellectual content, writing of the manuscript, R.S.: study conception, data analysis and interpretation, critical revision with the introduction of valuable intellectual content; T.A.N.: data collection, analysis and interpretation, drafting of the manuscript, critical revision with the introduction of valuable intellectual content; V.Z.: data collection, analysis and interpretation, drafting of the manuscript, critical revision with the introduction of valuable intellectual content; P.Y.: research supervision, critical revision with the introduction of valuable intellectual content, approval of the final version of the manuscript for publication.

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Внутригрудной нетуберкулёзный микобактериоз с эндобронхиальным поражением у ребёнка 11 лет с ВИЧ-инфекцией, диагностированной с помощью бронхоскопической биопсии, EBUS-TBNA и конфокальной лазерной эндомикроскопии

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Резюме

Диагностика внутригрудного нетуберкулёзного микобактериоза (НТМБ) является сложной задачей. Мы сообщаем о случае детского лёгочного НТМБ с эндобронхиальным поражением и лимфаденитом у ребёнка с ВИЧ-инфекцией, диагностированной с помощью бронхоскопической биопсии, EBUS-TBNA и зондовой конфокальной лазерной эндомикроскопии (pCLE). pCLE показал большое количество высоко флуоресцентных клеток и зоны плотности и дезорганизованных волокон эластина в альвеолярных областях. Для установления диагноза НТМБ необходима комбинация диагностических эндоскопических процедур.

Ключевые слова

биопсия, бронхоскопия, ребёнок, конфокальная лазерная эндомикроскопия, EBUS-TBNA, эндобронхиальная, ВИЧ-инфекция, нетуберкулёзный микобактериоз, оптическая биопсия

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Case Report

Repair of Type II Paraesophageal Hernia with Nissen Fundoplication in a Patient with Von Willebrand Disease and Spondylolisthesis – a Clinical Case Report

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Abstract

Hiatal hernias continue to be fairly common in clinical practice. However, the variety of different symptoms presented by patients may hinder establishing the ultimate diagnosis. Nevertheless, currently, the diagnosis of hiatal hernia can be easily established, based on barium swallow radiography. We would like to present a clinical case report of a patient with complex medical history, including von Willebrand disease, degenerative spinal disease, and chronic sinusitis, who was finally diagnosed with hiatal hernia and treated with a standard laparoscopic Nissen fundoplication. Our case focuses on the significance of comorbidities on patients' symptoms, which sometimes may mislead the therapeutic process.

Keywords

hiatal hernia, general surgery, paraesophageal hernia, Nissen fundoplication

INTRODUCTION

Hiatal hernia (HH) is defined as the condition when an abdominal organ, most commonly the stomach, is moved through the esophageal hiatus into the mediastinum and cannot be held up in the abdominal cavity. Consequently, the stomach is constantly pulled up by the esophagus while swallowing or in conditions of increased abdominal pressure. Thus, several conditions which may lead to the development of HH were reported, including obesity[1], pregnancy[2], genetic predispositions[3], collagen diseases[4] or even extensive physical training[5].

Clinically, four types of HH can be distinguished based on the topographic relations between gastroesophageal junction (GEJ) and diaphragm.[5] Type I, also called the sliding hernia, is the most common one and refers to the state when only the GEJ is displaced superiorly to the diaphragm, into the mediastinum; conversely, in type II hiatal hernia, the gastric fundus is displaced into the thoracic cavity and the GEJ remains in its anatomical location. Type III hiatal hernia is also called mixed type, due to the fact that it contains elements from both types I and II. Finally, type IV of this condition occurs when other organs such as the colon or the spleen move into the thorax from the abdominal cavity. Types II-IV are also known as the paraesophageal hernias (PEH).

HH is clinically significant due to its close relation to the pathophysiology of gastroesophageal reflux disease

(GERD).[6] It is important to highlight that patients with the coexistence of GERD and HH have worse overall prognosis and response to the medical treatment.[7] The patients with HH mainly present the typical GERD-related symptoms; however, the range of reported symptoms may vary. Therefore, we would like to introduce a case report of a patient presenting with type II PEH, which was accompanied by unusual symptoms and various comorbidities that made the diagnosis and treatment challenging.

CASE PRESENTATION

The case involves a 51-year-old obese male who initially presented with type 1 von Willebrand disease (vWD) and a long history of degenerative spinal disease in the form of spondylolisthesis at the L5-S1 level of the lumbar spine with bilateral spondylolysis at L5 and thoracic scoliosis. The patient reported hoarseness, cough, and rhinitis, which would appear after longer episodes of speaking and sitting. The symptoms he originally presented with were thought to be the consequence of chronic sinusitis; however, the patient subsequently started reporting exacerbation of spinal pain in the thoracolumbar region on the left side after long episodes of sitting, which would be relieved by walking. The computed tomography (CT) confirmed the known spinal findings as well as the degeneration of the intervertebral joints. The physical therapy and rehabilitation dismissed the reported ailments temporarily as pain symptoms started to happen, more frequently, and postprandially. The patient reported their localization in the left thoracolumbar segment, with radiation to the thorax and epigastrium. Finally, the pain appeared daily. Additionally, during the ailments, the patient reported the presence of heart palpitations, dyspnea, shallow breath, and dysphagia with belching. The symptoms were severe enough to cause difficulties in sitting and lying as the patient was forced to establish the standing position, keep a shallow breath, or take drugs such as paracetamol, metamizole, and/or drotaverine in order to relieve pain. Initially, the symptoms persisted for a dozen minutes. The physical examination revealed no abnormalities. Furthermore, laboratory results were also within the normal ranges. Ultimately, chest X-ray examination was performed and suggested the presence of HH ***(Fig. 1A)****. Interestingly, both the prior X-ray examination and previous CT did not detect the mentioned finding. Finally, the X-ray with contrast* ***(Fig. 1B)*** *of the upper part of the digestive tract and the chest CT* ***(Fig. 1C)*** *confirmed the presence of PEH measuring approximately 77×90×59 mm.*

Standard endoscopy of the upper part of the digestive tract revealed PEH with intact GEJ and no lesions in the mucosal lining of the esophagus and stomach were found. Subsequently, the patient was consulted with a hematologist for the type 1 vWD with familial occurrence, when the desmopressin test was performed. Desmopressin was administered intravenously in a dose of 0.3 mcg/kg and it provoked a relatively good hemostatic response. After completing the diagnostic evaluation, the patient was scheduled for surgery. In order to provide a hematological protection of the surgical treatment, the use of desmopressin in infusion was recommended. Ultimately, laparoscopic Nissen fundoplication was performed with the suture of diaphragm crura. The procedure went uneventful. The patient was discharged in good condition and reported complete recovery and relief of symptoms during the routine 1-year follow-up.

DISCUSSION

Paraesophageal hernia is a relatively common disorder with a slight female predominance.[8] In terms of etiology, PEH can be either congenital or acquired with a higher prevalence in elderly due to the age-related loss of elasticity of surrounding tissues and muscle weakness of the superior border of the abdomen. The most common obstructive symptoms are dysphagia, postprandial fullness, early satiety, vomiting and/or epigastric pain. The patient may also complain from chest pain or shortness of breath as a result of the compression of structures in the thoracic cavity caused by the expanding stomach. Less frequently reported symptoms are Cameron's ulcers, gastric volvulus,

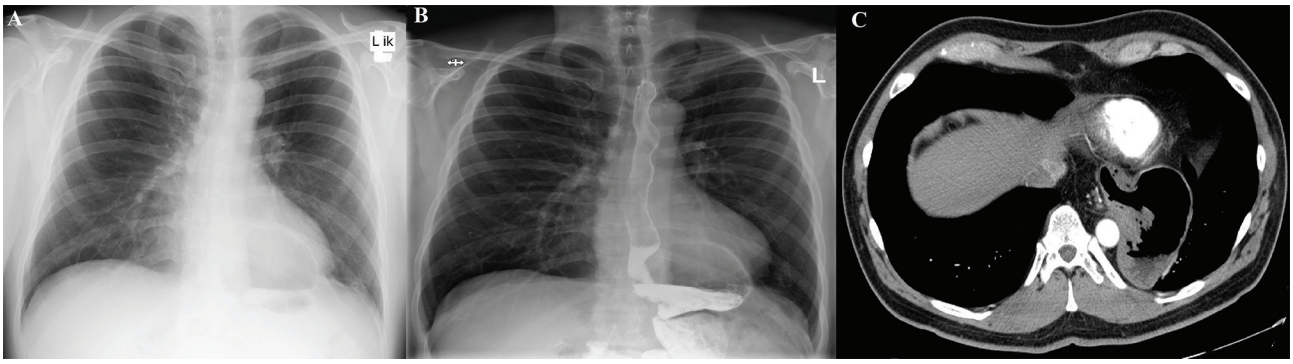

Figure 1. A. The chest X-ray examination in posteroanterior view revealing the presence of hiatal hernia, measuring 95×60 mm, seen as the brightening region which blurred the contour of the diaphragm; **B**. The chest radiograph with the barium swallow test in the posteroanterior view; **C**. Computed tomography of the chest in the axial projection revealing the hiatal hernia, measuring 77×90×59 mm with the significant part of the stomach in the thoracic cavity.

and iron-deficiency anemia. Interestingly, the presented patient did not report typical symptoms, as he complained mainly of spinal pain, although, the recent literature suggests that patients with degenerative spinal diseases, such as scoliosis, are at greater risk of developing HH.[9] According to SAGES guidelines, the preferred surgical approach for managing HHs is laparoscopic Nissen fundoplication.[10] Various literature reviews and studies advocate the superiority of laparoscopic fundoplication, over the open classical counterpart in terms of efficacy, mortality, and cosmetic outcomes.[11] Interestingly, a correlation between the increasing size of the HH and a higher risk of recurrence was also revealed.[12] Esophageal hiatal hernias have been reported to affect 10% to 50% of the population, increasing with age and with a slight female predilection; therefore, if these patients have upper dyspeptic symptoms, we should suspect them.[13,14]

CONCLUSION

Hiatal hernia in patients with complex medical history may mislead the diagnostic process. Thorough analysis of existing comorbidities' symptoms and additional investigations would enhance proper diagnosis for the right and timely treatment.

Author contributions

G.F.: conceptualization, writing the original draft; K.G.: writing the original draft; W.F.: writing the original draft; A.P: conceptualization, writing and editing; M.S.: conceptualization, supervision, writing and editing.

Conflict of Interest

The authors declare no conflict of interest.

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Пластика параэзофагеальной грыжи II типа с помощью фундопликации по Nissen у пациента с болезнью Von Willebrand и спондилолистезом – клинический случай

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Резюме

Хиатальные грыжи продолжают довольно часто встречаться в клинической практике. Однако разнообразие различных симптомов, предъявляемых пациентами, может затруднить постановку окончательного диагноза. Тем не менее, в настоящее время диагноз хиатальной грыжи можно легко установить на основании рентгенографии с приёмом бария. Мы хотели бы представить отчёт о клиническом случае пациента со сложной историей болезни, включая болезнь von Willebrand, дегенеративное заболевание позвоночника и хронический синусит, у которого наконец была диагностирована хиатальная грыжа и проведена стандартная лапароскопическая фундопликация по Nissen. В нашем случае основное внимание уделяется значимости сопутствующих заболеваний для симптомов пациентов, которые иногда могут ввести в заблуждение терапевтический процесс.

Ключевые слова

хиатальная грыжа, общая хирургия, параэзофагеальная грыжа, фундопликация по Nissen

folia medica

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Case Report

A Rare Clinical Case of Extra-gastrointestinal Stromal Pancreatic Tumor

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Abstract

Extra-gastrointestinal stromal tumors arising from the pancreas are extremely rare. To date, just over 30 cases have been described in the world literature. A clinical observation of a 67-year-old patient with dull epigastric pain and a large cystic solid neoplasm instrumentally identified as an extra-gastrointestinal stromal tumor of the head of the pancreas is presented. The volume of surgical intervention consisted of pancreatogastroduodenectomy and right-sided hemicolectomy, since tumor invasion into the transverse mesocolon was detected intraoperatively. The final diagnosis of extra-gastrointestinal stromal sarcoma of the head of the pancreas with invasion into the mesocolon pT4N0M0, stage IIIb was made on the basis of histopathology and immunohistochemistry results.

Extra-gastrointestinal stromal pancreatic tumors require careful differential diagnosis with other large abdominal masses. Timely diagnosis and use of modern treatment algorithms make it possible to avoid further disease progression.

Keywords

CD117, extra-gastrointestinal stromal tumor, immunohistochemistry, pancreas, targeted therapy

INTRODUCTION

Gastrointestinal stromal tumors (GIST) are the most common mesenchymal neoplasms of the gastrointestinal tract. They can occur in any segment, extending from the esophagus to the rectum, but in most cases, GIST are localized in the stomach and small intestine.[1-3] A group of stromal tumors similar to GIST in histological and immunohistochemical characteristics, but detected outside the gastrointestinal tract (mainly in the retroperitoneal space, omentum and mesentery), was called "extra-gastrointestinal stromal tumors" (EGIST).[3] There have been incidental cases of the of EGIST developing from the liver, kidneys, gallbladder, bladder, prostate, abdominal wall, pleura, mediastinum, etc.[2] EGISTs of the pancreas are extremely rare. To date, just over 30 cases have been described in the world literature.

CASE PRESENTATION

A 67-year-old female patient was routinely admitted to the Department of Hepatopancreatobiliary Surgery No. 50 of Botkin Hospital for additional examination and further

decision on treatment strategy. She had been having a dull pain in the epigastrium and a bad taste in her mouth for two months before hospitalization. A month before she had been admitted to the hospital, abdominal CT scan with IV contrast was performed, which revealed a mass in the epigastrium and mesogastrium on the right with a size of about 154×136×117 mm, intimately adjacent to the duodenum and transverse colon ***(Fig. 1)****.*

Upon admission, the patient did not have any symptoms, physical examination was unremarkable. Blood examination revealed mild anemia (hemoglobin, 99 g/l), biochemical indicators were without significant deviations, the main tumor markers levels were within the reference values ***(Table 1)****.*

During hepatobiliary ultrasound, a retroperitoneal mass in the right epi- and mesogastrium at the level of the head of the pancreas was found. It had an irregular shape with a cystic-solid structure and uneven clear margins, up to 150×135×125 mm in size. The central part of the mass was represented by heterogeneous liquid with an echogenic suspension. The walls of the cyst had increased echogenicity and were up to 10 mm in width. Septa were visualized in the cyst cavity. Doppler ultrasound revealed moderate vascularization along the margins. No signs of pancreatic or biliary hypertension were detected. Therefore, there was a volumetric partially cystic retroperitoneal mass on the right, most likely originating from the head of the pancreas. It was hard to differentiate between a cystic tumor and a post-necrotic cyst ***(Fig. 2)****.*

The patient underwent open surgery. Intraoperatively, a giant retroperitoneal mass was found in the region of the head of the pancreas and the uncinatum process. During the puncture of the mass, about 600 ml of hemorrhagic content

Table 1. Tumor markers levels in the blood serum

Tumor marker	Result	Unit of measurement	Reference values
AFP	3.58	ng/ml	0.2–10
CEA	384	ng/ml	0.2–5
CA 19-9	1.6	U/ml	0–35
CA 125	8,4	U/ml	0–35
CA 15-3	9.7	U/ml	0–31.3
CA 72-4	5.62	U/ml	0–6

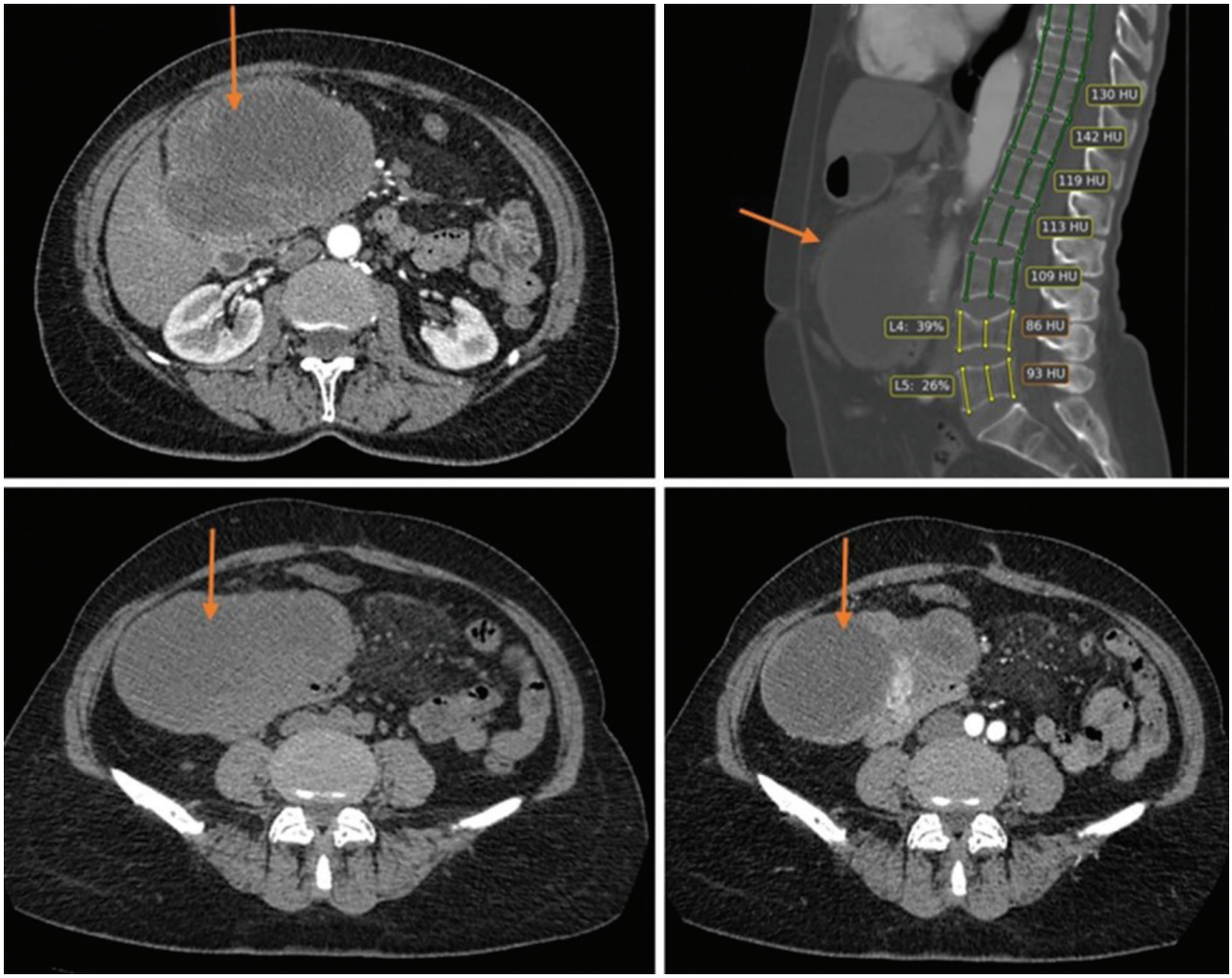

Figure 1. CT image of the mass formation of the abdominal cavity.

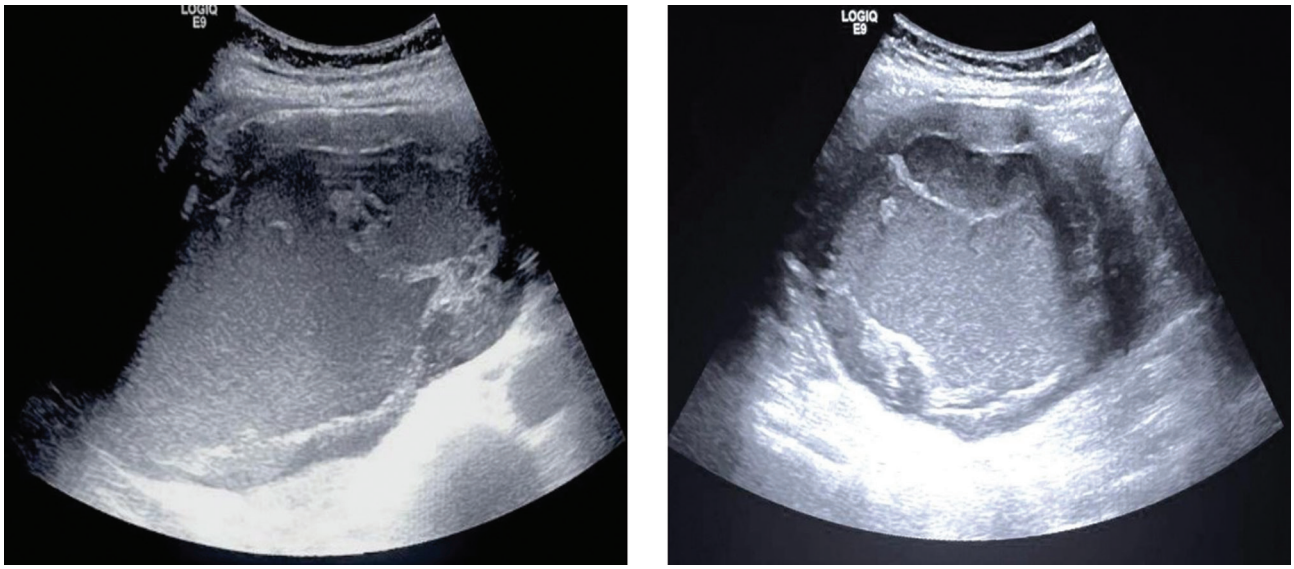

Figure 2. Ultrasound image of a retroperitoneal complex cyst.

was obtained. The adjacent visceral and parietal peritoneum walls were without signs of carcinomatosis. The tumor invaded the transverse mesocolon and compressed the duodenum. There were no signs of vascular invasion. Taking into account the localization of the mass and invasion into the transverse mesocolon, pancreatogastroduodenectomy was performed in combination with right-sided hemicolectomy, side-to-side ileotransversoanastomosis, pancreaticojejunoanastomosis, hepaticojejunoanastomosis, end-to-side entero-enteroanastomosis, and gastroenteroanastomosis ***(Fig. 3)****.*

During pathomorphological examination, the tumor node macroscopically was with cystic transformation. Its walls were grey, lobular, with foci of hemorrhages, up to 1.5-2.0 cm in width. Blood clots were seen in the cyst lumen. Microscopically, the tumor was represented by intertwining spindle and epithelial cells with edema, foci of hemorrhage and necrosis. Resection margins were without tumor growth (R_0). Immunohistochemical analysis (IHC) showed presence of CD34 and CD117 and absence of S100 and DOG1 markers. Ki67 proliferative activity index was 15%. Based on the intraoperative data, tumor morphology and immunophenotype, the final diagnosis was extra-gastrointestinal stromal sarcoma of the head of the pancreas with invasion into the mesocolon, epithelioid-spindle cell variant, $pT_4N_0M_0$ stage IIIb ***(Fig. 4)****.*

In the early postoperative period, no signs of fluid accumulation or inflammatory infiltrates in the abdominal cavity were detected; however, delayed food passage through the stomach was noted. Esophagogastroduodenoscopy identified

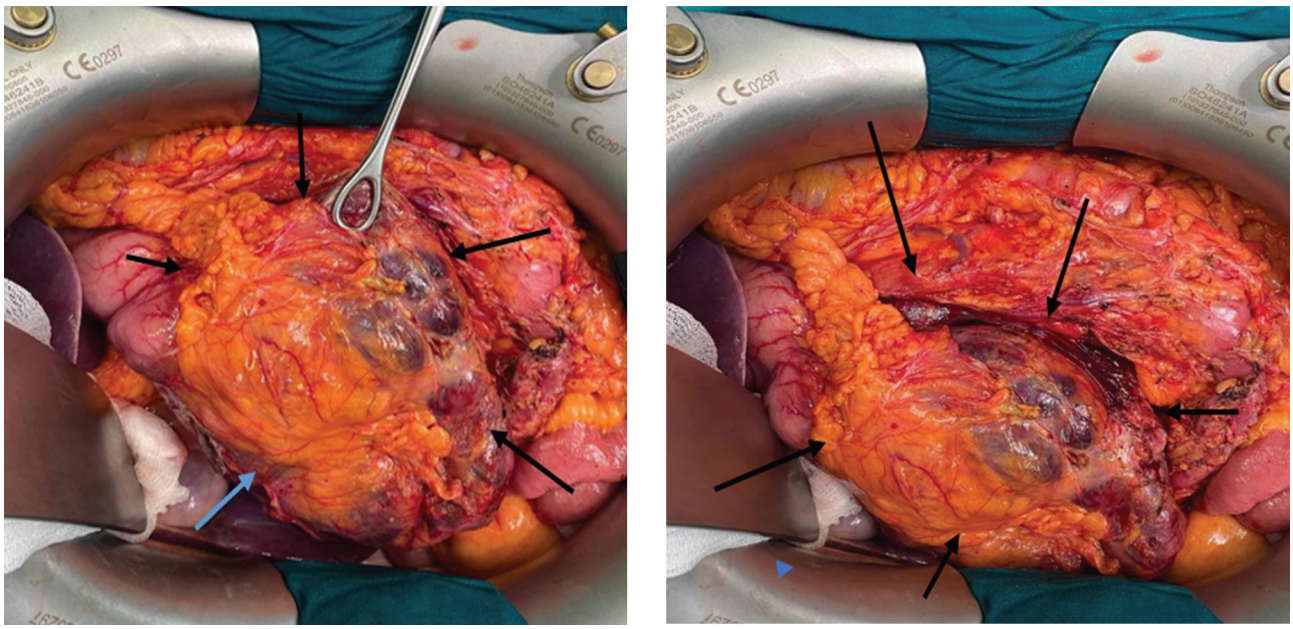

Figure 3. Intraoperative image of the tumor (arrows mark the tumor).

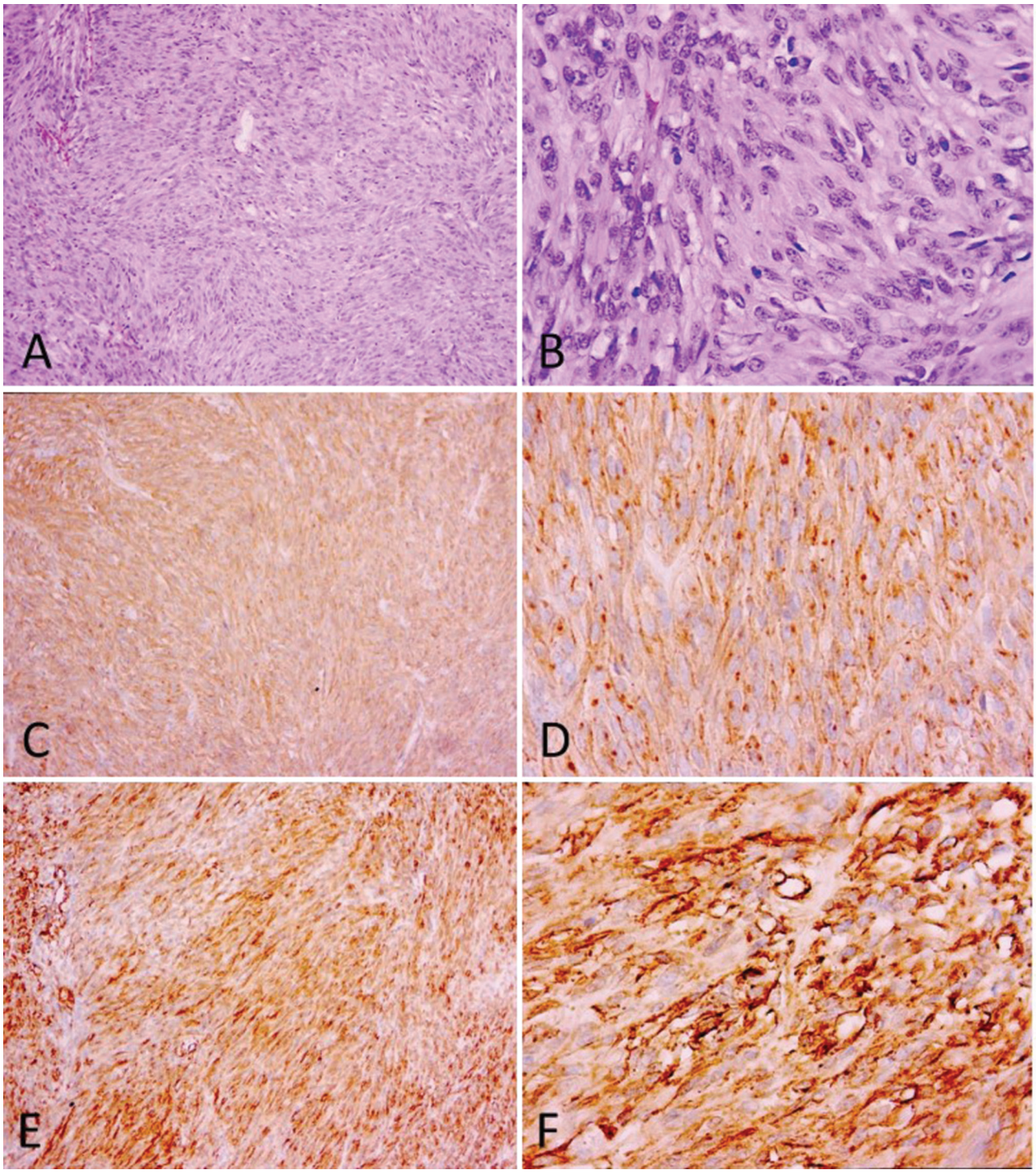

Figure 4. Tumor specimen histopathological image. **A**. H&E stain (×100); **B**. H&E stain (×400); **C**. CD117+ (×100); **D**. CD117+ (×400); **E**. CD34+ (×100); **F**. CD34+ (×400).

a deformity of gastroenteroanastomosis without signs of stenosis. After a failed attempt to endoscopically dilate the anastomosis area, a gastric stasis persisted and laparoscopic adhesiolysis was performed. The postoperative period was without any other complications. The patient was discharged 11 days after operation. At the 1-year follow-up examination, there was no evidence of relapse.

DISCUSSION

The term "gastrointestinal stromal tumors" was introduced in 1983 by MT Mazur and HB Clark, who for the first time identified a special subgroup of non-epithelial tumors of the gastrointestinal tract, differing in their immunohistochemical and ultrastructural characteristics from tumors

with true neurogenic and smooth muscle differentiation.[3,4] GIST are the most common mesenchymal tumors of the gastrointestinal tract, although they comprise only 1-3% of all primary neoplasms of this localization. These tumors most often occur in the stomach (60%–70%), but they also can be found in the small intestine (20%–25%), the large intestine (5%), and in the esophagus (<5%). EGISTs were first described in 2000. They are much less common than their gastrointestinal counterparts and account for less than 5% of all stromal tumors.[5]

GIST is believed to originate from Cajal interstitial cells, located between the circular and longitudinal muscle fibers of the walls of the gastrointestinal tract and regulating their spontaneous peristaltic activity.[4-5] The origin of EGIST currently remains controversial. Some authors believe that EGIST can be the result of extensive extramural growth of GIST, leading to their loss of contact with the wall of the gastrointestinal tract. Others suggest that EGISTs arise from Cajal and smooth muscle interstitial progenitor cells.[6] There are also publications suggesting the occurrence of EGIST from mesenchymal cells with the Cajal interstitial cell phenotype outside the gastrointestinal tract.[2]

In the vast majority of cases, the main triggering mechanism of stromal tumors development is a mutation of the C-KIT proto-oncogene which is located on chromosome 4 (4q11-4q13) and encodes the CD117 tyrosine kinase transmembrane receptor protein.[3]

The most selective IHC marker for distinguishing stromal tumors from true smooth muscle tumors is CD117, positive in 95% of cases. Expression of the CD34 hematopoietic stem cell receptor is detected in 60%–70% of cases.[7] Other mesenchymal markers periodically detected by IHC include DOG-1, smooth muscle actin, S-100, SMA, vimentin, desmin, and keratin.[8] A very important marker is the Ki-67 proliferative activity index, the expression level of which is directly proportional to the degree of tumor aggressiveness.[4]

GIST are divided into three main types: spindle cell (70%), epithelioid (20%) and mixed cell (10%).[1] Spindle cell tumors consist of elongated cells that form beam-like structures, have a less developed fascicular pattern, and are devoid of cytoplasmic eosinophilia. Epithelioid tumors are characterized by rounded or oval cells with eosinophilic cytoplasm. Mixed-cell forms are characterized by a combination of both spindle-cell and epithelioid-cell sites.[6]

Since GIST is mostly a spindle cell proliferation tumor, it must be differentiated from leiomyoma, leiomyosarcoma, schwannoma, fibrosarcoma, fibromatosis, inflammatory fibroid polyps, and other tumors of mesenchymal origin. Differential diagnosis is made on the basis of histopathological, immunophenotypic and molecular features. Immunohistochemical study with positive CD117 (C-Kit) confirms the diagnosis of GIST.[1] Analysis of mutations in the C-Kit and PDGFRA genes can be useful in cases where CD117 is negative, and can also be used to predict the therapeutic response to imatinib.[5]

The standard method of treating patients with localized and locally advanced forms of GIST is complete surgical resection with negative microscopic edges.[7] The choice of the optimal type of resection for EGIST of the pancreas depends on the localization of the mass. Pancreatoduodenectomy is performed for tumors of the head of the pancreas, and distal pancreatectomy – for tumors of the body and tail.[9] In small tumors with clear boundaries, duodenum-sparing resection of the pancreatic head or simple excision of the tumor is acceptable.[8] Systemic regional lymphadenectomy is usually not considered, since the incidence of lymphogenic metastasis in stromal tumors does not exceed 1%–3%. It is very important to prevent the rupture of the tumor capsule during surgery, as this can significantly worsen the prognosis of the disease.[4,10]

Stromal tumors have extremely low sensitivity to traditional chemotherapeutic drugs and radiotherapy: the response rates are less than 10% and 5%, respectively.[10,11] Targeted therapy with C-Kit tyrosine kinase inhibitors is now widespread. The most commonly used drug in this group is imatinib. Adjuvant imatinib therapy has been shown to reduce the risk of recurrence and increase the five-year survival rate after surgery.[9] Adjuvant targeted therapy for a year is recommended for all GIST patients with a high risk of progression. Neoadjuvant imatinib therapy is the gold standard for treating conditionally resectable, unresectable, and metastatic forms of gist. The purpose of preoperative treatment is to reduce the tumor mass, increase the resectability and frequency of organ-preserving operations, as well as reduce the risk of recurrence.[4,11]

The prognosis in patients with GIST depends on the biological behavior of the tumor. Fletcher et al. developed criteria for assessing the risk of aggressive behavior and GIST metastasis based on tumor size (cm) and the number of mitoses (50 in the field of view) according to histological examination. According to these criteria, GIST are divided into categories of very low (<2 cm, <5/50 per field of vision), low (2-5 cm, <5/50 per field of vision), intermediate (<5 cm, 6-10/50 per field of vision or 5-10 cm, <5/50 per field of vision), and high (>5 cm, >5/50 per field of vision or >10 cm, any number of mitoses) risk of metastasis.[5,10] Predictors of poor prognosis may also include a Ki-67 expression proliferation index greater than 10%, the presence of a tumor capsule rupture before or during surgery, vascular invasion, and the presence of foci of necrosis. GIST is more likely to metastasize by hematogenous means, with up to 90% of metastases found in the liver. Implantation metastases into the peritoneum and into the greater omentum are also possible.[4]

EGIST of the pancreas is an extremely rare tumor and requires careful differential diagnosis with other large tumors of the abdominal cavity. Diagnostic modalities such as CT with intravenous contrast and ultrasound of the abdominal organs, make it possible to visualize the tumor. Nevertheless, the diagnosis must be confirmed on the basis of a morphological examination of the removed specimen with immunohistochemical analysis for specific markers.

It is impossible not to pay attention to the discrepancy between the general satisfactory condition of the patient and the severity of the disease. The patient presented with extremely scarce, nonspecific clinical symptoms with no signs of biliary obstruction or deviations of laboratory tests. There is no doubt that the patient belongs to the group of high risk of recurrence, taking into account the large size of the tumor (15 cm), invasive growth in the mesentery of the transverse colon, the proliferation index of Ki-67 expression – 15%, the presence of foci of necrosis. In this regard, the patient is required to undergo adjuvant targeted therapy with imatinib for a year and intensive follow-up.

CONCLUSIONS

The presented clinical observation clearly demonstrates the importance of timely diagnosis of an extra-gastrointestinal pancreatic tumor, often characterized by an aggressive course, and the use of modern treatment algorithms, which makes it possible to avoid further progression of the disease, as well as to reduce mortality and improve the quality of life of patients.

Ethics

Written informed consent was obtained from the patient to publish the cases report.

Author contributions

S.A., B.Z., T.M., D.D., A.I., Sh.T., I.N., F.A., Ch.Z., and C.S. conceived and designed the analysis; S.A., B.Z., T.M., D.D., A.I., Sh.T., I.N., F.A., Ch.Z., and C.S. collected the data; A.I., Ch.Z., and C.S. contributed data or analysis tools; Ch.Z. and C.S. drafted the manuscript; S.A., B.Z., T.M., D.D., A.I., Sh.T., I.N., F.A., Ch.Z., and C.S. edited the final version of the manuscript.

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Competing Interests

The authors have declared that no competing interests exist.

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Редкий клинический случай экстрагастроинтестинальной стромальной опухоли поджелудочной железы

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Резюме

Внегастроинтестинальные стромальные опухоли, возникающие из поджелудочной железы, встречаются крайне редко. На сегодняшний день в мировой литературе описано чуть более 30 случаев. Представлено клиническое наблюдение больного 67 лет с тупыми болями в эпигастрии и крупным кистозным солидным новообразованием, инструментально идентифицированным как внегастроинтестинальная стромальная опухоль головки поджелудочной железы. Объём оперативного вмешательства включил панкреатогастродуоденэктомию и правостороннюю гемиколэктомию, поскольку интраоперационно была выявлена инвазия опухоли в поперечно-ободочную кишку. На основании результатов гистопатологии и иммуногистохимии установлен окончательный диагноз: экстрагастроинтестинальная стромальная саркома головки поджелудочной железы с инвазией в среднетолстую кишку pT4N0M0, стадия III b.

Внегастроинтестинальные стромальные опухоли поджелудочной железы требуют тщательной дифференциальной диагностики с другими крупными образованиями брюшной полости. Своевременная диагностика и использование современных алгоритмов лечения позволяют избежать дальнейшего прогрессирования заболевания.

Ключевые слова

CD117, внегастроинтестинальная стромальная опухоль, иммуногистохимия, поджелудочная железа, таргетная терапия.

Frieze of the healing family (Front Cover)

One of the most beautiful reliefs of the healing cults in the Greco-Roman pantheon is the unique "Frieze of the Healing Family" exhibited in the Archeology Museum in Plovdiv.
It was excavated in the foundations of an old, ruined Turkish mosque in 1921. The correct identification of all figures was performed by Professor Zapryanov in 1964 in the Department of Social Medicine. The frieze, according to him, used to adorn a Roman valetudinaria - a military hospital - off the walls of the east entrance of the ancient city which was called Trimontium by the Romans in the late III century. It weighs about 3000 kg and is 2.80 m long and 1.08 m high. The figures on it are framed in a wide rim; it bears the personified images of the Moon (on the left) and the Sun (on the right).*
Presented on the frieze are (from left to right): Jaso and Panacea - Asclepios' daughters, Telesphor - the fortunate genius of the healing process, Asclepios - the god of healing art, Hygeia - his daughter, Epione - Asclepios' wife, Machaon and Podaleirios - his sons worshipped as military physicians.
All figures, except Panacea, are entirely in full face which is very rare in a general composition picture. The frieze's sculptor depicted in great detail the figures' anatomic features, clothes and peculiar attributes. All deities in the composition are on a par with the only association seen between Panacea and Asclepios (Panacea touches a bundle of herbs next to Telesphor's cowl with her left hand, while pouring the cure all (panacea) in Asclepios' bowl).

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* Folia Medica 1964; 6(3): 152 - 156