İSTANBUL MEDICAL JOURNAL
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The target population of the journal includes specialists in all fields of medicine, academicians and relevant health care professionals.

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Table 1. Limitations for each manuscript type

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Review Article	5000	250	50	6	10 or total of 20 images
Case Report	1000	200	15	No tables	10 or total of 20 images
Letter to the Editor	500	No abstract	5	No tables	No media

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Tables should be included in the main document, presented after the reference list, and they should be numbered consecutively in the order they are referred to within the main text. A descriptive title must be placed above the tables. Abbreviations used in the tables should be defined below the tables by footnotes (even if they are defined within the main text). Tables should be created using the "insert table" command of the word processing software and they should be arranged clearly to provide easy reading. Data presented in the tables should not be a repetition of the data presented within the main text but should be supporting the main text.

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When a drug, product, hardware, or software program is mentioned within the main text, product information, including the name of the product, the producer of the product, and city and the country of the company (including the state if in USA), should be provided in parentheses in the following format: "Discovery St PET/CT scanner (General Electric, Milwaukee, WI, USA)"

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Journal Article: Rankovic A, Rancic N, Jovanovic M, Ivanović M, Gajović O, Lazić Z, et al. Impact of imaging diagnostics on the budget – Are we spending too much? Vojnosanit Pregl 2013; 70: 709-11.

Book Section: Suh KN, Keystone JS. Malaria and babesiosis. Gorbach SL, Barlett JG, Blacklow NR, editors. Infectious Diseases. Philadelphia: Lippincott Williams; 2004.p.2290-308.

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Books with a Single Author: Sweetman SC. Martindale the Complete Drug Reference. 34th ed. London: Pharmaceutical Press; 2005.

Editor(s) as Author: Huizing EH, de Groot JAM, editors. Functional reconstructive nasal surgery. Stuttgart-New York: Thieme; 2003.

Conference Proceedings: Bengisson S. Sothemin BG. Enforcement of data protection, privacy and security in medical informatics. In: Lun KC, Degoulet P, Piemme TE, Rienhoff O, editors. MEDINFO 92. Proceedings of the 7th World Congress on Medical Informatics; 1992 Sept 6-10; Geneva, Switzerland. Amsterdam: North-Holland; 1992. pp.1561-5.

Scientific or Technical Report: Cusick M, Chew EY, Hoogwerf B, Agrón E, Wu L, Lindley A, et al. Early Treatment Diabetic Retinopathy Study Research Group. Risk factors for renal replacement therapy in the Early Treatment Diabetic Retinopathy Study (ETDRS), Early Treatment Diabetic Retinopathy Study Kidney Int: 2004. Report No: 26.

Thesis: Yılmaz B. Ankara Üniversitesindeki Öğrencilerin Beslenme Durumları, Fiziksel Aktiviteleri ve Beden Kitle İndeksleri Kan Lipidleri Arasındaki Ilişkiler. H.Ü. Sağlık Bilimleri Enstitüsü, Doktora Tezi. 2007.

Manuscripts Accepted for Publication, Not Published Yet: Slots J. The microflora of black stain on human primary teeth. Scand J Dent Res. 1974.

Epub Ahead of Print Articles: Cai L, Yeh BM, Westphalen AC, Roberts JP, Wang ZJ. Adult living donor liver imaging. Diagn Interv Radiol. 2016 Feb 24. doi: 10.5152/dir.2016.15323. [Epub ahead of print].

Manuscripts Published in Electronic Format: Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis (serial online) 1995 Jan-Mar (cited 1996 June 5): 1(1): (24 screens). Available from: URL: http:/ www.cdc.gov/ncidodlElD/cid.htm.

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Case Reports

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EDITORIAL

Dear Colleagues and Researchers,

I am honored to have been selected as the new Editor in Chief of the İstanbul Medical Journal. I want to thank our previous Editor in Chief, Dr. Tevfik Fikret Çermik, for his great effort over the years and success about getting registered in an international index. I also want to thank to associate editors and all previous Editors in Chief for supporting the journal with commitment.

As Editor in Chief, I will try to achieve a high-quality, and unbiased peer-review conducted promptly and provide a high standard of editing and publishing for every paper submitted. I will also try to ensure that the journal represents a wide range of academic and clinical interests with high-quality articles and to be widely recognized at an international level and be accepted in Pubmed over time.We hope to receive your contribution.

Kind regards,

MD, Feray AKBAŞ

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Health Care Transitions for Children with Sickle Cell Disease: Interventions, Perspectives of Health Care Providers and Caregivers

Orak Hücreli Hastalığı Olan Çocuklarda Sağlık Bakım Geçişleri: Müdahaleler, Sağlık Bakımı Sağlayıcıları ve Bakıcılarına Bakış Açıları

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ABSTRACT

The incapacitating episodic complications associated with the Sickle Cell disease (SCD) limit the opportunities of those SCD patients in education, societal roles, career options, and quality of life, leading to an increase in under-5 mortality. The United Nations included the reduction of newborns and children under-5 mortality among the Sustainable Development Goals. Healthcare providers and caregivers are often involved in the process, have their own perceptions, and challenges. This paper aimed to review relevant studies regarding aspects of health care providers, caregivers, and intervention programs toward strengthening the health care transition in children with SCDs. Literature searched was conducted into PubMed to identify relevant studies. Several intervention programs have been developed and tested, including the transtheoretical model, self-efficacy, theory of planned behavior, and bioecological model, among others. The available literature demonstrates the efficacy of psychosocial, therapeutics, and technological interventions for overcoming SCD-related complications. It was found that the use of wireless and information technology in health care transitions for children with SCD has been gaining more attention. More well-designed studies and holistic approaches are needed to improve the health care transition in children with SCD.

Keywords: Transition care, sickle cell disease, pediatric care, transition care intervention, providers

ÖZ

Orak Hücre hastalığı (OHH) ile ilişkili aciz olan epizodik komplikasyonlar, OHH hastalarının eğitimdeki fırsatlarını, toplumsal rolleri, kariyer seçeneklerini ve yaşam kalitesini sınırlandırmakta ve 5 yaş altı ölüm oranlarının artmasına neden olmaktadır. Birleşmiş Milletler, Sürdürülebilir Kalkınma Hedefleri arasında yenidoğanların ve 5 yaş altı ölüm oranlarının azaltılmasını içeriyordu. Sağlık hizmeti sağlayıcıları ve bakıcıları genellikle sürece katılır, kendi algılarını ve zorluklarını yaşarlar. Bu yazıda, OHH'li çocuklarda sağlık hizmeti geçişini güçlendirmeye yönelik sağlık hizmeti sağlayıcıları, bakıcılar ve müdahale programlarının yönleriyle ilgili çalışmaları gözden geçirmeyi amaçladık. Aranan literatür ilgili çalışmaları tanımlamak için PubMed'de gerçekleştirildi. Transteorik model, öz yeterlik, planlı davranış teorisi ve biyoekolojik model gibi birçok müdahale programı geliştirildi ve test edildi. Mevcut literatür, OHH ile ilgili komplikasyonların üstesinden gelmek için psikososyal, terapötik ve teknolojik müdahalelerin etkinliğini göstermektedir. OHH'li çocuklar için sağlık hizmetlerinde telsiz ve bilgi teknolojilerinin kullanımının daha fazla dikkat çektiği tespit edildi. OHH'li çocuklarda sağlık hizmeti geçişini iyileştirmek için daha iyi tasarlanmış çalışmalara ve bütünsel bir yaklaşıma ihtiyaç vardır.

Anahtar Kelimeler: Geçiş bakımı, orak hücre hastalığı, çocuk bakımı, geçiş bakımı müdahale, sağlayıcılar

Introduction

Sickle Cell disease (SCD) is a chronic, inherited hemoglobin disorder associated with lifelong severe and life-threatening complications in neonates, pediatrics, and transitioning young adults (1). These chronic severe complications include anemia, episodic intense pains, stroke, priapism, infections, organ failure, tissue damage, increased morbidity, and premature death while transiting from pediatric to adults. The incapacitating episodic complications associated with the SCD limited the opportunities of those SCD patients in education, societal roles, career options, and quality of life (QoL) (2).

Globally, SCD affects approximately 300.000 newborn babies each year. The traits of SCD have been postulated to confer resistance against malaria, especially among the heterozygous carriers, leading to a higher prevalence in malaria-endemic areas due to selection pressure that promotes SCT for survival (1,3-5). The prevalence and impacts of SCD vary across different parts of the world, with the highest percentage (40%)

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being reported in sub-Saharan Africa, the eastern part of Saudi Arabia, and Central India (2). It has been estimated that 90% of SCD cases are in developing countries, and more than 90 percent of babies born with SCD in developing countries do not make it to adulthood due to limited resources (6-8). For example, more than half of babies born with SCD in Africa, and central and western India die before adulthood (1). In contrast, more than 90 percent of people with SCD reach adulthood in the United States due to medical advancements and better healthcare (2,6).

The United Nations included the reduction of newborns and children under-5 mortality due to communicable and non-communicable diseases among the Sustainable Development Goals (SDGs). Under the SDGs program, all participating countries are expected to reduce under-5 mortality of not more than 25 deaths per 1.000 live births (9). Bridging the disparities in transition health care in children with SCDs between the developed and developing worlds would go a long way towards achieving the goals of reducing preventable under-5 mortality in the world. Therefore, the objective of this paper is to briefly review and synthesize literature regarding reported perspectives of health care providers, caregivers, and intervention programs toward strengthening the health care transition in children with SCDs.

Intervention and Programs to Address Transition Care

Transition care in SCD patients has been regarded as a dynamic, multidimensional, deliberate, and active process that ensures uninterrupted affordable and accessible desired health care services. The primary goal of interventions is to prevent and reduce complications, mortality, and improve QoL among people living with the SCD. Therefore, any transition program must adequately prepare pediatric SCD patients to achieve the ultimate goals of uninterrupted quality transition care from pediatrics to adulthood. Several intervention programs have been developed and tested based on the public health and psychological conceptual frameworks, including the transtheoretical model, self-efficacy, theory of planned behavior, and bioecological model, among others. The available literature demonstrates the efficacy of psychosocial, therapeutics, and technological interventions for overcoming SCD-related complications (10). Empirical evidence on the psychological conceptual approaches such as cognitive-behavioral techniques, behavioral change, and social support programs towards improving SCD-related complications have been reported (11). Cognitive-behavioral techniques include cognitive-behavioral therapy, relaxation, cognitive coping strategies, and biofeedback, hypnosis, and interruption strategies. Education and education-related behavioral modification interventions have been regarded as behavioral change programs. The psychosocial intervention approach is sometimes nested in social network support, including support groups, peers, family members, and friends to explore individuals' social networks.

Cognitive-behavioral Interventions

A few studies explore the Cognitive-behavioral Intervention approaches towards transition health care intervention programs. For example, a qualitative research was conducted in patients with SCD (n=14; 14-24 years old) and providers (n=10) to evaluate the feasibility of the Social-ecological Model of Adolescent and Young Adult Readiness to Transition (SMART) model for transition intervention program in adolescents and young adults (AYA) with SCD. The investigators reported promising applicability of the social-ecological framework, such as SMART, to improve transition readiness in SCD (12). Accurately, both the providers and young adults with SCD reported several factors that influence the transition process, including episodic and seasonal variability of SCD disease, SCD-related stigma, misconceptions and knowledge gap about the SCD in community, interactions between SCD patients and family members, friends and other components of social networks, knowledge, and skills regarding symptoms and self-management, goal-oriented psychosocial functioning and developmental transition, providers' skills and perspectives of individuals living with SCD (12).

Additionally, another SMART and AYA with SCD models-based qualitative study was conducted among healthcare providers (n=13 SCD experts) to evaluate transition success metrics and determinants of transition success from pediatric to adult healthcare for AYAs with SCD (13). The investigators conducted 60 minutes semi-structured qualitative interviews to explore metrics and facilitators of transition success from the providers' perspectives. A complication-free stable transition, healthcare utilization, continuation care, and QoL are the yardsticks measuring for the success of the health care transition program.

Behavioral Change and Education Interventions

Multiple studies explored behavioral change and education interventions in the transition from pediatric to adults with SCD. A pre-post and retrospective cohort study with intervention, comparison group, and historical groups was conducted to investigate the effectiveness of transition coordinator program in improving fulfillment after first adult care appointment, integration into adult care by establishing a relationship with the adult care provider, and transition process in the coordinated SCD adolescents compared to the non-coordinated group. The intervention components included a care-coordinator structured pediatric SCD program targeted at pre-adult care at 18th birthday (including discussion and assessment of readiness for transition). Other elements include pilot testing transition activities and involving stakeholders such as parents and providers. This pilot testing was created to provide real-world experience to adolescents with SCD in identifying and establishing care with adult providers. Compared to the control group, the authors reported statistically significant improvement in the adult clinic attendance, service utilization, fulfilling transition, and cost-effectiveness for the intervention group (14). Similarly, another pre-post study aimed to investigate the effects of the Duke SCD Transition Program in improving transition readiness and integration into adult care. The investigators reported a significant improvement in SCD knowledge scores, but not in the Sickle Cell Transfer readiness scores among adolescents. Further findings demonstrated gaps in SCD knowledge and concerns about moving to adult care among adolescents with SCD. The difference in knowledge and worries about transition could result in healthcare transition-related stress among transition pediatric to adults with SCD and could lead to a negative impact on health outcomes (15). Stakeholders in the health care transition of pediatric to adults with SCD should educate individuals with SCD patients about diseases,

management while addressing the raised concerns. A few programs have explored Bandura's social learning theory in an intervention aimed at changing behavior in transition pediatric to adults living with SCD. Correctly, researchers applied the self-efficacy component of Bandura's theory in transitions. The transition from pediatric to adults is not static, so self-efficacy, as defined by Bandura, is the belief in the ability to control behaviors to achieve specific outcomes depending on the situation (16). A prospective cohort study (n=4141 adolescents and their families) was conducted to investigate the effectiveness of self-efficacy on transition in SCD patients (17). The investigators used a Sickle Cell Self-Efficacy scale instrument to assess the level of adjustment among transition SCD patients at the beginning and end of the intervention program (one-year interval). The adjustment level was determined based on pain management, physical and mental symptoms, and health care utilization. These domains were compared with self-efficacy before the intervention and after the intervention to determine to evaluate the relationship between self-efficacy and SCD adjustment. Findings demonstrated significant improvement in physical and psychological symptoms, fewer physician visits among participants with a better self-efficacy after one year (17). This positive correlation reflects the effectiveness of self-efficacy in improving SCD-related health outcomes in adults. This study was limited to adult populations and may not give similar findings when replicated in pediatric SCD patients. Similarly, another study reported significant relationships between self-efficacy, spirituality, and QoL among adults with SCD (18). Furthermore, another study used the hospital-based program to investigate the effectiveness of an educational intervention on the functional ability of the providers (19). The intervention was rooted in a theoretical framework from David Kolb's theory of experiential learning (20). This conceptual framework spanned the cycle of the learning process; concrete-based learning, abstract-based concepts, reflective learning, and active exploration. The investigators used the Youth Acute Pain Functional Ability Questionnaire to evaluate both the providers and SCD patients at the hospital before and after the educational intervention. There was no statistically significant difference between pre and post educational intervention (19).

Technology-based Interventions

The use of wireless and information technology in health care transitions for children has been gaining more attention. Considering that mobile phones, computers, games, and other devices using wireless technology are readily accessible to the 81-88% of teens, wireless technology could be a correct approach for addressing SCD related complications (21). An increasing body of evidence explores the use of wireless-based operating devices such as mobile phones to provide various interventions among transition adolescents with SCD. Findings from this study showed significant improvement in symptom management, knowledge, and communication skills between people living with SCD and providers (22). Another study that enrolled 37 transition adolescents and engaged them in text messaging reported a significant definite reduction in SCD-related complications, medication adherence, screening, patient education, and prompt pain and symptom management (23). Other findings from this study include psychosocial support and referral services utilization among children and adolescents living with SCD. Furthermore, in 2012, Jacob et al. (24) conducted a survey among children and adolescents 10 to 17 years of age aimed at investigating the use of smartphones in improving symptoms reporting and communication between participants and providers. The investigators found significant improvements in positive behaviors such as self-monitoring, patient-provider interaction opportunities, and symptom reporting. They also concluded that their smartphone-based e-Diary documentation program could be a cost-effective approach to remove barriers to access to health care, facilitating communication, and appropriate self-management among children and adolescents living with SCD. A randomized clinical trial study conducted among 160 youth aged 12-18 years living with SCD reported improvement in pain self-management and functioning after web and smartphone-based intervention. The smartphone-based intervention was delivered in four components; self-management skills, daily symptom tracking, goal setting, and social community. Another health information-based intervention study reported promising benefits of information technology towards improving transition care in SCD patients (25). The investigators used a qualitative and quantitative approach to improve transitions of care for patients with SCD. The researcher conducted an environmental scan into the literature to understand what it takes to prepare for and transit from pediatric to adults, focus groups, and key informant interviews among the participants. The participants include SCD patients, providers, caregivers, and IT developers.

Perspectives of Health Care Providers

The views of the health care providers, essential stakeholders to transition care, are critical. Healthcare institutions often provide transition SCD patients with access to adult provider information and promote their services. However, there are significant variations in assessment and practices associated with the transition of patients' readiness, self-management, and independence among individuals living with SCD. In 2011, Sobota et al. (26) conducted a qualitative study to evaluate transitions practices on patient transition readiness, self-management, logistics, preparation for the transition, and independence of SCD patients across 45 selected pediatric sickle cell clinics. The investigators found that the most significant challenge identified by most of the participated clinics is difficulty in accessing an appropriate adult provider with requisite skills in SCD to transfer pediatric patients with SCD. Also, over 60% of the involved sickle cell clinics engaged parents, have documented targets for self-management skills, and review the transition readiness of individuals living with SCD as a team. Furthermore, another qualitative study reported that pediatric healthcare providers' perception was negative about transferring pediatric SCD patients to adult care due to the understanding that pain and complication management in adult care is not as good as in pediatric care. Additionally, it was reported that healthcare providers noted the considerable variation in how patients sought care and wish to be treated with disease and complications (27). The available literature demonstrated that the pressing challenge most cited by health care providers is difficulty in identifying an appropriate adult provider with expertise in SCD. There is limited evidence regarding the perspectives of health care providers about the transition in pediatric to adults living with SCD. Well-designed epidemiological studies should be conducted

to explore the views of health care providers about transition care in the population living with SCD.

Perspectives of Caregivers

The inputs from caregivers, including parents and family members, are critical to a successful transition among AYA with SCD. The involvement of caregivers has been linked to transition readiness (28). Family members take less responsibility and participation in young adults compared to pediatrics and create a gap in health care and supports (29). A descriptive, correlational study (n=60) aimed to evaluate transition readiness of caregivers and transition individuals with SCD found that parents perceived to be highly responsible for all the healthcare needs for their transition children, but not the young adults with SCD. The investigators also reported significant positive associations between parent involvement in their SCD children transition responsibility and parent's perception of overall transition readiness. However, parent involvement was negatively correlated with the perspectives of an individual with SCD in the study. Therefore, for the better transition process, the responsibility of individuals with SCD should be increased while reducing parent involvement in transitions from pediatric to adults (30). Furthermore, another study (n=65) was conducted to investigate how healthy siblings of individuals with SCD cope psychosocially with SCD-related health and family functioning. The researchers found an increased negative psychosocial stress, such as emergency visits among primary caregivers of family members with SCD (31). Similarly, another study reported that the behavior of parents, family members, and social networks of individuals living with SCD significantly influence clinic visits and hospitalizations (32). These interrelationships in the system of caregivers could have implications in the design and implementation of the transition's intervention program. In 2014, Porter et al. (33) conducted a qualitative study using focus groups to determine the perspectives of caregivers on the transition from pediatric to adult care. The investigators concluded that views of family members are critical in the design and planning of transition from pediatric to adolescents with SCD. They also reported that siblings and caregivers are significantly concerned about medication adherence of family members living with SCD. These findings further highlighted the importance of involving family members in developing and delivery of a successful transition program.

Future Direction and Recommendations

Great efforts and resources have been applied in health care transitions, especially in the developed world. However, there are several ways to improve transition care and transition programs among children living with SCDs. The identified communication gap between the providers and individuals with SCD should rectify by broadening and incorporating step-by-step approaches for pain management and alleviation of wrong perceptions among the stakeholders involved in health care transition. For example, a clear plan with the division of responsibility should be targeted toward improving direct communication between adult and pediatric providers (34). Another area to address in future research is the difficulty in identifying an appropriate adult provider to transit children with SCD. More deliberate corporative efforts are required from both the pediatric and adult providers towards identifying adult providers who are willing to actively corporate in transitioning individuals with SCD from pediatric to adult health care smoothly. This corporative effort between pediatric and adult providers should be maintained for at least three months after the transition from pediatric to adult health care. The pediatric health care provider could follow up on the transitioning individual with SCD after being transitioned, and the adult providers should consult with a pediatric health care provider to ensure a smooth transition and established care after the transition. Some researchers proposed a more holistic approach to health care transitions. For example, transition programs should consider incorporating vocational and educational needs that occur at the same time as health care transitions (35-37). This synchronization could offer the opportunity for an individual with SCD to acquire life skills toward symptoms management, improving health, QoL, and ability to cope with increasing independence as individual transit from pediatric to adult health care. The incorporation for holistic transition health care would require the recruitment of diverse transition teams that include family members, physicians, nurses, mental health professionals, social workers, and public health practitioners (38,39). Offering transition programs, as part of day camps or through local community organizations, would increase knowledge of health care transition and transition programs leading to improved stakeholder participation and corporation toward holistic transition care. Moreover, the compressive pre-transition assessment was recommended to be one of the criteria for evaluating transition readiness. The synchronization of the traditional age-based health care transition with the pre-transition assessment would develop better transition plans toward making informed decisions about transition care. The pre-transition evaluation should evaluate the family structure, educational status, developmental, and efficacy of individuals with SCD, and should be reviewed annually to ensure benchmarks are being met (36). As part of the pre-transition stage, the scheduling portion of pediatric visits without adults could improve self-efficacy and independent of transitioning children with SCD (34). Organizing separate informal meetings between adult providers and individuals with SCD was suggested to enhance transition health care (38). Another promising recommendation is incorporating wireless and information technology into transition care. For example, smartphones and video modules have been explored to promote self-efficacy and identify patient comprehension of the transition process (40). Similarly, the use of web-based e-Diary before, during, and after a stable transition may improve pain management because most adolescents are using web-based wireless information technology (24). Further research is needed on the outcomes associated with a lack of transition care in the developing world as well as the effects of delaying transition care. There have been needs for consistent measures of transitions across the health system. The regular measures will allow for a better understanding of the transition needs of individuals with SCD and help identify solutions to any challenges. It will also allow for better tracking and long-term assessment of transition care. Incorporate automated message alerts about medication adherence and appointments to remind individuals with SCD about care needs. Significant percentages of adolescents are using wireless technology, including adolescents with SCD. This wireless technology has come to stay, and health care transition stakeholders should fully explore this aspect towards improving healthcare transition

success, especially among individuals living with SCD. This study is limited by its comprehensive review of nature, which may not represent all the published studies in this area. However, efforts were placed to provide a good summary of what is available in the literature. Moreover, only studies published in the English language were used in this mini-review, and therefore, relevant studies published in other languages are not captured here.

Conclusion

This paper summarized perspectives of health care providers and caregivers, and interventions to assist with transition care for those with SCD and highlighted recommendations to improve transition care for those with SCD. It was demonstrated that more well-designed studies are needed to improve health care transition in children with SCD, as most of the available evidence was from smaller sample studies and surveys. Changes from pediatric to adult care do not just affect individuals with SCD. Healthcare providers and caregivers are often involved in the process, but a more holistic approach is needed in the health care transition of SCD patients. Understanding the perspectives of each of these individuals provides a greater understanding of transition care.

Key Findings

- Researchers and providers used different measures of transition need among transitioning SCD patients that call for concerted efforts for the use of uniform measures of transition needs.

- It was also demonstrated that health care providers lack the necessary knowledge to provide transitioning individuals, including providers that were interested in providing care.

- Available studies conducted to investigate the association between health outcomes and lack of transition care in the developing world as well as the effects of delaying transition care.

- Incorporation of automated message alerts system about medication adherence and appointments to remind individuals with SCD about care needs demonstrate promising approach towards improving health and QoL. Especially that a significant percentage of adolescents are using wireless technology, including adolescents with SCD.

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Ethics

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Evaluation of the Effect of *NQO1 C609T (rs1800566)* Gene Variations in Philadelphia-negative Myeloproliferative Neoplasms in Turkish Population

Philadelphia-negatif Miyeloproliferatif Neoplazilerde *NQO1 C609T (rs1800566)* Gen Varyasyonlarının Etkisinin Türk Toplumunda Değerlendirilmesi

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ABSTRACT

Introduction: Philadelphia-negative myeloproliferative neoplasm (MPN) is a hematopoietic stem cell disorder consisting of essential thrombocythemia (ET), polycythemia vera (PV), and primary myelofibrosis (PMF) associated with myeloid cell proliferation without differentiation and maturation defects. It is characterized by hypercellular bone marrow and an increase in one or more cell lines in the peripheral blood. In the hematopoietic stem cell, janus kinase 2 (JAK2), which is a cytoplasmic tyrosine kinase, remains constantly phosphorylated (active) as a result of the V617F somatic mutation in the pseudokinase region. Even if the phosphorylated JAK2 does not receive a stimulus, it performs signal transmission and causes continuous gene expression. This explains the excessive increase in one or more blood cell lines. NAD(P)H quinone oxidoreductase-1 (NQO1) is a phase 1 enzyme that prevents the formation of reactive and toxic semiquinone metabolites by reducing two electrons in one step. The C609T polymorphism of the *NQO1* gene leads to loss of enzyme activity due to the enzyme becoming unstable. While enzyme activity is not observed in individuals with both mutant alleles, moderate activity is observed in heterozygous individuals. Studies have reported a relationship between NQO1 C609T polymorphism and various cancer types. In our study, it was aimed to investigate the possible relationship between NQO1 C609T polymorphism and MPN development.

Methods: Our study group consisted of 119 MPN patients and 122 healthy controls. DNA isolation was performed from peripheral blood taken from the study groups. The JAK2 V617F mutation was detected using Real-time polymerase chain reaction (PCR), and *NQO1 C609T* gene polymorphism was detected using the PCR- restriction fragment length

ÖZ

Amaç: Philadelphia-negatif miyeloproliferatif neoplazi (MPN); farklılaşma ve olgunlaşma kusuru olmaksızın, miyeloid hücre çoğalması ile ilişkili esansiyel trombositemi (ET), polisitemi vera (PV) ve primer miyelofibrozisten (PMF) oluşan hematopoetik kök hücre bozukluğudur. Hipersellüler kemik iliği ve periferik kanda bir veya daha fazla hücre serisinin artması ile karakterizedir. Hematopoietik kök hücrede, sitoplazmik tirozin kinaz olan janus kinaz 2 (JAK2), psödokinaz bölgesindeki V617F somatik mutasyonunun bir sonucu olarak sürekli fosforile (aktif) kalır. Fosforile JAK2 bir uyarıcı almasa bile, sinyal iletimi gerçekleştirir ve sürekli gen ekspresyonuna neden olur. Bu durum bir veya daha fazla kan hücre serisinin aşırı artışını açıklamaktadır. Nikotinamid adenin dinükleotid fosfat kinon oksidoredüktaz-1 (NQO1) tek basamakta iki elektron indirgenmesini sağlayarak reaktif ve toksik semikinon ara metabolitlerinin oluşumunu engelleyen faz 1 enzimidir. *NQO1* geninin C609T polimorfizmi, enzimin kararsız hale gelmesinden dolayı enzim aktivitesinin kaybına yol açar. İki alleli de mutant olan bireylerde enzim aktivitesi görülmezken, heterozigot bireylerde orta düzeyde aktivite gözlenir. Yapılan çalışmalarda NQO1 C609T polimorfizmi ile çeşitli kanser tipleri arasında ilişki olduğu bildirilmiştir. Çalışmamızda NQO1 C609T polimorfizmi ile MPN gelişimi arasındaki olası ilişkinin araştırılması amaçlanmıştır.

Yöntemler: Çalışma grubumuzu 119 MPN hastası ve 122 sağlıklı kontrol oluşturdu. Çalışma gruplarından alınan periferik kandan DNA izolasyonu yapıldı. Janus kinase 2 (JAK2) V617F mutasyonu gerçek zamanlı polimeraz zincir reaksiyonu (PZR), *NQO1 C609T* gen polimorfizmi PZR-sınırlayıcı enzim

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polymorphism method. SPSS 21.0 was used for statistical analysis.

Results: No statistically significant difference was found between the patient and control groups in terms of NQO1 genotype distributions (p>0.05). When cases with ET, PMF, and PV were compared in terms of frequency of JAK2 V617F mutation, the rate in PV was higher (respectively; 62.5%, 61.5%, 78.6%). There was no relationship between JAK2 mutation positivity and NQO1*2 polymorphism.

Conclusion: According to the results obtained from our study, there is no relationship between NQO1*2 polymorphism and MPN. Variants of the NQO1 enzyme, which is essential in detoxification and activation of procarcinogens, did not increase the formation of the JAK2 V617F mutation, which is common in MPN patients. It is thought that the results should be supported by increasing the number of patient and control groups.

Keywords: Myeloproliferative neoplasm, MPN, JAK2 V617F mutation, *NQO1 C609T* gene polymorphism

parça uzunluk çeşitliliği yöntemi kullanılarak tayin edildi. İstatistiksel analizlerde SPSS 21.0 paket programı kullanıldı.

Bulgular: Hasta ve kontrol grubu arasında NQO1 genotip dağılımları açısından istatistiksel olarak anlamlı fark bulunmamıştır (p>0,05). ET, PMF ve PV tanılı olgular JAK2 V617F mutasyonunun sıklığı açısından karşılaştırıldığı zaman PV'deki oran daha yüksek saptanmıştır (sırasıyla; %62,5, %61,5, %78,6). JAK2 mutasyonu pozitifliği ile NQO1*2 polimorfizmi arasında ilişki saptanmamıştır.

Sonuç: Çalışmamızdan elde ettiğimiz sonuçlara göre, NQO1*2 polimorfizmi ile MPN arasında ilişki bulunmamaktadır. Prokarsinojenlerin detoksifikasyonu ve aktivasyonu için gerekli olan NQO1 enziminin varyantları, MPN hastalarında yaygın olan JAK2 V617F mutasyonunun oluşumunu artırmamıştır. Sonuçların hasta ve kontrol grubu sayısının artırılmasıyla desteklenmesi gerektiği düşünülmektedir.

Anahtar Kelimeler: Miyeloproliferatif neoplazi, MPN, JAK2 V617F mutasyonu, *NQO1 C609T* gen polimorfizmi

Introduction

Philadelphia-negative (Ph-) myeloproliferative neoplasms (MPNs) are a group of clonal multipotential hematopoietic stem cell diseases characterized by overproduction of mature and fully functional blood cells in one or more cell types, as well as thrombotic events and leukemia transformation. They consist mainly of polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF) (1).

Our knowledge of the pathogenesis of MPNs has been dramatically expanded since the janus kinase 2 (JAK2) V617F mutation was discovered in 2005. In the period after the JAK2 V617F mutation was found, more critical fundamental deviations were identified, such as repetitive changes in the thrombopoietin receptor (MPL) gene, exon 12 mutations in the *JAK2* gene, and finally different types of mutations in the calreticulin gene. Advanced studies have shown that the JAK2 V617F mutation is higher than 95% in PV and approximately 50-60% in ET or PMF (2-4).

The JAK2 V617F mutation, the somatic single-point mutation in the *JAK2* gene in patients with myeloproliferative neoplasia, is a tyrosine kinase mutation, and this mutation occurs in 50-95% of Ph- MPN cases (5). In 2006, mutations in the 515th codon of MPL were identified in 3-8% of cases diagnosed with ET and PMF (6). In 2007, an additional mutation was identified in JAK2 exon 12 in several cases with PV that did not carry the JAK2 V617F allele (7). All of these mutant alleles cause the gain of function due to the activation of tyrosine kinase-dependent cellular signal pathways, especially the JAK-STAT pathway (8). However, activation in the JAK-STAT pathway has also been proven in cases that do not carry the JAK2 or MPL mutation. This evidence indicates that there are still unknown mutations in other genes associated with this pathway, and this is still of great interest to researchers. Also, the cellular and molecular mechanisms involved in the pathophysiology of MPNs have not been fully elucidated yet. Although these mutations cannot fully explain the phenotypic heterogeneity of MPNs, further genetic changes still await identification in approximately 20% of ET and PMF cases (7,8).

The detoxification enzyme NAD(P)H quinone oxidoreductase 1 (NQO1) is a phase 1 enzyme expressed in a wide range of tissue, including the bone marrow in the human body, including the epithelium of various organs. The most important feature of this enzyme is to prevent the formation of reactive and toxic semiquinone intermediate metabolites by reducing two electrons in a single step (9). NQO1 protects cells from free radicals and toxic oxygen metabolites from single-electron reductions catalyzed by cytochrome P450 and other enzymes (10). Also, NQO1 has been shown to play a direct role in protecting against oxidative stress by preventing the redox cycle and free radical formation (11). There are more than 93 single nucleotide polymorphisms (SNPs) discovered in the *NQO1* gene located on the chromosome in 16q22.1. A change in the amino acid sequence of the protein occurs with the C/T change (C609T) (rs1800566) at position 609, the most studied SNP of the NQO1 cDNA encoding the NQO1 enzyme (P187S) (10). Compared to the original type, heterozygous individuals (C/T or NQO1*1/*2) have intermediate activity, while homozygotes (T/T or NQO1*2/*2) are insufficient in NQO1 activity (12). In the presence of this polymorphism, the enzyme's ability to detoxify carcinogens is reduced, which may increase the likelihood of malignant changes in susceptible individuals.

Described as an anti-cancer enzyme, NQO1 plays a protective role in carcinogenesis by modifying internal exposure to bioactive carcinogens. In some studies, NQO1 C609T polymorphism has been associated with the risk of childhood and adult acute lymphoblastic leukemia (ALL) and acute myeloid leukemia (AML) (13-16).

Based on all this information, in our study, we aimed to investigate whether there is a relationship between NQO1 C609T polymorphism and MPN development.

Methods

The study included 119 patients (64 ET, 13 PMF, and 42 PV) who were diagnosed at the İstanbul University İstanbul Faculty of Medicine, Department of Hematology between 1995-2013 and met the 2008 "World Health Organization (WHO)" criteria (17) and 122 healthy controls. The control group consisted of volunteers without hematological

malignancy in their family and with a mean age similar to the patient group. In this study, which was accepted by the Ethics Committee of İstanbul University İstanbul Faculty of Medicine (decision no: 2012/634-1037), an informed consent form was obtained from the subjects before the study. The study was supported by the İstanbul University Scientific Research Fund (project no: BAP/23924). DNA was isolated from the peripheral blood samples with two cc EDTA taken from patients during their routine check-ups between 2012 and 2013 (MagnaPure Compact DNA Isolation Kit, Roche). JAK2 V617F mutation was determined using real-time-polymerase chain reaction (RT-PCR), and *NQO1*2* gene polymorphism was determined using PCR -restriction fragment length polymorphism method.

JAK-2 V617F Real-time-Polymerase Chain Reaction Analysis

The JAK2 V617F assay was performed using the manufacturer's recommended protocol ("JAK2 MutaScreenTM Kit Reference scale" Ipsogen, Luminy Biotech, Marseille, France).

NQO1 Gene Region Analysis

For *NQO1* gene region

Forward primer: 5'-AGTGGCATTCTGCATTTCTGTG-3',

Reverse primer: 5'-GATGGACTTGCCCAAGTGATG-3',

PCR protocol: Following the initial denaturation at 94 °C for 2 minutes; 35 cycles including 45 seconds denaturation at 94 °C, 45 seconds binding at 58 °C, 45 seconds elongation at 72 °C, and finally 5 minutes at 72 °C (18). Genotyping was performed by imaging the obtained PCR products at 37 °C with Hinf1 restriction enzyme and imaging under UV in 2% agarose gel.

Statistical Analysis

The results obtained from the patient and control groups were statistically evaluated with SPSS 21.0 program (SPSS Inc., Chicago, IL, USA). The differences of the parametric data between the two groups were evaluated with the Student's t-test, and the parametric differences between the three groups were evaluated by one-way analysis of variance. The rates were compared with the chi-square test. The results are shown as mean ± standard deviation (SD). The 95% confidence interval, odds ratio, and p value were determined. Results with a p value of <0.05 were considered significant. Fisher's exact test was used when the minimum expected value (MEV) was ≤5, and Pearson's chi-square was used when MEV was ≥25.

Results

Our study cohort included a total of 119 MPN patients, including 64 MP, 13 PMF, and 42 PV patients, and 119 healthy controls. One of our ET patients (CC genotype) and one of our PMF patients (CT genotype) had AML transformation. Clinical information of our patient group is given in Table 1. The mean age of diagnosis of the patients was 55.44 (SD: 14.25), and the age of the patients was higher in the PMF group than the ET group ($p<0.05$). Blood samples were taken for a mean of 6.18 (SD: 5.66) years after the diagnosis. The mean leukocyte count of patients was $10.99x10^9$/L (SD: 5.61), Hemoglobin (Hb) level was 14.34 g/dL (SD: 3.42), platelet count was $744.07x10^9$/L (SD: 421.25), and LDH level was 511.27 U/L (SD: 264.97). Leukocyte count was statistically significantly higher in the PMF group and PV group than in the ET group ($p<0.001$ and $p=0.014$, respectively). Hb level was higher in the ET group than the PMF group ($p<0.001$) and was significantly higher in the PV group than ET

Table 1. Characteristic features of patients diagnosed with essential thrombocythemia, polycythemia vera, and primary myelofibrosis

	ET	PMF	PV	Total
n (%)	64 (53.80%)	13 (10.90%)	42 (35.30%)	119 (100%)
Gender				
Female (%)	27 (42.19%)	8 (61.54)	20 (47.62%)	55 (46.22%)
Male (%)	37 (57.81%)	5 (38.46%)	22 (52.38%)	64 (53.78%)
Mean age (mean ± SD)	53.97±14.31	63.15±11.32[a]	55.29±14.51	55.44±14.25
Disease duration, years (mean ± SD)	6.32±5.64	6.07±6.17	5.96±5.69	6.18±5.66
Leukocyte ($X10^9$/L) (mean ± SD)	9.65±4.26	14.24±9.35[b]	12.30±4.89[c]	10.99±5.60
Hb (g/dL) (X ± SD) (mean ± SD)	13.63±2.02[b]	10.66±2.40	17.79±3.39[b]	14.34±3.42
Platelet ($X10^9$/L) (mean ± SD)	931.66±372.93[b]	551.44±430.27	436.34±274.93	744.07±421.25
LDH (U/L) (mean ± SD)	421.76±181.68	776.00±392.16	566.80±244.93	511.27±264.97
Splenomegaly group				
Splenomegaly (-) (%)	67.40%	0	27.60%	44.80%
Splenomegaly (+) (%)	32.60%	100%[b]	72.40%[b]	55.20%
Bone marrow reticulin fibrous degree	100%	100%	100%	100%
0	20.93%	0	4.54%	12.90%
1	58.14%	0	54.56%	48.10%
2	20.93%	8.33%	18.18%	18.20%
3	0	75%	18.18%	16.90%
4	0	16.67%	4.54%	3.90%
Use of hydroxyurea (%)	82.80%	76.90%	76.20%	79.80%

[a]: $p<0.05$, [b]: $p<0.001$, [c]: $p=0.014$, ET: essential thrombocythemia, PMF: primary myelofibrosis, PV: polycythemia vera, SD: standard deviation, Hb: hemoglobin, LDH: lactate dehydrogenase

and PMF groups (p<0.001). Platelet count was statistically significantly higher in the ET group than PMF and PV groups (p<0.001 and p<0.001, respectively). Reticulin fibrosis levels were higher in the PMF group than in ET and PV groups. It was observed that reticulin fibrosis levels were increased in ascending order in ET, PV, and PMF groups, respectively. When the PMF group was compared with the ET group, the frequency of splenomegaly was observed to be at high frequency (Fischer's exact test, p<0.001). All PMF patients had splenomegaly. The frequency of splenomegaly was higher in the PV group than in the ET group (Pearson X^2=11.291; p<0.001). There was no difference between the patient groups in terms of hydroxyurea use (p>0.05). JAK2 V617 mutations were detected in 40 (62.5%) ET patients, 8 (61.5%) PMF patients and 33 (78.6%) PV patients (Table 2). Although JAK2 mutation was higher in the PV group than the PMF group, no significant difference was observed (p=0.08). JAK2 V617 mutation distribution was similar between PV and ET and PMF and ET patient groups (p>0.05).

In the statistical evaluation, no relation was found between the presence of splenomegaly and the patient subgroups in terms of the presence of the JAK2 mutation. When NQO1 genotype distribution was examined, rapid metabolizer CC genotype was observed in 42 ET patients (65.6%), 9 PMF (69.2%) patients, and 25 PV (59.5%) patients. Intermediate metabolizer CT genotype was detected in 20 ET (31.3%) patients, four PMF (30.8%) patients and 12 PV (28.6%) patients. The slow metabolizer TT genotype was observed in two ET (3.1%) patients and five PV (11.9%) patients but was not found in PMF patients. When the patients were compared in terms of NQO1*2 polymorphism, no difference was observed between the ET, PMF, and PV groups in terms of genotypes (p>0.05). CC genotype was observed in 85 (69.7%) controls. CT genotype was found in 35 (28.7%) controls, and the TT genotype was found in two (1.6%) controls. When the entire patient group and the control group were examined in terms of genotype distributions, there was no statistically significant difference (p>0.05) (Table 3). In the entire patient group and patient subgroups, it was observed that NQO1 alleles were not related to age, gender, disease duration, leukocyte, platelet, Hb, and bone marrow reticulin fibrosis levels (p>0.05). In the PV group, the platelet count was significantly higher in those carrying the NQO1 T allele (p=0.047). No difference was observed in other patient subgroups.

According to the distribution of NQO1 polymorphism in patient subgroups and total patient group, the presence of JAK2 mutation was examined (Table 4). In terms of the co-existence of NQO1 polymorphism and JAK2 mutation, the patient and control groups were similar (p>0.05). No difference was observed among the patient subgroups (p>0.05).

Discussion

Classic Ph- MPNs are a family of clonal chronic hematological malignancies, including PV, ET, and PMF (19). Transformation of

Table 2. JAK2 V617F mutation distribution of patients diagnosed with essential thrombocythemia, primary myelofibrosis, and polycythemia vera

	ET, n (%)	PMF, n (%)	PV, n (%)	Total, n (%)
JAK2 V617F Mutation				
Yes	40 (62.5%)	8 (61.5%)	33 (78.6%)	81 (68.1%)
No	24 (37.5%)	5 (38.5%)	9 (21.4%)	38 (31.9%)

ET: essential thrombocythemia, PMF: primary myelofibrosis, PV: polycythemia vera, JAK2: janus kinase 2

Table 3. Distribution of NAD(P)H quinone oxidoreductase-1 (C>T) polymorphism genotypes between patient and control groups

Genotype	MPN group (n=119)				Control group (n=122)
	ET (n=64)	PMF (n=13)	PV (n=42)	Total (n=119)	
RM (CC)	42 (65.6%)	9 (69.2%)	25 (59.5%)	76 (63.9%)	85 (69.7%)
IM (CT)	20 (31.3%)	4 (30.8%)	12 (28.6%)	36 (30.2%)	35 (28.7%)
SM (TT)	2 (3.1%)	0 (0%)	5 (11.9%)	7 (5.9%)	2 (1.6%)

MPN: myeloproliferative neoplasm, ET: essential thrombocythemia, PMF: primary myelofibrosis, PV: polycythemia vera, RM: Rapid metabolizer, IM: intermediate metabolizer, SM: slow metabolizer

Table 4. Relationship between NAD(P)H quinone oxidoreductase-1 (C>T) polymorphism and JAK2 V617F mutation in patient groups

NQO1 (C>T)		JAK2 V617F mutation		Total, n (%)
		(+) n (%)	(-) n (%)	
ET	CC	17 (70.8%)	25 (62.5%)	42 (65.6%)
	CT+TT	7 (29.2%)	15 (37.5%)	22 (34.4%)
PMF	CC	2 (40%)	7 (87.5%)	9 (69.2%)
	CT+TT	3 (60%)	1 (12.5%)	4 (30.8%)
PV	CC	5 (55.6%)	20 (60.6%)	25 (59.5%)
	CT+TT	4 (44.4%)	13(39.4%)	17 (40.5%)
Total	CC	24 (63.2%)	52 (64.2%)	76 (63.9%)
	CT+TT	14 (36.8%)	29 (35.8%)	43 (36.1%)

NQO1: NAD(P)H quinone oxidoreductase-1, JAK2: janus kinase 2, ET: essential thrombocythemia, PV: polycythemia vera, PMF: primary myelofibrosis

MPNs into AML is characterized by a 20% blast in the bone marrow or peripheral blood, according to the WHO, and is one of the most feared complications of MPN. Approximately 5-10% of all MPNs progress to AML within ten years after diagnosis, which occurs in 1% of ET cases, 4% of PV cases, and 20% of PMF cases. Although the risk factors leading to this transformation have not been entirely determined, advanced age (>60 years) and exposure to chemotherapy are known to increase the risk of transformation. The molecular basis of this progression has not yet been clarified and remains attractive as a current research area (20,21).

In our study, it was aimed to investigate the prevalence of NQO1 C609T polymorphism, which causes loss of enzyme activity and which may be a risk factor in the etiology of specific hematopoietic malignancies in the Turkish community of MPN patients.

In *NQO1* gene defective mice, the increase in myeloid cells in the peripheral blood, bone marrow, and granulocytes significantly causes myeloid cell hyperplasia (22). NQO1-/-g-radiation mice have also been shown to develop the myeloproliferative disease, including loss of spleen follicular structure and bone marrow hypercellularity due to the infestation of granulocytes and megakaryocytes in the blood granulocytes and bone marrow myeloid cells. These results provide direct evidence that mice with NQO1 deficiency are highly prone to developing the myeloproliferative disease (23). The association between NQO1 C609T polymorphism and the risk of acute leukemia has been reported from the studies conducted since 2000, but these data are still insufficient. In their study with 82 patients with different types of hematopoietic malignancies and 99 healthy controls in 2011, Lozic et al. (24) reported that NQO1 C609T polymorphism was more common in adult patients with myeloid disorder compared to adult controls (p=0.0267). The results of the meta-analysis study conducted by Cuiping Li and Yang Zhou (25). in 2014 show that NQO1 C609T polymorphism is a significant genetic risk factor in ALL. As a result of another meta-analysis study, NQO1 C609T polymorphism was found to be associated with increased ALL risk (26). According to the study results of Ouerhani et al. (27), the NQO1 C609T polymorphism has been reported concerning the increased risk of ALL in the Tunisian population. According to the results of Smith et al. (28), null or low NQO1 activity caused by the inheritance of the C609T allele has been reported to be associated with an increased risk of de novo acute leukemia in adults. It has also been reported that there is a statistically significant relationship between NQO1 C609T polymorphism and the risk of chronic myelocytic leukemia (29).

As a result, in most of the studies, NQO1 C609T polymorphism has been associated with increased hematopoietic malignancies. However, the data of the only study conducted by Sirma et al. (30) in the Turkish population on this issue provide evidence that the NQO1 C609T polymorphism does not play a role in the etiology of *de novo* pediatric acute leukemia. We think that these different results between studies may result from differences in ethnic origin and sample sizes.

Conclusion

Our study is the first study to examine the relationship between NQO1 C609T polymorphism and MPN in the Turkish population. Our results are that there is no relationship between NQO1*2 polymorphism and MPN. It is also inferred that the NQO1 enzyme variants, which are important in the activation of detoxification and procarcinogens, do not increase the formation of the JAK2 V617F mutation, which is common in MPN patients. We think that the results should be supported by increasing the number of patient and control groups.

Ethics Committee Approval: In this study, which was accepted by the Ethics Committee of İstanbul University İstanbul Faculty of Medicine (decision no: 2012/634-1037).

Informed Consent: Informed consent form was obtained from the subjects before the study.

Peer-review: Externally peer-reviewed.

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The Evaluation of Thrombospondin 1 Levels in Patients with Acromegaly

Akromegali Hastalarında Thrombospondin 1 Düzeylerinin Değerlendirilmesi

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ABSTRACT

Introduction: Acromegaly is a rare disease caused by overproduction of growth hormone (GH) secreted from a benign pituitary adenoma. It has been shown in many studies that the incidence of cancer is increased in patients with acromegaly with increased GH and insulin-like growth factor-1 (IGF-1). Especially, colon cancer is more common. Thrombospondin-1 (TSP-1) is a glycoprotein that is thought to play an important role in cancer formation. This study aimed to evaluate circulating TSP-1 levels in patients with acromegaly.

Methods: This study was a case-control study. Fifty-two patients with active acromegaly with GH <1 and IGF-1 values higher than age were included in the study. Twenty-six age and gender-matched volunteers without acromegaly were included as the control group. Height, weight, and biochemical evaluations of all volunteers and acromegaly patients were performed. Serum TSP-1 levels were evaluated by ELISA.

Results: The median age and gender distribution of the two groups were similar (p=0.313, p=0.148, respectively). Fasting plasma glucose and lipid parameters were not different between the groups. GH and IGF-1 were higher in patients with acromegaly (p=0.001 for both). Although TSP-1 levels were lower in the acromegaly group, the differences were not statistically significant (p=0.183). There was no correlation between TSP-1 and GH (p=0.265) and IGF-1 (p=0.131).

Conclusion: It is known that the prevalence of cancer, especially colon cancer, is increased in patients with acromegaly. It has been shown in many studies that decreased TSP-1 level plays a role in cancer development. In particular, its association with somatostatin receptors suggests that it may play an important role in the future follow-up of acromegaly and treatment options. We think that studies evaluating TSP-1 levels in patients with acromegaly should be performed, especially in large patient groups diagnosed with colon cancer. Our study is the first to evaluate the level of TSP-1 in patients with acromegaly.

Keywords: Acromegaly, thrombospondin, colon cancer

ÖZ

Amaç: Akromegali genellikle iyi huylu hipofiz adenomundan salgılanan büyüme hormonunun (BH) aşırı üretimi sonucu ortaya çıkan nadir bir hastalıktır. Akromegali hastalarında artmış BH ve insülin benzeri büyüme faktörü-1 (IGF-1) ile kanser görülme sıklığının da arttığı birçok çalışmada gösterilmiştir. Özellikle kolon kanserleri daha sık görülmektedir. Trombospondin-1 (TSP-1) kanser oluşumunda önemli bir rol oynadığı düşünülen bir glikoproteindir. Bu çalışmada akromegali hastalarında dolaşımdaki TSP-1 düzeylerinin değerlendirilmesi amaçlanmıştır.

Yöntemler: Bu bir olgu kontrol çalışmasıdır. Çalışmamıza takip altında olan BH <1 olan ve yaşa göre beklenen IGF-1 değeri yüksek olan aktif akromegali olan 52 hasta dahil edildi. Kontrol grubu olarak yaş ve cinsiyet yönünden benzer, akromegali tanısı olmayan 26 gönüllü alındı. Tüm gönüllüler ve akromegali hastalarının boy, kilo ve biyokimyasal değerlendirmeleri yapıldı. Serum TSP-1 düzeyleri ELISA ile değerlendirildi.

Bulgular: İki grupta medyan yaş ve cinsiyet dağılımı benzerdi (sırasıyla; p=0,313, p=0,148). Açlık plazma glukozu ve lipid parametrelerinde gruplar arasında anlamlı fark yoktu. GH ve IGF-1 akromegali hastalarında daha yüksekti (Her ikisi de, p=0,001). TSP-1 düzeyleri akromegali grubunda düşük olsa da, farklar istatistiksel olarak anlamlı değildi (p=0,183). TSP-1 ile BH (p=0,265) ve IGF-1 (p=0,131) arasında korelasyon saptanmadı.

Sonuç: Akromegali hastalarında kanser ve özellikle de kolon kanseri prevalansının arttığı bilinmektedir. Azalmış TSP-1 düzeyinin kanser gelişiminde rol oynadığı birçok çalışmada gösterilmiştir. Özellikle somatostatin reseptörleri ile ilişkili olması gelecekte akromegali takibinde ve tedavi seçenekleri konusunda önemli bir rol oynayabileceğini düşündürmektedir. Akromegali hastalarında TSP-1 düzeyinin değerlendirildiği ve özellikle kolon kanseri tanısı almış olan geniş hasta gruplarında değerlendirildiği çalışmalar yapılması gerektiğini düşünüyoruz. Çalışmamız akromegali hastalarında TSP-1 düzeyinin değerlendirildiği ilk çalışma olması yönünden değerlidir.

Anahtar Kelimeler: Akromegali, thrombospondin, kolon kanseri

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Introduction

Acromegaly is a clinical disease caused by excessive growth hormone (GH) secretion. The most common cause of acromegaly is GH secreting benign adenoma in the anterior pituitary. The onset of acromegaly is insidious and generally slow. The most classic clinical feature of acromegaly is acral growth with the growth of hands and feet and coarse facial features. The clinical features of acromegaly are caused by increased GH and resulting in liver-derived insulin-like growth factor-1 (IGF-1) secretion (1). The prevalence of acromegaly ranges from 2.8-13.7, with an annual incidence of 0.2-11.1 cases/100.000 individuals. However, the actual incidence is estimated to be higher (2,3). The most common cause of morbidity and mortality in acromegalic patients is cardiovascular complications and cancer. There are three treatment methods in the management of acromegaly, including surgery, medical, and radiotherapy. Treatment targets in acromegaly are keeping serum IGF-1 levels within age- and gender-appropriate range values and GH levels below <1.0 ng/mL (4). In patients with uncontrolled and delayed diagnosis of acromegaly, colon, thyroid, breast, and prostate cancers are more common, but many organs are reported to have increased cancer risk (5). In a meta-analysis in which 23 studies were evaluated, an increased risk was observed in colorectal, breast, thyroid, gastric, and urinary system cancers in acromegaly patients without treatment response (6). It has been reported that acromegaly patients in whom target values are not reached or who are not treated may have an increased risk for hyperplastic polyps, adenomas, and cancer in the digestive system and especially in the colon (7,8). In patients with acromegaly, high serum IGF-1 levels were associated with the presence of colorectal neoplasms, increased prevalence of colorectal adenoma, and adenocarcinoma (9,10).

The extracellular matrix (ECM) is a complex supporting structure between the cells in mammalian tissue. ECM consists of proteins that are reshaped continuously due to the balance between synthesis, storage, and disintegration. Changes in the interaction between cells and ECM may facilitate the formation and progression of cancer cells (11). Thrombospondins (TSP-1 to -5) are glycoproteins secreted into the ECM (12). The first identified glycoprotein is TSP-1. TSP-1 is a 450-kDa protein that is considered an inhibitor of angiogenesis. TSP-1 plays an essential role in pathological angiogenesis, inflammation, and cancer formation. Studies have shown that TSP-1 inhibits cell proliferation and induces apoptosis. It is thought that TSP-1 performs some anti-angiogenic functions via receptor CD36 (13).

Somatostatin (SST) is a 14-amino acid peptide hormone released from the D-cells of the islets of the pancreas and the hypothalamus. It is also known as a factor that inhibits the release of GH from the pituitary gland. SST is also an inhibitory hormone in the pancreas and gastrointestinal tract. In addition to exocrine and endocrine functions, it has a vasoconstrictor effect and an inhibitory effect on intestinal absorption (14). SST receptors (SSTRs) are numbered between 1 and 5. Each receptor is more densely present in different tissues. Especially, SSTR2 and SSTR5 are more common in pituitary adenomas, and many endocrine tumors (15). SST analogs and SSTR antagonists used in the medical treatment of acromegaly are particularly useful on these SSTR2 and SSRT5 (4). In a study by Laklai et al. (16) including patients with pancreatic cancer, decreased SSTR2 activity was shown in patients with low TSP-1. Low SSTR2 activity may also be effective in the treatment response of acromegaly patients. Decreased levels of TSP-1 may make it challenging to control IGF-1 and GH values in acromegaly. Uncontrolled acromegaly increases the risk of malignant diseases.

In this study, we aimed to evaluate the TSP-1 level, which is thought to be an effective molecule in cancer formation and progression in acromegaly patients. This is the first study in the literature to evaluate the level of TSP-1 in acromegaly patients.

Methods

This study included 52 uncontrolled acromegaly patients with high IGF-1 values according to age or with random GH level >1 who were followed in the outpatient clinic of Endocrinology and Metabolic Diseases Clinic of our hospital between September 1, 2017, and September 1, 2018. Also, 26 age- and gender-matched controls without acromegaly were included. Participants with a history of malignant disease, pregnancy, and those under 18 years of age were not included in the study.

Ethical Evaluation

The Ethics Committee of University of Health Sciences, İzmir Tepecik Training and Research Hospital approved this study on 17.08.2017 (decision no: 7). Our study was consistent with the Declaration of Helsinki. Written consent was obtained from all participants.

Biochemical Evaluation

Blood samples were taken from all volunteers following 10 hours of fasting by venous puncture technique with ethylene diamine tetraacetic acid without the need for anticoagulants for biochemical tests. Serum samples were obtained after centrifugation of blood samples at 4.000 rpm for 10 minutes. Obtained serum samples were put into polypropylene tubes and stored at -80 °C until biochemical analysis. Serum GH and IGF-1 levels were measured by chemiluminescent immunometric assay (Immulite XPi, Siemens, Germany). The normal range of IGF-1 was age-dependent (21-25 years: 116-358; 26-30 years: 117-329; 31-35 years: 115-307; 36-40 years: 109-284; 41-45 years: 101-267; 46-50 years: 94-252; 51-55 years: 87-238; 56-60 years: 81-225; 61-65 years: 75-212 years, 66-70 years: 69-200; 71-75 years: 64-188; 76–80 years: 59-177 ng/mL). Serum concentrations of TSP-1 were determined by solid-phase ELISA (Quantikine ELISA human TSP-1 immunoassay kit, R&D systems, Minneapolis, MN, USA). The sensitivity limit of the test was 0.355 ng/mL. The intra-variability and inter-assay coefficients of the test were below 6.7% and 6.2%, respectively.

Statistical Analysis

All data were analyzed using SPSS version 15 (SSPS, Inc., Chicago, Illinois, USA). An alpha level of 0.05 was used to determine statistically significant differences. Shapiro-Wilk tests were performed to evaluate the normality of distribution. Results were reported as mean ± standard deviation (SD) for normally distributed continuous variables, and as minimum, maximum, and median for non-normally distributed variables. Variables between acromegaly and control groups were compared by independent t-test. The non-normally distributed variables

were compared by the Mann-Whitney U test between acromegaly and control group. Non-parametric Spearman test was used for correlation analysis. In this study, $p<0.05$ was considered statistically significant.

Results

A total of 78 volunteers, including 52 active acromegaly patients and 26 controls, were included in the study. There was no difference between sex and gender distributions in both groups. There was no difference between the groups in terms of rates of type 2 diabetes and hypertension. Demographic and anthropometric data are shown in Table 1. While GH and IGF-1 levels were higher ($p<0.001$, both) in patients with acromegaly, there was no significant difference in other biochemical parameters. Although the median value of the TSP-1 level was lower in the acromegaly group compared to the control group, no statistically significant difference was observed ($p=0.183$). The results are presented in Table 2. There was no significant correlation between TSP-1 and GH ($p=0.265$) and IGF-1 ($p=0.131$). There was no significant correlation between TSP-1 and other parameters. The correlation analysis results between the TSP-1 level and demographic, anthropometric, and biochemical parameters are shown in Table 3.

Discussion

Colorectal neoplasms are known to be a common complication of acromegaly. In a meta-analysis, 701 acromegaly patients and 1573 non-acromegaly controls were evaluated, and it was found that the risk of colon cancer was 4.3 times higher in acromegaly patients compared to the control group (8). However, data on the prevalence of colorectal neoplasms in patients with acromegaly are limited. Also, different factors

Table 1. Comparison of demographic and anthropometric characteristics between acromegaly and control groups

	Acromegaly, n=52	Control, n=26	p
Gender			
Male/Female	27/25	9/17	0.148
Age			
Mean ± SD*, years	45.77±12.37	45.07±14.4	0.313
Type 2 diabetes			
Yes/No	25/27	11/15	0.81
Hypertension			
Yes/No	20/32	10/16	0.60
Systolic blood pressure, mm/Hg**, median (min-max)	119 (92-147)	125 (105-160)	0.093
Diastolic blood pressure, mm/Hg**, median (min-max)	80 (65-105)	147.5 (65-100)	0.284
Weight, kg**, median (min-max)	88.7 (54-158)	79 (55.4-127.1)	0.042
Body mass index, kg/m2**, median (min-max)	30.6 (20.83-50.2)	26.21 (20.35-44.71)	0.179
Waist circumference, cm**, median (min-max)	95 (70-126)	94 (75-118)	0.380

*SD: standard deviation, **min: minimum, max: maximum

Table 2. Comparison of biochemical parameters between acromegaly and control groups

	Acromegaly (n=52) Median (min-max)	Control (n=26) Median (min-max)	p
Glucose, mg/dL	104 (77-338)	96 (77-220)	0.284
LDL, mg/dL	115 (60-208)	123.5 (84-246)	0.309
HDL, mg/dL	49 (20-104)	48 (32-97)	0.867
Triglyceride, mg/dL	134 (31-314)	149 (54-532)	0.539
Total cholesterol, mg/dL	189 (139-307)	218 (155-364)	0.088
Urea, mg/dL	29 (12-86)	27 (16-52)	0.925
Uric acid, mg/dL	4.8 (2.60-7.43)	5.15 (3.20-8.80)	0.245
Creatinine, mg/dL	0.9 (0.6-1.7)	0.9 (0.7-1.3)	0.168
AST, U/L	18 (11-34)	22 (12-45)	0.765
ALT, U/L	14 (7-32)	19 (10-56)	0.243
TSH, mIU/L	1.0 (0.35-3.34)	1.34 (0.37-5.84)	0.137
IGF1, µg/L	408 (95-1357)	210 (60-290)	0.001*
Growth hormone, µg/L	1.9 (0.08-40)	0.115 (0.05-0.90)	0.001*
Thrombospondin-1, ng/mL	840.8 (122.2-9445)	1275.05 (179.8-7418)	0.183

*SD: standard deviation, min: minimum, max: maximum, HDL: high-density lipoprotein, LDL: low-density lipoprotein, ALT: alanine aminotransferase, AST: aspartate aminotransferase, TSH: thyrotropin-stimulating hormone, IGF-1: insulin-like growth factor 1

Table 3. Correlation analysis between thrombospondin-1 levels and independent variables

	r	p
Age, years	0.199	0.75
Growth hormone	-0.127	0.265
IGF-1	0.199	0.131
Weight, kg	0.098	0.401
Body mass index, kg/m^2	0.056	0.357
Waist circumference, cm	0.171	0.142
Glucose, mg/dL	0.028	0.802
LDL, mg/dL	0.111	0.335
HDL, mg/dL	-0.090	0.427
Triglyceride, mg/dL	0.088	0.438
Total cholesterol, mg/dL	0.160	0.157
Urea, mg/dL	0.019	0.868
Uric acid, mg/dL	0.121	0.299
Creatinine, mg/dL	0.046	0.688
AST, U/L	0.003	0.977
ALT, U/L	0.032	0.776
TSH, mIU/L	-0.110	0.335

HDL: high-density lipoprotein, LDL: low-density lipoprotein, ALT: alanine aminotransferase, AST: aspartate aminotransferase, TSH: thyrotropin-stimulating hormone, IGF-1: insulin-like growth factor 1

have been suggested in the etiopathogenesis of colorectal neoplasms in patients with acromegaly. High serum IGF-1 levels were associated with increased colorectal adenoma. Increased IGF-1 and IGF-binding protein 3 ratio were associated with the prevalence of colon adenocarcinoma (9,10). Among tumor angiogenesis inhibiting agents, TSP-1 is the most well-known molecule. Low TSP-1 levels have been reported in ovarian cancer (17), gastric cancer (18), breast cancer (19), lung cancer (20), renal cell carcinoma (21), and pancreatic cancer (16). Also, a low TSP-1 level was associated with increased tumor invasion and metastasis rate, and it was thought to be effective in the prognosis of malignancies. These studies suggest that low or incomplete expression of TSP-1 is associated with advanced-stage cancer. Also, decreased expression of TSP-1 is associated with poor prognosis in colorectal carcinoma (22-24). In a study by Jo et al. (23), it was observed that TSP-1 was strongly expressed in healthy colon epithelial cells, whereas TSP-1 loss was observed in early colonic adenomas and TSP-1 was not detected in epithelial cells of invasive colon cancer. In a study performed in patients with colon cancer treated with bevacizumab, Marisi et al. (25) found that high TSP-1 level was associated with better clinical outcome. This study suggests that increased circulating levels of TSP-1 can also be used as a marker for evaluating treatment response. In our study, although the median value of the TSP-1 level was lower in acromegaly patients compared to the control group, no statistically significant difference was observed. Low TSP-1 level and SST relationship may make it challenging to control IGF-1 and GH values in acromegaly. In our study, no significant correlation was found between TSP-1 and IGF-1 and GH levels.

Although our study was the first to evaluate TSP-1 levels in acromegaly patients, there were some limitations. First, the sample size was relatively small. Secondly, all patients with a history of cancer were not included in our study in the selection process. Thirdly, this study was carried out in a single tertiary center.

Conclusion

Our study is the first study in the literature to evaluate the level of TSP-1 in acromegaly. It has been shown in many studies that low TSP-1 levels play a role, especially in the development of colorectal cancer. TSP-1 can be used as a marker for assessing treatment response in the future in acromegaly patients and for monitoring increased cancer risk. It may be more appropriate to perform studies evaluating the TSP-1 level with uncontrolled acromegaly patients who were diagnosed with colon cancer.

Ethics Committee Approval: The Ethics Committee of University of Health Sciences, İzmir Tepecik Training and Research Hospital approved this study on 17.08.2017 (decision no: 7).

Informed Consent: Written consent was obtained from all participants.

Peer-review: Externally and internally peer-reviewed.

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Evaluation of the Functional and Radiological Outcomes of Serial Casting as an Initial Treatment of Congenital Scoliosis

Konjenital Skolyozun İlk Tedavisi Olarak Seri Alçılama Tekniğinin Fonksiyonel ve Radyolojik Sonuçlarının Değerlendirilmesi

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ABSTRACT

Introduction: It has been reported that serial casting is an effective treatment method in early-onset idiopathic scoliosis, but its role in congenital scoliosis has not been clarified. This study aimed to evaluate the efficacy of serial castings in young children with congenital scoliosis and to discuss whether serial casting can be used effectively to delay surgical procedures.

Methods: Patients between the ages 2 and 5 years with congenital scoliosis who had a Cobb angle above 25 degrees and had not undergone any surgical treatment between 2016 and 2019 were included in this study. Cast changes were performed at 3-month intervals. Radiographic evaluations were performed on posteroanterior and lateral orthoroentgenograms in the cast on the first day after the cast application and at the last follow-up visit.

Results: A total of 10 patients (6 female, 4 male) with long congenital curves with mixed type formation or segmentation anomalies were included in the study. The mean number of cast applications was 4 for each patient (range: 3 to 6). The initial casting age was 3.2 (range: 2-4 years). The mean follow-up period was 15.1 months (range: 12-23 months). The mean precasting Cobb angle was 61.9±13.7 degrees (range: 38-76 degrees), which was reduced to 43.4±12.8 degrees (range: 24-58 degrees) after the initial casting, and it was 48.4±12.6 degrees (range: 28-63 degrees) at the latest follow-up. The mean precasting T1-T12 length was 223±27.3 mm (range: 176-271 mm). After the initial cast application, T1-T12 length was 241.8±27.5 mm (range: 189-285 mm). At the last follow-up, the average T1-T12 length was 254±27.6 mm (198, 290 mm).

Conclusion: In early-onset scoliosis, even when growth-friendly methods were occupied, spontaneous fusion may develop. Serial casting under anesthesia allows for lengthening by controlling the progression of the deformity. This method can provide more time for the patient to delay surgical interventions.

Keywords: Congenital scoliosis, serial casting, non-surgical scoliosis treatment

ÖZ

Amaç: Erken başlangıçlı idiyopatik skolyozda seri alçılama uygulamalarının etkili bir tedavi yöntemi olduğu literatürde bildirilmiştir; ancak bu yöntemin konjenital skolyozdaki rolü yeteri kadar araştırılmamıştır. Bu çalışmanın amacı, konjenital skolyozlu çocuklarda seri alçılama tekniğinin etkinliğini değerlendirmek ve seri alçılamanın cerrahi prosedürleri geciktirmek için etkili bir şekilde kullanılıp kullanılamayacağını tartışmaktır.

Yöntemler: 2016-2019 yılları arasında 2-5 yaş aralığında etiyolojisi konjenital skolyoz olan ve Cobb açısı 25 derece üzerinde olup herhangi bir cerrahi tedavi geçirmemiş hastalar çalışmaya dahil edildi. Rutin alçı değişiklikleri 3 aylık aralıklarla yapıldı. Alçı uygulamasından sonraki gün ve son takipte alçıda alınan standart ayakta posteroanterior ve lateral radyografilerde spinal ölçümleri yapıldı.

Bulgular: Çalışmaya 6 kız, 4 erkek toplam 10 hasta dahil edildi. Ortalama 4 kez alçılama yapıldı (minimum: 3-maximum: 6). İlk alçılama yaşı 3,2'ydi (minimum: 2- maximum 4). Takip süremiz ortalama 15,1 ay (minimum: 12, maximum: 23) idi. Alçılama öncesi ortalama Cobb açısı 61,9±13,7 derece (38-76), ilk alçılama sonrası 43,4±12,8 derece (24-58), son takipte 48,4±12,6 derece (28-63) olarak değerlendirildi. Alçılama öncesi T1-T12 uzunluğu 223±27,3 mm (176-271 mm) idi. İlk alçılama sonrası T1-T12 uzunluğu 241,8±27,5 mm (189-285) idi. Son takipte, ortalama T1-T12 uzunluğu 254±27,6 mm (198, 290 mm) olarak bulundu.

Sonuç: Erken başlangıçlı skolyozda büyüme dostu cerrahi yöntemlerde dahi cerrahi prosedürler nedeniyle anatomik yapılar zarar görmekte ve sponton füzyon gelişebilmektedir. Anestezi altında seri alçılama, eğriliğin ilerlemesini kontrol altında tutarak boy uzamasına imkan vermekte ve cerrahi müdahaleleri geciktirmekte güvenli ve etkili bir zaman kazanımı sağlamaktadır. Hastaya herhangi bir cerrahi girişim yapılmadığı için cerrahi girişimlerin büyüme üzerine olumsuz etkileri olmadığından boy uzamasını engellemeden hastalara zaman kazanımı sağlanabilmektedir.

Anahtar Kelimeler: Konjenital skolyoz, seri alçılama, cerrahi dışı skolyoz tedavisi

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Introduction

Treatment of progressive scoliosis of the immature spine presents several challenges. It has been shown that the rapid growth period of the thoracic spine is from birth to the age of 5 years, with a 50% increase in the spine length during this period (1). If left untreated during this period, progressive curves may result in significant thoracic deformity, resulting in life-threatening cardiopulmonary pathologies (2).

It is accepted in long-term studies that, fusion applied before five-years of age may have adverse effects on pulmonary function via disrupting the growth process of the thoracic cage, thus decreased thoracic spinal height is correlated with reduced forced vital capacity and is associated with low quality of life (3). Therefore, growth-friendly methods have gained popularity in most early-onset progressive scoliosis types, and in congenital deformities to control the progression of curves and to preserve natural growth (4-6). Various growing instruments have been developed which maintain spine alignment while allowing the spine and thoracic cage to grow. For this purpose, conventional and magnetically controlled growing rods and vertical expandable prosthetic titanium rib aim to delay definitive fusion surgery (7). In these methods, complications such as rod fracture, infection, crankshaft phenomenon, rigid and incompatible thoracic wall formation can be observed (7,8). Therefore, non-invasive procedures like serial casting are accepted as an alternative treatment modality in early-onset scoliosis (2). In the literature, it has been shown that serial casting can be used to delay surgery by preventing risks and complications associated with recurrent surgical procedures in growth-friendly methods. Despite the current evidence confirming that the initiation of casting at an early age is an important factor in the success of the treatment, the effect of the curvature etiology on treatment outcomes is not clearly defined in the literature (2).

This study aimed to evaluate the efficacy of serial castings in young children with congenital scoliosis and to discuss whether serial casting can be used effectively to delay surgical procedures.

Methods

Ten patients with congenital scoliosis who underwent serial casting in a single center between 2016 and 2019 were included in the study. Patients under the age of 5-years with a Cobb angle above 25 degrees with congenital scoliosis (falls into three categories; failure of formation, failure of segmentation and mixed), who had not undergone any surgical treatment, and who were followed for at least 12 months were included in the study. A minimum number of three casts were applied to all patients. Patients whose families refused casting treatment and who had a progression of the curvature more than 10 degrees in the course of casting were excluded from the study. Written consent was obtained from the parents of all patients.

Cast application was performed under general anesthesia on a modified Cotrel frame. Mehta modification of the Cotrel-Morel technique was used in cast application (9). The apex points of the kyphotic deformities were well padded to prevent skin lesions. The deformity is gently corrected by traction, derotation, and lateral pressure. The cast is molded over the rib hump to flatten it. An anterior window is made to relieve the chest and abdomen while preventing the lower ribs from rotating. No activity restriction was performed in patients. Casts were changed every 12 weeks. Radiographic evaluations were performed on posteroanterior and lateral orthoroentgenograms in the cast on the first day after the cast application and at the last follow-up visit.

The obtained data included age, Cobb angles of congenital coronal curves (angle formed by the intersection of two lines, one parallel to the endplate of the superior end vertebra and the other parallel to the endplate of the inferior end vertebra), sagittal deformity magnitude, coronal balance (measuring the distance between the central sacral vertical line and the plumb line), sagittal balance (measuring the distance between the posterosuperior aspect of the S1 vertebral body and the plumb line), thoracic height (The T1-T12 height was measured on full-length posteroanterior radiographs of the spine using the vertical distance from the middle of), number of cast applications, follow-up time and complications.

The study was approved by Metin Sabancı Baltalimanı Bone Diseases Training and Research Hospital Ethics Committee (decision no: 254, date: 12.11.2018).

Statistical Analysis

SPSS 15.0 for Windows (IBM Corporation, Chicago, IL, USA) was used for statistical analyses. The descriptive statistic was expressed as numbers and percentages for categorical variables. The mean and the standard deviation were used as a numerical variable for normally distributed data. The mean of T1-S1 length, Cobb angle, Kyphosis angle, coronal and sagittal balance at the preoperative period, early postoperative period, and the late postoperative period were analyzed with Repeated measures ANOVA with a Greenhouse-Geisser correction. Post hoc tests using Bonferroni correction determined differences among preoperative, early postoperative, or late postoperative period. A p value of <0.05 was considered to indicate significance. Inter-observer agreement was assessed using the "κ" statistical test. A kappa value between 0.8 and 1 was considered a perfect agreement.

Results

A total of 10 patients (6 female, 4 male) with long congenital curves with mixed type formation or segmentation anomalies were included in the study. The mean number of cast applications was 4 for each patient (range: 3 to 6). The initial casting age was 3.2 (range: 2-4 years). The mean follow-up period was 15.1 months (range: 12-23 months). In two patients, the decision to perform a growing rod was made. In one of them, curve progression exceeded more than 10 degrees within the cast, and for the other, the parents of the patient refused further cast application.

The mean pre-casting Cobb angle was 61.9±13.7 degrees (range: 38 to 76 degrees), which was reduced to 43.4±12.8 degrees (range: 24 to 58 degrees) after the initial casting, and it was 48.4±12.6 degrees (range: 28-63 degrees) at the latest follow-up. When the pre-casting and after the initial casting values have been compared, we found that there was a statistically significant improvement ($p=0.001$), but statistical significance was impaired when initial correction magnitude and last

follow up values were compared (p=0.275). The mean pre-casting T1-T12 length was 223±27.3 mm (range: 176-271 mm). After the initial cast application, T1-T12 length was 241.8±27.5 mm (range: 189-285). At the last follow-up, the mean T1-T12 length was found to be 254±27.6 mm (range: 198-290 mm). When the pre-casting, initial cast application, and last follow up values were compared, we found that statistically significant elongation was achieved (p=0.001).

The mean pre-casting kyphosis angle was 28.8±8.2 degrees (range: 20-45 degrees). It was reduced to 25.2±5.1 degrees (range: 15-32 degrees) after the initial cast application and was measured as 26.7±6.2 degrees (range: 17-38 degrees) at the last follow-up. We observed no significant change in the kyphosis angle (p=0.242).

The mean pre-casting after initial casting and last follow-up lumbar lordosis angles were 37.1±11.4, 34.3±12.2, and 34.8±12.5, respectively (p=0.799). We did not observe a statistically significant improvement in coronal and sagittal balance values (p=0.622 and p=0.066) (Table 1).

None of the patients had a neurological deficit or thoracic wall deformity. Two patients had mild skin irritation and improved with local wound care. In one patient, the cast treatment was temporarily terminated due to pneumonia and continued after the infection was resolved. The treatment of patients continues. Curvature was kept under control during one-year period, and no surgical treatment was required (Figure 1). Only in one patient, scoliosis progressed, and cast treatment was discontinued.

Discussion

Congenital spinal deformities have a broad spectrum ranging from mild asymptomatic curves to deformities that are concomitant with neurological and cardiopulmonary pathologies. The course of the deformity varies according to the localization and type of malformation and the age of the patient (10). Although the studies are limited, patients with congenital anomalies have been reported to have limited lung capacity, which can cause severe disability and even death if left untreated (5). Conservative treatment methods in congenital scoliosis are thought to be ineffective due to the rigid nature of the deformity but may have a corrective effect only on compensatory curves. Therefore, it has been reported that surgical treatment should be preferred if there is a high risk of progression in this patient group (5,11). In selected patients with congenital scoliosis, convex hemiepiphysiodesis has been used as a growth-friendly surgical method for many years, but it has been shown that hemiepiphysiodesis may have unpredictable outcomes (12). However, hemiepiphysiodesis is still an invasive procedure regardless of technique and may not always result in the resolution of the deformity.

Various methods have been described for the cast application in scoliosis. The casting was first proposed 50 years ago by Cotrel and Morel for the treatment of scoliosis. Cotrel and Morel's EDF (elongation, derotation, flexion) correction technique is a frequently used conservative treatment modality (13). Cotrel and Morel stated that the technique is suitable not only to prevent progression but also to regress structural vertebral and thoracic deformities (13). One of the most commonly used methods is the Risser casting method applied from three points (14). This technique cannot adequately intervene in rotational abnormalities and can cause significant rib deformations and chest constriction, especially in younger children with the flexible bone (15). Due to the problems that emerged in growth-friendly surgical treatment modalities, serial casting treatment has gained popularity once more, and it has been shown that serial casting can provide successful results in idiopathic and non-idiopathic deformities (2,16). Fletcher et al. (16) reported that spinal growth was sustained during the casting treatment in 12 idiopathic and 17 non-idiopathic scoliosis patients, and 39 months delay for the surgery had been achieved and 72.4% of the patients avoided growing rod surgery. In their series of 39 patients including 17 non-idiopathic scoliosis patients, Baulesh et al. (2) concluded that non-idiopathic deformities had less resolution of the deformity when compared to idiopathic deformities, on the other hand, the thoracic growth was sustained and the initial surgical procedure for the growing rod was delayed by an average of two years. In our study, the mean pre-casting T1-S1 length was 223±27.3 mm (range: 176-271 mm). After initial casting, T1-S1 lengths were 241.8±27.5 mm (range: 189-285). At the last follow-up, the mean T1-S1 length was measured to be 254±27.6 mm (range: 198-290 mm). When we compared pre-casting, initial casting, and last follow-up values, we concluded that the growth was similar to other studies in the literature and was statistically significant (p<0.05).

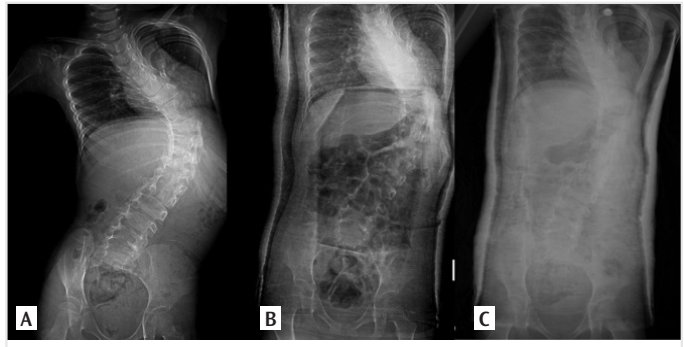

Figure 1. A) Twenty-eight month-old girl congenital scoliosis pre-casting posteroanterior radiography, B) after initial cast application, C) at five cast application

Table 1. General overview of the patient data

	Preoperative	Initial casting	Last follow up	p
T1-T12 length (mm)	223±27.3	241.8±27.5	254±27.6	<0.001
Cobb angle (degree)	61.9±13.7	43.4±12.8	48.4±12.6	<0.001
T3-T12 kyphosis angle (degree)	28.8±8.2	25.2±5.1	26.7±6.2	0.242
L1-S1 lumbar lordosis (degree)	37.1±11.4	34.3±12.2	34.8±12.5	0.799
Coronal balance (mm)	17.5±17.11	15.9±7.6	12.9±5.9	0.622
Sagittal balance (mm)	47.5±26.1	31.1±27.6	35.1±23.3	0.066

In their study of 16 early-onset scoliosis (EOS) patients (8 idiopathic, 8 syndromic), Waldron et al. (17) stated that the Cobb angle decreased from 73 degrees to 45 degrees, and progression to surgery was observed in 31% of the patients and complications due to casting in 19%. In their study, Demirkiran et al. (5) stated that the correction was obtained after the first cast application and was maintained during the treatment period, and the majority of the correction was provided during the first cast application, and the magnitude of the deformity correction was similar to the growing rod application. In our study, Cobb angle was 61.9±13.7 degrees (range: 38-76 degrees), which was corrected to 43.4±12.8 degrees (range: 24-58 degrees) after the initial casting, and it was 48.4±12.6 degrees (range: 28-63 degrees) at the latest follow-up. When we compared pre-casting and early post-casting values, we found that there was a statistically significant improvement ($p<0.05$), but we found that statistical significance was impaired when we compared initial casting and last follow-up values ($p=0.275$). Although the improvement achieved with the first casting was somewhat lost in the subsequent casts, the present correction could be preserved and saved us time for surgical treatment. In only one patient, due to the progression of the curvature, we switched to a growing rod, and we did not encounter any severe complications.

A potential disadvantage of the casting treatment is that it may have adverse effects on pulmonary function due to the advanced deformity of the rib cage, especially in patients with congenital scoliosis. Despite the deterioration of pulmonary parameters after the initial cast application, it was reported in the literature that it returned to the baseline values after the second cast application (18). In our study, we did not evaluate pulmonary functions before and after cast applications, but in our patient group, cast application was well tolerated in terms of pulmonary functions, and we did not encounter any problems. In one patient, the cast treatment was temporarily terminated due to pneumonia and continued after the infection had resolved. Two patients had mild skin irritation due to the insufficiency of skin and subcutaneous tissues. Between the cast changes, one week of rest and local care was given, and the skin irritation was resolved without any problems.

Although modern cast application requires general endotracheal anesthesia, it is far from surgical trauma, infection risk, and neurological complications that surgical treatment methods may cause. With cast treatment, the goal is to delay the surgery until sufficient vertebral growth has been achieved for satisfactory respiratory function (4). The purpose of treatment in EOS is to control the progression of deformity to delay or eliminate the need for spinal fusion without compromising normal spinal, thoracic, and pulmonary growth (6). Serial casting is an effective growth protection technique and is less invasive than the growth-friendly surgical techniques described. We estimate that most of the children in this study will be followed up with a growth-preserving surgical method following serial cast treatment. Regardless of the severity of the etiology of the deformity, we assume that serial cast treatment should be considered as an alternative method to delay growth-sparing surgery. The limitation of our study is the low number of patients and incomplete cast treatments.

Conclusion

We concluded that serial casting treatment is an effective alternative for delaying surgical interventions and avoiding surgical risks in congenital scoliosis patients.

Ethics Committee Approval: The study was approved by Metin Sabancı Baltalimanı Bone Diseases Training and Research Hospital Ethics Committee (decision no:254, date: 12.11.2018).

Informed Consent: Written consent was obtained from the parents of all patients.

Peer-review: Externally peer-reviewed.

Author Contributions: Surgical and Medical Practices - S.E., D.K., A.A.; Concept - D.K.; Data Collection and/or Processing - A.Ç.; Analysis and/or Interpretation - A.Ç.; Literature Search - S.E., E.A.Ü.; Writing Manuscript - S.E., E.A.Ü.

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The Predictive Role of Neurobiochemical Markers in Multiple Sclerosis

Multiple Sklerozda Nörobiyokimyasal Belirteçlerin Prediktif Rolü

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ABSTRACT

Introduction: Multiple sclerosis (MS) is the most common, chronic, inflammatory, demyelinating disease of the central nervous system. We aimed to evaluate the levels of some neurobiochemical markers in order to evaluate their predictive role in MS.

Methods: Fifty-one patients with a diagnosis of MS and 37 healthy subjects were included in the study. The patients with MS were diagnosed by a skilled neurologist based on the medical history and physical examination according to revised McDonald criteria. Neuron-specific enolase (NSE) and S100B levels were measured by electrochemiluminescence immunoassay. Glial fibrillary acidic protein (GFAP) and myelin basic protein (MBP) were measured by quantitive sandwich enzyme immunoassay technique with a commercially available ELISA kit.

Results: There was a significant difference in NSE levels between the patient and the control groups. No significant difference was determined between the patient and the control groups in terms of S100B, MBP, and GFAP levels. S100B levels were positively correlated with Expanded Disability Status scale scores.

Conclusion: Our findings indicated that NSE levels are significantly lower in MS patients. However, NSE levels should not be used alone at discriminating the disease. Multifactorial evaluation should be done during the diagnosis and follow-up of MS.

Keywords: Multiple sclerosis, GFAP, MBP, NSE, S100B

ÖZ

Amaç: Multiple skleroz (MS), merkezi sinir sisteminin en sık görülen, kronik, enflamatuvar ve demiyelinizan hastalığıdır. Bu çalışmada MS'deki prediktif rollerini değerlendirmek için bazı nörobiyokimyasal belirteçlerin seviyelerinin değerlendirilmesi amaçlanmıştır.

Yöntemler: Çalışmaya MS tanısı olan 51 hasta ve 37 sağlıklı birey dahil edilmiştir. MS tanısı, hastalara tıbbi öykü ve revize edilmiş McDonald kriterlerine göre yapılan fizik muayene temelinde uzman bir nörolog tarafından konmuştur. Nöron spesifik enolaz ve S100B düzeyleri elektrokemilüminesan immünoassay ile ölçülmüştür. Glial fibriller asidik protein (GFAP) ve miyelin bazik protein (MBP), ticari olarak temin edilebilen ELISA kiti ile sandviç enzim immün yöntem ile ölçülmüştür.

Bulgular: Hasta ve kontrol grubu arasında NSE düzeylerinde anlamlı bir fark bulunmuştur. Hasta ve kontrol grubu arasında S100B, MBP ve GFAP düzeyleri açısından anlamlı fark saptanmamıştır. S100B seviyeleri Genişletilmiş Özürlülük Durum ölçeği skorları ile pozitif olarak koreleydi.

Sonuç: Bulgularımız NSE düzeylerinin MS hastalarında anlamlı derecede düşük olduğunu göstermiştir. Ancak, NSE düzeyleri hastalığın ayırıcı tanısında tek başına kullanılmamalıdır. MS'nin tanısı ve takibinde multifaktöriyel değerlendirme yapılmalıdır.

Anahtar Kelimeler: Multiple skleroz, GFAP, MBP, NSE, S100B

Introduction

Multiple sclerosis (MS) is the most common, chronic, inflammatory, demyelinating disease of the central nervous system (CNS) that usually appears in young adults (1,2). Autoimmune response to self-antigens destroys the axons and myelin sheath and causes the formation of characteristic plaques of MS in the white matter of CNS (3). Clinical appearance changes according to the localization of inflammation, demyelination, axonal, and neuronal loss (2,4). Three major forms for MS were described: relapsing-remitting MS (RRMS) that there is a period of recovery after the symptoms, secondary progressive MS (SPMS) that

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irreversible and progressive destruction occurs after the remission, and primary progressive MS (PPMS) that progressive disability is presented from disease onset. Also, patients usually present initially with clinically isolated syndrome (CIS) defined as a first neurological episode and followed by subacute clinical events, and the symptoms spontaneously remit (2,5).

The destruction of the neuronal tissue of CNS in MS according to the demyelination and axonal degeneration causes the release of proteins such as the calcium-binding protein S100B, neuron-specific enolase (NSE), myelin basic protein (MBP) and glial fibrillary acidic protein (GFAP). S100B is one of the calcium-binding proteins, usually exists in astroglial cells. Elevated levels in both serum and cerebrospinal fluid (CSF) can be detected according to the CNS damage (6). NSE is a glycolytic enzyme, found in neuron cytoplasms and neuroendocrine cells. It is released by damaged neurons, and increased levels are found in both serum and CSF (7). MBP is one of the major proteins of the myelin sheath, and demyelination causes elevated levels of MBP in serum and CSF (8). GFAP exists in the glial cells of the CNS and composes the major protein of the astrocytic cytoskeleton (9).

In the current study, we aimed to evaluate the levels of some neurobiochemical markers in order to evaluate their predictive role in MS.

Methods

Patients

Fifty-one patients who admitted to Neurology Department of Ankara Numune Training and Research Hospital with a diagnosis of MS and 37 healthy subjects were included in the study. Informed consent was obtained from all participants included in the study. The patients with MS were diagnosed by a skilled neurologist based on the medical history and physical examination according to revised McDonald criteria (10). The patients were divided into four groups according to their clinical presentation as CIS (n=4), RRMS (n=36), SPMS (n=8) and PPMS (n=3). The disability status was assessed using the Expanded Disability Status scale (EDSS) score (11). The magnetic resonance imaging (MRI) scans of patients were recorded, and the lesions were categorized into four groups according to their location as periventricular, supratentorial, infratentorial, and spinal.

All study procedures were approved by the Ethics Committee of Ankara Numune Training and Research Hospital (decision no: 2012/498).

Blood Samples and Measurement

Venous blood samples were collected in vacutainer tubes and centrifuged at 1300 g for 10 minutes. The sera were separated and stored at -80 °C until analysis.

NSE and S100B levels were measured by electrochemiluminescence immunoassay technique in Cobas E601 analyzer (Roche Diagnostics, Germany). Detection range of NSE assay was 0.050-370 ng/mL. Detection range of S100B assay was 0.005-39 ng/mL. Intermediate precision of S100B assay was 2.8%, 2.0% and 2.4% in concentrations of 0.08 ng/mL, 0.24 ng/mL and 2.13 ng/mL respectively. Intermediate precision of NSE assay was 4.4%, 3.9% and 4.4% in concentrations of 2.58 ng/mL, 9.32 ng/mL and 88.0 ng/mL.

GFAP was measured by a quantitive sandwich enzyme immunoassay technique with a commercially available ELISA kit (Uscn Life Science Inc, PRC). The detection range of the assay was 0.312-20 ng/mL. Intra and inter-assay precision were <10% and <12% respectively.

MBP was measured by a commercially available ELISA kit (Uscn Life Science Inc, PRC) using a quantitive sandwich enzyme immunoassay technique. The detection range of the assay was 15.6-1000 pg/mL. Intra and inter-assay precision were <10% and <12% respectively.

Statistical Analysis

The findings of this study were analyzed with "Statistical Package for Social Sciences for Windows" (SPSS version 18) software. The conformity of continuous variables to normal distribution was tested with the Kolmogorov-Smirnov test. The descriptive statistics of continuous variables were expressed as mean ± standard deviation with normal distribution and median (minimum-maximum) with non-normal distribution. The presence of a statistically significant difference between the groups in terms of continuous variables was examined with Student's t-test for parametric and Mann-Whitney U test for non-parametric variables. The presence of a correlation between the groups was searched with Spearman's rho tests. Chi-square test was used for comparison of qualitative data. The area under curve (AUC) was calculated with a receiver operating characteristic (ROC) analysis for statistically significant parameters. P<0.05 was considered the threshold of statistical significance for all tests.

Result

Fifty-one MS patients (38 males, 13 females) and 37 control subjects (25 males, 12 females) were included in the study. The mean age of the patient group was 36.39±9.8 years, and the mean age of the control group was 40.45±12.37 years. No significant difference was found in terms of age between the patient and the control groups. There was a significant difference in NSE levels between the patient and the control groups (p=0.039). No significant difference was determined between the patient and the control groups in terms of S100B, MBP, and GFAP levels (p>0.05) (Table 1).

Table 1. Comparison of neuron-specific enolase, S100B, glial fibrillary acidic protein and myelin basic protein levels of patient and control groups

	Patient group (n=51)	Control group (n=37)	p
NSE (ng/mL)	10.21 (1.29-106.60)	11.26 (7.75-34.62)	0.039
S100B (ng/mL)	0.036 (0.01-0.21)	0.037 (0.02-0.23)	0.477
GFAP (ng/mL)	4.50±3.47	5.48±2.81	0.862
MBP (pg/mL)	0.27 (0.16-0.50)	0.27 (0.19-0.37)	0.162

NSE: neuron-specific enolase, GFAP: glial fibrillary acidic protein, MBP: myelin basic protein

There was a statistically significant correlation between NSE levels and S100B levels (r=0.316; p=0.003). No significant correlation was found between NSE levels and age, GFAP, and MBP levels (Table 2).

The patients were divided into four subgroups according to their clinical phenotype. Seven point eight percent of 51 patients had CIS, 70.6% had RRMS, 15.6% had SPMS, and 3.9% had progressive relapsing MS.

The disability status was evaluated according to EDSS. The patients were divided into two groups according to their EDSS scores. Group 1 with EDSS scores of 1 to 4.5 referred to patients who can walk without any aid and Group 2 with EDSS scores of 5 to 9.5 were defined by the impairment to walking, based on measures of impairment in eight functional systems. S100B levels were positively correlated with EDSS scores (r=0.282, p=0.045).

The lesions of MS patients were categorized into four groups according to their MRI scans and localization. Forty-seven patients had periventricular lesions, 46 patients had supratentorial lesions, 28 had infratentorial lesions, and 24 had spinal lesions. MBP levels were statistically different in patients with supratentorial lesions and without supratentorial lesions (p=0.007).

ROC analysis was performed for serum NSE levels in MS patients (Figure 1). AUC value for NSE was 0.63 (Figure 1). Classifying the accuracy of a diagnostic test was evaluated according to the point system: 0.90-1: excellent, 0.80-0.90: good, 0.70-0.80: fair, 0.60-0.70: poor, 0.50-0.60: fail. NSE was "poor" at distinguishing MS patients from healthy subjects.

Table 2. Correlation between neuron-specific enolase levels and age, S100B, glial fibrillary acidic protein, myelin basic protein

NSE (ng/mL)	r	p
Age	-0.003	0.978
S100B (ng/mL)	0.316	0.003
GFAP (ng/mL)	0.151	0.160
MBP (pg/mL)	0.171	0.112

NSE: neuron-specific enolase, GFAP: glial fibrillary acidic protein, MBP: myelin basic protein

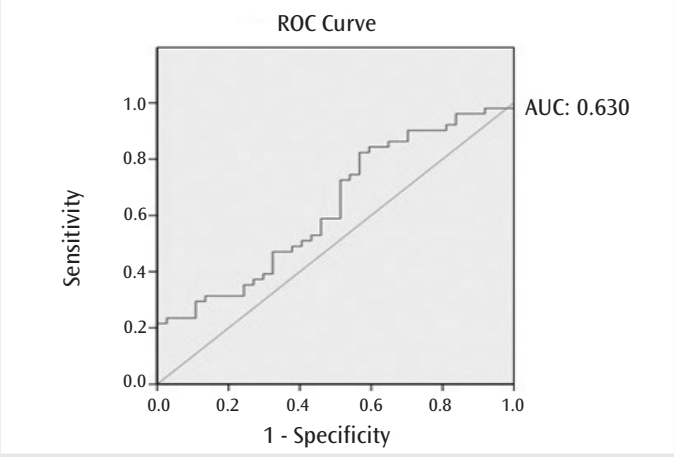

Figure 1. Receiver operating characteristic curve analysis evaluating serum neuron-specific enolase levels in multiple sclerosis patients

ROC: receiver operating characteristic, AUC: area under curve

Discussion

In the current study, we evaluated the levels of NSE and S100B, GFAP, and MBP levels in patients with MS. Serum NSE levels were statistically significantly lower in the MS group. Serum S100B, GFAP, and MBP levels were not different between the groups. ROC curve analysis showed that serum NSE levels might be a discriminative factor in MS.

In this study, we found similar results with Hein Nee Maier et al. (12) in terms of significantly lower plasma NSE levels of the patients than the healthy control group. In previous studies, NSE levels were higher in patients after traumatic brain injury, stroke, and intracerebral hemorrhage according to the neuronal cell damage (13). We also expected NSE levels to be higher in the patient group as it is known that neuronal loss is one of the reasons for neurological impairment in MS (14). In our study, the patients were in a steady-state of chronic disease, and neuronal loss is usually seen in the early phases of the disease. So, we believe that lower concentrations of NSE levels were associated with a decrease in neuronal loss. Koch et al. (15) also studied NSE levels in MS patients and found lower NSE levels in MS patients, especially in the progressive disease course. They indicated that lower levels of NSE might be related to reduced neuronal activity. Jongen et al. (16) also studied NSE levels in RRMS and SPMS patients and reported that tissue damage was more evident between relapses than in progressive phase, and thus NSE levels were higher in patients with RRMS diagnosis. It is unclear that lower plasma NSE levels in MS patients are associated with a reduced neuronal activity or neuronal loss.

S100B is a marker of glial damage, and increased levels were associated with cerebral damage and/or disruption of the blood-brain barrier (BBB). In previous studies, S100B levels were increased in patients with traumatic brain injury, global hypoxia and cerebral ischemia, stroke (7,17-19). In patients with cerebral ischemia, it was supported that S100B is released during the acute phase of the event (20). The short half-life of S100B and this hypothesis can explain the similar concentrations of S100B levels in patients and the control subjects as none of the patients were in the acute phase of the disease. However, we found a positive correlation between S100B levels and the disability status of the patients. The patients with higher disability scores had higher S100B levels.

We found a positive correlation between NSE and S100B levels. A limitation of our study was the fact of the release of S100B and NSE can be originated from non-neuronal tissues: S100B can be released from fat tissue, and NSE can be found in neuroendocrine cells. Also, both of the markers can be released after trauma and inflammation. We tried to minimize these effects by including the patients in clinically steady-state and the control group without any acute or chronic illnesses.

GFAP is one of the major intermediate filament proteins of astrocytes (21). These filaments form astrogliosis and the major dominant protein in chronic MS lesions (22). In previous studies, GFAP levels were shown to be increased in acute damages of brain cells like traumatic brain injury and hydrocephalus (7,23). In studies with MS patients, GFAP levels were found to be significantly elevated in comparison with the control subjects, and GFAP was offered to be a potential biomarker of disease severity of MS (9,24). In contrast with these studies, we did not find

any difference between patient and control groups in terms of GFAP levels. GFAP is rapidly and remarkably released from brain cells after severe acute brain injury, ischemia, and slowly and mildly elevation of GFAP can be seen in chronic neurological diseases (25,26). GFAP passes through systemic circulation via disrupted BBB. As we studied GFAP levels from the sera of patients, it can be the reason that we did not find elevated levels of GFAP in stable MS patients.

MBP is the major component of the myelin sheath and is essential to the demyelination process. CNS inflammation, BBB breakdown, and the resulting demyelination and neuronal damage and loss are characteristics of MS (27). We did not find any difference between patient and control groups. However, patients with supratentorial lesions had statistically higher MBP levels. In contrast with our study, several studies found elevated MBP levels in patients with MS suggesting a biochemical marker of MS disease activity (8,28). MBP levels increase in CSF following the injury and pass through systemic circulation after the break down of the BBB. The dilution effect can cause lower MBP concentrations according to the larger blood volume. This hypothesis may explain our MBP results.

One of the limitations of our study was that we did not study CSF samples of subjects in terms of biochemical markers. Also, our patients were in the steady-state of the disease; patients in acute or subacute state were not evaluated.

Conclusion

Evaluating several neurobiochemical marker levels can suggest the state or prognosis of the disease. Our findings indicated that NSE levels were significantly lower in MS patients. However, NSE levels should not be used alone for distinguishing the disease. It should be combined with symptoms, physical and neurological examination, MRI scans, and other markers.

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Management and Clinical Outcomes of Iatrogenic Injury Secondary to Endoscopic Retrograde Cholangiopancreatography

Endoskopik Retrograd Kolanjiopankreatografi Sonrasındaki İşleme Bağlı Yaralanmaların Tedavisi ve Klinik Sonuçlarımız

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ABSTRACT

Introduction: Perforation secondary to endoscopic retrograde cholangiopancreatography (ERCP) is a rare complication but a problematic one because of associated morbidity and mortality. In our study, we aimed to define correct timing for surgery, to analyze and present our results on suitable methods that can be used in the surgical management of perforation secondary to ERCP done for various indications.

Methods: The data were collected from 19 patients who underwent surgery for perforation secondary to ERCP. We retrospectively analyzed clinical and demographic characteristics with the treatment outcomes of these patients.

Results: The mean age of the patients was 57 years (range: 16-92). The ERCP procedure was for therapeutic purposes in all the patients. Perforation mostly occurred during sphincterectomy, as was seen in 12 patients (63%). The patients underwent surgical intervention at a mean of 42.5 hours (range: 3-192) after perforation. Postoperative mortality occurred in seven patients (36.8%). The mean hospitalization period was 16.5 days (range: 11-49).

Conclusion: Duodenal perforation is an ERCP-related complication that carries high mortality and morbidity risks, even in experienced tertiary centers. When perforation is suspected, these patients should immediately be referred to experienced centers/units for further management. Careful scrutiny of clinical and radiological findings is critical in choosing the appropriate surgical intervention.

Keywords: Endoscopic retrograde cholangiopancreatography, perforation, surgical treatment

ÖZ

Amaç: Endoskopik retrograd kolanjiyo pankreatografi (ERCP) sonrası perforasyon nadir görülen bir komplikasyondur; ancak morbidite ve mortalitesi nedeniyle yönetimi problemlidir. Çalışmamızda, ameliyat için doğru zamanlamayı tanımlamayı, çeşitli endikasyonlar için yapılan ERCP sonrası perforasyonun cerrahi tedavisinde kullanılabilecek uygun yöntemler üzerine sonuçları analiz etmeyi ve sunmayı amaçladık.

Yöntemler: ERCP'ye bağlı perforasyon nedeniyle ameliyat edilen 19 hastanın verileri toplandı. Bu hastaların tedavi sonuçları ile klinik ve demografik özellikleri retrospektif olarak incelendi.

Bulgular: Hastaların yaş ortalaması 57 (16-92) idi. ERCP prosedürü tüm hastalarda tedavi amaçlı uygulanmıştı. Hastaların 12'sinde (%63) perforasyon sfinkterektomi sırasında meydana geldi. Hastalara perforasyon sonrası ortalama 42.5 (3-192) saat sonra cerrahi girişim uygulandı. Postoperatif 7 (%36,8) hastada mortalite gözlendi. Ortalama hastanede kalış süresi 16,5 gün (11-49) idi.

Sonuç: Duodenal perforasyon, deneyimli merkezlerde bile yüksek mortalite ve morbidite riskleri taşıyan ERCP ilişkili bir komplikasyondur. Perforasyondan şüphelenildiğinde, bu hastalar derhal ileri tedavi için deneyimli merkezlere/birimlere yönlendirilmelidir. Uygun cerrahi müdahalenin seçiminde klinik ve radyolojik bulguların dikkatli bir şekilde incelenmesi çok önemlidir.

Anahtar Kelimeler: Endoskopik retrograd kolanjiyopankreatografi, perforasyon, cerrahi tedavi

Introduction

Endoscopic retrograde cholangiopancreatography (ERCP) has been used in managing diseases of the biliary system and pancreas since its introduction in 1974 (1). With an increased scope of use, the reported complication rate ranges from 5-10% and includes pancreatitis, hemorrhage, and perforation (2). Perforation secondary to ERCP is a rare complication but a problematic one because of associated morbidity and mortality. Early suspicion and directed diagnostic imaging are vital for proper diagnosis and effective clinical management. Non-surgical interventions like endoscopic repair and stenting are options if the injury

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is detected during the procedure. In some cases, monitoring the patient without any intervention is sufficient. However, based on the type of injury and its clinical outcomes, surgery is inevitable in some cases (3). The type of surgical intervention varies considerably depending on the location of the perforation, its size, and time from perforation to initiation of the treatment. There is no consensus on a uniform procedure of choice.

In this study, we aimed to define the correct timing for surgery, to analyze and present results on suitable methods that can be used in the surgical management of perforation secondary to ERCP.

Methods

In this study, we retrospectively analyzed the files of 19 patients who underwent surgery for perforation secondary to ERCP done between January 1999 and January 2019. This study was approved by the Başkent University Medical and Health Sciences Research Board Ethical Committee (decision no: KA19/84). Informed consent was obtained from all patients before ERCP and before surgical intervention for ERCP-related perforation. The demographic and clinical characteristics of the patients, ERCP indications, location and type of perforation, diagnostic methods, the clinical course of the condition, timing for the operation, the surgical procedure applied, hospitalization period, postoperative complications, and treatment outcomes were analyzed. Based on the surgery notes and computerized tomography (CT) reports, it was noted that Stapfer classification was used to grade the ERCP perforations (Table 1) (4).

The vital signs and the physical examination of the patients, leukocytosis, the presence of peritoneal free air, or fluid on CT and/or abdominal X-ray were also analyzed. The patients were categorized into two distinct groups: The early surgical group included the patients in whom perforation was diagnosed during the procedure and surgery was performed within 6 hours, whereas the rest of the patients were classified as the late surgical group.

Statistical Analysis

The SPSS 17.0 program was used to statistically analyze the demographic and clinical data of patients as well as to interpret the outcomes.

Results

Eleven (58%) of the 19 patients were female, while 8 (42%) were male, with a mean age of 57 years (range: 16-92). One patient was under 18 years of age and was operated on by a pediatric surgeon. Eight of the patients had severe comorbidities such as hypertension, diabetes, and coronary artery disease. One patient had cardiopulmonary arrest during the ERCP procedure and was taken to theatre for operation after resuscitation and stabilization of vitals. Thirteen patients (68%) underwent ERCP procedure at our center, whereas the remaining six patients (32%) were referred to our center for further management after ERCP-related injury at other centers. The ERCP procedure was for therapeutic purposes in all the patients, including 14 (74%) for choledocholithiasis and five (26%) for biliary stent placement for various reasons. Perforation mostly occurred during sphincterectomy, as was seen in 12 patients (63%). Perforation occurred in three patients (16%) during stent placement and in two patients (10%) during manipulation of the endoscope. There was no data about the stage at which the perforation occurred in two referred patients (10%). In six patients, perforation was diagnosed during the ERCP procedure and they underwent surgery within six hours. While in the remaining 13 patients, perforation was diagnosed later during the post-ERCP clinical follow-up, hence they underwent surgery late. In these 13 patients (68%), there was abdominal tenderness, fever, and tachycardia. There was subcutaneous emphysema in five patients (26%). There was leukocytosis in 11 patients (58%) at diagnosis. These 11 patients had retroperitoneal or intraperitoneal free air on CT imaging. There were intraperitoneal and retroperitoneal abscess and free fluid in the delayed cases. The patients underwent surgical intervention at a mean of 42.5 hours (range: 3-192) after perforation. According to Stapfer classification, the most common perforation was type 1 that was seen in ten patients (52.6%), while type 2 was seen in five patients (26.3%), and type 3 was seen in one patient (5.3%). Type 4 perforation was seen in three patients (15.8%) (Table 2). In six of the ten patients with type 1 perforation, the site was localized and repaired with primary sutures. In three patients who presented late, primary repair was not plausible due to inflammation and tissue fragility. In one case of advanced cholangiocelullar carcinoma, primary duodenostomy was preferred, as the primary repair was not adequate. One patient with periampullary tumor underwent pancreaticoduodenectomy. All clinical data, surgical procedures utilized, and postoperative results are as shown in detail in Table 3.

Postoperative mortality occurred in seven patients (36.8%). Five patients (26.3%) died due to sepsis-related multiorgan failure on Days 2., 3., 4., 5., and 8., whereas one patient had cardiac arrest due to myocardial infarction (MI) during ERCP procedure and died on Day 3 for cardiac-related reasons. One patient with preexisting hepatic insufficiency had decompensated failure and died on postoperative Day 10. The remaining 12 patients were eventually discharged from the hospital on mean postoperative Day 16.5. However, during perioperative management, two patients developed an intraabdominal abscess, one patient had sepsis, one patient had cardiac arrest during surgery, one patient had

Table 1. Stapfer classification of endoscopic retrograde cholangiopancreatography-related perforations

Perforation type	Localization
Type 1	Lateral or medial duodenal wall, away from the ampulla
Type 2	Periampullary vateri injury
Type 3	Biliary tree or pancreatic duct injury
Type 4	Only free air in the retroperitoneal space

Table 2. Patient distribution according to type of injury

Type	Patient number (n=19)	Ratio (%)
Type 1	10	52.6
Type 2	5	26.3
Type 3	1	5.3
Type 4	3	15.8

hemorrhage at the gastroenterostomy site, and one patient developed surgical site infection (Table 3).

Discussion

ERCP-related complications are very low when the procedure is done by experienced endoscopists. There is a reported complication rate of 4-16% (5,6). Duodenal perforation is the most common cause of mortality and morbidity among these complications (7). Perforation is more common in ERCP done for therapeutic reasons with a mean occurrence rate of 1% and related mortality of around 50% in cases that require surgical intervention (7,8). In our study, all patients underwent therapeutic ERCP with a mortality rate of 36% after surgical intervention.

Based on etiology and perforation, drainage, or conservative medical treatment options are other management options (9). However, in some cases, based on worsening clinical condition or change in imaging findings, surgical intervention may be necessary. In our study, perforation was not detected during the procedure in 13 patients (68.4%). The decision for surgery was made during in-patient follow up due to worsening clinical conditions of these patients. The most important step in the management of ERCP related perforations is determining which patients require surgery and the correct timing of the intervention (10). Besides the detection of perforation during the procedure, patient's clinical condition and CT findings play a vital role in deciding surgical intervention. In our study, patients had three out of four clinical signs, such as fever, tachycardia, leukocytosis, and abdominal tenderness, as described by Knudson et al. (11) for ERCP-related perforation.

A study highlighted that physical examination was more valuable than radiological findings as intraperitoneal free air can be managed conservatively just as in peptic ulcer perforation (12). CT findings for perforation may include duodenal wall thickening, intramural air, retroperitoneal adipose tissue contamination or collection, extravasation of contrast, and intraperitoneal, retroperitoneal or subcutaneous free air (13). In our study, perforation was detected early in six patients, and they underwent immediate surgical intervention. In these patients who

Table 3. Demographic and clinical characteristics of patients

	G/A	Comorbidity	Diagnosis	Reason of Injury	Type	Surgical therapy	Complication	Day	Result
1	M/79	CAD + DM	Choledocholithiasis	Cannulation	2	CE+T-Tube+GE	Sepsis	20	Discharged
2	F/72	HT	Choledocholithiasis	Cannulation	1	CE+T-Tube+GE	Sepsis	5	Exitus
3	M/75	COPD	Choledocholithiasis	Endoscope	1	PS+CE+T-Tube+C	None	11	Discharged
4	F/92	HT	Choledocholithiasis	Cannulation	1	PS+CE+T-Tube+C	Wound inf.	30	Discharged
5	F/36	None	Chronic pancreatitis	Stenting	1	PS+GE	Sepsis	3	Exitus
6	M/61	None	Pancreatic cancer	Stenting	2	Whipple procedure	IA abscess	30	Discharged
7	K/69	None	Choledocholithiasis	Cannulation	2	PS+CE+T-Tube+C	None	22	Discharged
8	F/61	None	Bile Fistulae	Stenting	3	CE+T-Tube+GE	Sepsis	2	Exitus
9	M/78	CAD	Biliary tree cancer	Endoscope	1	DO+GE	MI	3	Exitus
10	M/61	None	Choledocholithiasis	Cannulation	4	CE+T-Tube+GE	Sepsis	8	Exitus
11	F/51	None	Choledocholithiasis	Cannulation	1	PS+CE+T-Tube+C	None	18	Discharged
12	F/16	None	Choledocholithiasis	Cannulation	2	CE+T-Tube+GE+C	None	21	Discharged
13	M/46	HT + HBV	Choledocholithiasis	Cannulation	1	PS+CE+T-Tube+C+GE	MOF	10	Exitus
14	F/23	None	Choledocholithiasis	Cannulation	1	CE+T-Tube+GE	None	19	Discharged
15	M/56	None	Choledocholithiasis	Cannulation	1	CE+T-Tube+GE	IA abscess	13	Discharged
16	F/81	HT	Choledocholithiasis	Cannulation	1	CE+T-Tube+C+GE+BTV	Sepsis	4	Exitus
17	M/29	None	Choledocholithiasis	Unknown	4	CE+T-Tube+GE	HIE	32	Discharged
18	F/42	None	Choledocholithiasis	Cannulation	2	CE+T-Tube+GE+C	Bleeding	13	Discharged
19	F/48	DM	Mirizzi syndrome	Unknown	4	CE+T-Tube+GE+C	None	49	Discharged

G/A: gender/age, M: male, F: female, CAD: coronary artery disease; DM: diabetes mellitus, HT: hypertension, COPD: chronic obstructive pulmonary disease, HBV: hepatitis B virus, CT: computerized tomography, CE: choledochal exploration, GE: gastroenterestomy, PS: primary suture DO: duodenostomy, C: cholecystectomy, BTV: bilateral truncal vagotomy, IA: intraabdominal, MI: myocardial infarction, MOF: multi-organ failure, HIE: hypoxic ischemic encephalopathy

Table 4. Mortality and complication rate

	Early surgery (<6 hours), n=6 patients	Late surgery (>6 hours), n=13 patients
Mortality	1 patient (16.6%)	6 patients (46.1%)
Complications	1 patient (16.6%) Wound infection	5 patients (38.4%) IA abscess: 2 patients Sepsis: 1 patient Bleeding from GE: 1 patient HIE: 1 patient

GE: gastroenterostomy, HIE: hypoxic-ischemic encephalopathy, IA: intraabdominal

received surgical intervention within six hours, mortality rate was 16.6%, as only one patient died. This patient had cholangiocellular carcinoma and died due to MI during treatment. Infection was observed in just one patient. Eleven of the 13 delayed surgery patients required a CT to fully diagnose the perforation, whereas, in two patients, clinical examination was sufficient to decide to operate. Mortality occurred in six patients (46.1%) in this group at a rate observed to be comparably higher than the early group (38.4%). The mean hospitalization period was 17.5 days for the early group but 16 days for the late group. There was no statistically significant difference noted in the hospitalization periods. The mortality and complication rates of the individual groups are as shared in Table 4.

In line with published data, our study underscores the importance of early diagnosis and timely intervention. As much as the choice of surgical procedure is dependent on size or type of perforation and extent of inflammation, the main aim of the procedure should be to close the defect and divert gastric content away from the duodenum. Pylorus should be excluded from gastrojejunostomy diversion to inhibit the activation of pancreatic enzymes (14). External drainage of the bile, where possible, will also hasten to heal. In our cases, the patients received definitive intervention for the underlying pathology that necessitated ERCP by choledochotomy and gallstone extraction followed by T-tube placement. External drainage of bile was therefore provided by T-tube. In delayed cases with extensive intraabdominal infection and cases of periampullary malignancy, pyloric exclusion, together with duodenostomy and/or gastrojejunostomy, was carried out. One patient with resectable periampullary tumor underwent a Whipple procedure.

Although postoperative mortality due to ERCP-related perforations varies depending on the patient's age, comorbidities, and timing of surgery, this rate is reported as 9-30% (15,16). Mortality is higher in elderly patients and cases of delayed surgical intervention. In our study, mortality was found to be 36.8%, which is higher than reported rates. This can be attributed to delayed intervention in the three referred cases and advanced age together with comorbidities in the others. Six patients had no postoperative complications. Six patients with complications such as surgical site infections, intraabdominal abscess, and sepsis responded well to medical therapy. These patients were discharged from the hospital on postoperative Day 23 on average. There is no consensus on the surgical procedure of choice for patients with ERCP related perforations. Perforation site, the timing of intervention, and the presence of intraabdominal infection should be considered in decision-making. All studies published on this topic are retrospective in nature and include a limited set of patients. Our study is no exception to this limitation as it is also retrospective and contains a limited number of patients.

Because early diagnosis and intervention reduce mortality and morbidity, patients should be closely monitored after the ERCP procedure. More importantly, difficult ERCP procedure cases, patients with malignancy-related indications for the procedure, and those with suspect clinical course after the ERCP should be monitored for perforation.

Conclusion

In summary, duodenal perforation is an ERCP-related complication that carries high mortality and morbidity risks, even in experienced tertiary centers. Careful scrutiny of clinical and radiological findings is very important in choosing the appropriate surgical intervention.

Ethics Committee Approval: This study was approved by the Başkent University Medical and Health Sciences Research Ethical Committee (decision no: KA19/84).

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Endoscopic Approach to Esophageal Leiomyomas: Single Center Results

Özofagus Leyomiyomlarında Endoskopik Yaklaşım: Tek Merkez Sonuçları

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ABSTRACT

Introduction: Leiomyoma is the most common esophageal benign lesion. There are many treatment methods from endoscopic treatment methods to surgery in the treatment of leiomyoma. In this study, we evaluated the results of our patients who underwent endoscopic mucosal (EMR) and submucosal dissection (ESD) due to esophageal leiomyoma.

Methods: A total of 18 patients who underwent EMR or ESD protocol with the diagnosis of esophageal leiomyoma were included in the study and age, gender, radiological imaging results, endoscopic ultrasonography results, treatment method, pathology results, and outpatient follow-up data of the patients were recorded following retrospective examination of the patient files.

Results: The mean age of the patients was 52.7±13.36 years, and the ratio of female/male was 1/1.25. Incidental lesions were found during endoscopic procedures in six patients (33.3%) due to dyspeptic complaints and in three patients (16.7%) due to dysphagia and during computed tomography in the remaining nine patients (50%) for various reasons. For treatment, three patients underwent EMR, and 15 patients underwent ESD. One patient had a hemorrhage controlled by endoscopic intervention, and no other complications were observed after treatment.

Conclusion: It should be kept in mind that ESD and EMR, which are among the endoscopic treatment methods in the treatment of esophageal leiomyoma, could be safely applied in experienced hands.

Keywords: Esophagus, leiomyoma, endoscopic mucosal resection, endoscopic submucosal dissection

ÖZ

Amaç: En sık gözlenen özofagus benign lezyonları leiomiyomlardır. Leiomiyomların tedavisinde endoskopik tedavi yöntemlerinden cerrahiye kadar birçok tedavi yöntemi bulunmaktadır. Biz de bu çalışmamızda özofagus leiomiyomu sebebiyle endoskopik mukozal (EMR) ve submukozal rezeksiyon (ESD) yapılan hastalarımızın sonuçlarını değerlendirdik.

Yöntemler: Özofageal leiomiyom tanısı ile EMR veya ESD protokolü uygulanan toplam 18 hasta çalışmaya dahil edilmiş olup hastaların hastane dosyalarının retrospektif olarak incelenmesi sonucunda hastaların yaşları, cinsiyetleri, radyolojik görüntüleme sonuçları, endoskopik ultrasonografi sonuçları, uygulanan tedavi yöntemi, patoloji sonuçları ve hastaların poliklinik takip notları kayıt altına alınmıştır.

Bulgular: Hastaların ortalama yaşı 52,7±13,36 yıl olup kadın/erkek oranı 1/1,25'tir. Hastaların 6'sında (%33,3) dispeptik şikayetler nedeniyle yapılan tetkiklerde, 3'ünde (%16,7) disfaji nedeniyle yapılan endoskopik girişimler sırasında, diğer 9 hastada (%50) ise çeşitli sebeplerle çekilen bilgisayarlı tomografiler sırasında insidental olarak lezyonlar bulunmuştur. Tedavi için 3 hastaya EMR, 15 hastaya ise ESD uygulanmıştır. Tedavi sonrası 1 hastada endoskopik müdahale ile durdurulan kanama mevcut olup başka bir komplikasyon gözlenmemiştir.

Sonuç: Özofagus leiomiyomlarının tedavisinde endoskopik tedavi yöntemlerinden olan ESD ve EMR'nin tecrübeli ellerde güvenle uygulanabileceği akılda bulundurulmalıdır.

Anahtar Kelimeler: Özofagus, leiomiyom, endoskopik mukozal rezeksiyon, endoskopik submukozal rezeksiyon

Introduction

Benign lesions of the esophagus are rare lesions and constitute less than 1% of all esophageal lesions, and 2/3 of these lesions are leiomyomas, and the rest are polyps and cysts (1). Although esophageal leiomyomas are seen at any age from the age of 20 to the age of 80, they are most frequently observed between the ages of 40-50 and show similar morphological features as the leiomyomas observed in other organs. Leiomyomas generally originate from muscularis propria and can grow up to 30 cm intraluminally and extraluminally (2-4).

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There are many options in the treatment of benign esophageal lesions, from endoscopic interventions to surgical treatment (1,5). While thoracotomy was the preferred treatment method in the 1990's, later thoracoscopy and laparoscopy replaced it as the more frequently applied treatments (6-9). The developments in endoscopy in the last few decades have caused an increase in the frequency of using endoscopic treatments (10). Endoscopic mucosal resection (EMR) and endoscopic submucosal dissection (ESD), which are among the endoscopic treatment options, can be used safely in experienced hands in benign and early-stage malignant tumors (5,10,11).

In this study, we evaluated the results of our endoscopic treatments for benign esophageal lesions.

Methods

Our study started after obtaining approval from the University of Health Sciences, İstanbul Training and Research Hospital Local Clinical Research Ethics Committee (decision no: 2017, date:11/10/2019), and 38 patients who underwent standard EMR or ESD protocol with the diagnosis of an esophageal mass in the endoscopy unit between 2013-2017 constitute the universe of our study. Informed consent was obtained from all patients before the study. The patients with a pathological diagnosis of leiomyoma were included in the study. Patients with malignant and other benign esophageal pathologies or patients with incomplete data in hospital records were excluded from the study.

The ages, genders, endoscopic ultrasonography (EUS) results, treatment methods, pathology results, and outpatient follow-up data of 18 patients included in the study were recorded. Age and tumor size analysis of the patients were calculated as mean and standard deviation (SD).

Statistical Analysis

Statistical analysis of the data of our study was performed with SPSS version 21.0. Continuous data of the patients were given as mean ± SD, and categorical data were reported as a percentage.

Results

The mean age of 18 patients in our study was 52.7±13.36 years, and female/male (F/M) ratio was 1/1.25. Lesions were detected during endoscopic procedures due to dyspeptic complaints in six patients (33.3%) and dysphagia in three patients (16.7%). In nine patients (50%), incidental lesions were found during thoracoabdominal computed tomography (CT). All patients underwent EUS and thoracoabdominal CT before treatment. In only two of the patients, an endoscopic biopsy was performed to clarify the diagnosis of leiomyoma in CT and EUS, and the pathology results were leiomyoma. Considering the treatment methods, EMR was applied in three patients, and ESD was applied in 15 patients, and the mean size of the excised lesions was 11.1±6.26 mm. The mean diameter of the lesions of patients with symptomatic leiomyoma was 23.3 mm. Considering the location of the lesions, there was one lesion in the cervical esophagus, four lesions in the abdominal esophagus, and 13 lesions in the thoracic esophagus (Table 1).

When the post-treatment complications were evaluated, hemorrhage developed in one patient and was taken under control by using endoscopic cauterization and 9 mm "through the scope clip" (Quick clip®, Olympus, Hamburg, Germany). Perforation and mortality were not observed in any patient. Malign transformation or recurrence was not observed in the mean follow-up of 3.8±1.85 years.

Discussion

The successful surgical operation was performed approximately 65 years after esophageal leiomyomas were described by Wirshow in the 19th century (12,13). With the popularization of minimally invasive interventions over the years, thoracoscopic interventions and then endoscopic interventions were initiated in the leiomyoma treatment. While the F/M ratio was reported to be equal in some studies, this ratio was reported as 1/1.9 in some studies (1,14). In our study, leiomyomas were found more frequently in men. The mean age of diagnosis in studies was reported to be in the 4th-5th decade, and our study had a similar mean age with literature (1,10,14).

Leiomyomas are usually incidentally detected due to being slow-growing tumoral lesions on the esophageal wall (15,16). The most common symptoms are dysphagia, chest and retrosternal pain, regurgitation, epigastric pain, dyspnea, and weight loss. In a study, it was reported that there was a correlation between the size of leiomyoma and the presence of symptoms, and the mean tumor diameter in symptomatic patients was reported to be 5.3 cm (4). In our study, endoscopy was performed in three patients due to dysphagia, and the mean tumor size of these patients was 23.3 mm. Leiomyomas are usually located in the middle 2/3 part of the esophagus (17). In our study, in accordance with the literature, leiomyomas were detected in the thoracic esophagus in the majority of patients.

Table 1. Results of patients with esophageal leiomyomas

Gender	Age	ESD/EMR	Diameter (mm)	Pathology	Location
M	31	ESD	6	Leiomyoma	Abdominal
F	51	ESD	25	Leiomyoma	Thoracic
F	57	ESD	15	Leiomyoma	Thoracic
M	51	ESD	8	Leiomyoma	Thoracic
M	48	EMR	8	Leiomyoma	Thoracic
M	61	EMR	7	Leiomyoma	Cervical
F	65	ESD	20	Leiomyoma	Thoracic
M	35	ESD	10	Leiomyoma	Thoracic
F	52	EMR	16	Leiomyoma	Thoracic
F	61	ESD	12	Leiomyoma	Thoracic
M	60	ESD	5	Leiomyoma	Thoracic
M	58	ESD	8	Leiomyoma	Thoracic
M	55	ESD	10	Leiomyoma	Thoracic
F	72	EMR	5	Leiomyoma	Abdominal
M	63	ESD	6	Leiomyoma	Thoracic
M	70	ESD	8	Leiomyoma	Abdominal
F	20	ESD	7	Leiomyoma	Thoracic
F	40	ESD	25	Leiomyoma	Abdominal

ESD: submucosal dissection, EMR: endoscopic mucosal, F: female, M: male

Chest X-ray, barium esophagography, endoscopy, EUS, CT, and magnetic resonance are among the imaging methods used in the diagnosis of esophageal leiomyomas (1,18-20). The chest X-ray shows a mediastinal mass, and barium esophagography shows a filling defect. Leiomyomas are seen as homogeneous, round or lobulated soft tissue masses in tomography (20). In endoscopy, they are observed as free-moving masses under the intact mucosa. EUS is one of the most useful methods for diagnosis. In this method, the mass can be distinguished as intramural, homogeneous, hypoechoic, and well-circumscribed (19,20).

In cases where leiomyoma is suspected, if the mucosa is intact during endoscopy, it is recommended to avoid biopsy due to the possibility of being non-diagnostic and increasing surgical complications (1,18,21). However, it is not always possible to distinguish leiomyomas from other submucosal lesions despite all imaging methods (21). In our study, ESD was applied to two patients after diagnosis by endoscopic biopsy because of the suspicion of imaging methods.

EMR and ESD have complications due to being invasive procedures. Iatrogenic injuries, which have an essential place in esophageal perforations, can be observed during endoscopic treatments of leiomyomas (22). Compared with EMR, hemorrhage, and perforation rates are higher in ESD. Many of these complications are associated with surgeon-dependent factors (23,24). Depending on the experience, hemorrhage and perforations can be managed with clips and coagulation (25). In our study, hemorrhage occurred in only one patient who underwent ESD, and it was stopped using both coagulation and clips, and no patient had perforation.

Conclusion

As a result, while choosing the treatment to be used in the treatment of esophageal leiomyomas, ESD and EMR performed with endoscopic methods can be successfully applied with low complication rates in experienced hands instead of treatment methods with higher morbidity such as surgery.

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Ethics Committee Approval: Our study started after obtaining approval from the University of Health Sciences, İstanbul Training and Research Hospital Local Clinical Research Ethics Committee (decision no: 2017, date:11/10/2019).

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Hemoglobin A1c Measurement Using Point of Care Testing

Hasta Başı Test Cihazları Kullanarak Hemoglobin A1c Ölçümü

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ABSTRACT

Introduction: Glycated hemoglobin is a sensitive predictor of the long-term complications of diabetes. Hemoglobin A1c (HbA1c) measurements can be performed using point of care testing (POCT) devices, and comparing POCT devices with high-performance liquid chromatography (HPLC) is essential. This study aimed to compare the HBA1c results of POCT devices with HPLC in diabetic patients with and without hemoglobinopathy.

Methods: Twenty-six diabetic patients with hemoglobinopathy and 51 diabetic patients without hemoglobinopathy are included in this study. HbA1c analyzes were performed using Tri-stat POCT analyzer (Trinity Biotech, Ireland) from venous blood and capillary blood, Premier Hb 9210 (Trinity Biotech, Ireland) HPLC method only from venous blood of patients.

Results: HbA1c levels did not differ statistically in patients without hemoglobinopathy between HPLC and capillary blood POCT, HPLC and venous blood POCT, capillary blood POCT, and venous blood POCT, p=0.392, p=0.167, and p=0.288), respectively. HbA1c levels also did not differ statistically in patients with hemoglobinopathy between HPLC and venous blood POCT (p=0.076).

Conclusion: Tri-stat POCT analyzer results did not differ statistically in comparison to HPLC results in patients. POCT devices approved by the ministry and accepted by the authorities can be used safely in HbA1c analyzes. Clinicians may prefer both methods in patients with type 2 diabetes who need close follow-up, and whose treatment regimen will be affected by this follow-up.

Keywords: HbA1c, HPLC, POCT, hemoglobinopathy

ÖZ

Amaç: Hemoglobin A1c (HbA1c) diyabetin uzun dönem komplikasyonlarını hassas olarak belirlemektedir. HbA1c ölçümleri hasta başı test cihazları (HBTC) ile yapılabilmekte olup, bu HBTC'lerin yüksek performanslı sıvı kromatografi (HPLC) ile kıyaslanması önem arzetmektedir. Bu çalışmada hemoglobinopatisi olan ve olmayan hastaların HbA1c sonuçlarının HBTC HbA1c cihazı (Tri-stat Analyzer, Trinity Biotech) ve HPLC (Hb Premiere) ile karşılaştırılması amaçlanmıştır.

Yöntemler: Hemoglobinopatili 26 diyabetik, hemoglobinopatisiz 51 diyabetik hasta çalışmaya alındı. Hastaların HbA1c analizleri Tri-stat HBTC kullanılarak (Trinity Biotech, Ireland) venöz ve kapiller kandan, Premier Hb 9210 (Trinity Biotech, Ireland) HPLC kullanılarak sadece venöz kandan çalışılmıştır.

Bulgular: Hemoglobinopatisi olmayan hastaların HbA1c düzeyleri karşılaştırıldığında HPLC - kapiller kan HBTC arasında (p=0,392), HPLC - venöz kan HBTC arasında (p=0,167) ve venöz kan HBTC - kapiller kan HBTC arasında (p=0,288) istatistiksel olarak anlamlı bir fark saptanmamıştır. Hemoglobinopatisi olan hastaların HbA1c düzeyleri karşılaştırıldığında HPLC - venöz kan HBTC arasında da (p=0,076) istatistiksel olarak anlamlı bir fark saptanmamıştır.

Sonuç: Hastalarda, Tri-stat HBTC'yi HbA1c sonuçları HPLC ile karşılaştırıldığında istatistiksel olarak farklı değildi. Bakanlıkça onaylı ve otoriteler tarafından kabul gören HBTC'yi HbA1c analizlerinde güvenle kullanılabilir. Klinisyenler, yakın takibe ihtiyaç duyan ve tedavi rejimi bu takipten etkilenecek tip 2 diyabetli hastalarda her iki yöntemi de tercih edebilirler.

Anahtar Kelimeler: HbA1c, HPLC, HBTC, hemoglobinopati

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Introduction

Regular monitoring of glycated hemoglobin subfraction A1c (HbA1c) in people with diabetes and treatment with glucose-lowering medications to improve glycemic control can reduce the risk of developing complications (1). In the past, the recommended method for the diagnosis of diabetes was through the repeated measurement of fasting plasma glucose or an oral glucose tolerance test (2). More recently, the measurement of the glycated fraction (HbA1c) of HbA1c has been recommended to diagnose diabetes (3), in addition to monitoring glycemic control. Glycated hemoglobin is a sensitive predictor of the long-term complications of diabetes. High HbA1c levels are strongly linked to increased risk of cardiovascular disease, nephropathy, and retinopathy (4,5) and predict most of the excess mortality risk in men with diabetes (6).

The traditional method of testing for glycemic control in primary care involves sending blood samples for laboratory testing and waiting several days for results. In parallel with the developments of nanotechnology in biomedical applications, point of care testing (POCT) of HbA1c is available using a non-invasive, quick and easy analysis. POCT devices are especially useful in emergency departments, in general practitioner's offices or in distant locations to reference laboratories. POCT can also improve the life quality in chronic patients (7,8).

This study aimed to compare HBA1c results of the POCT HbA1c device (Tri-stat Analyzer, Trinity Biotech) with high-performance liquid chromatography (HPLC) (Hb Premiere) in diabetic patients with and without hemoglobinopathy.

Methods

Twenty-six diabetic patients with- and 51 patients without hemoglobinopathy who admit to University of Health Sciences, Bakırköy Dr. Sadi Konuk Training and Research Hospital, outpatient clinic, were enrolled. The American Diabetes Association (ADA) criteria were used to diagnose diabetes (9).

All patients signed an informed consent form before being enrolled, and the study protocol was approved by the University of Health Sciences, Bakırköy Dr. Sadi Konuk Training and Research Hospital Local Ethical Committee (decision no: 2014/231). There was no statistically significant difference between age and sex distributions of the two groups.

HbA1c analyzes were performed using Tri-stat POCT analyzer (Trinity Biotech, Ireland) from venous blood and capillary blood, Premier Hb 9210 (Trinity Biotech, Ireland) HPLC method only from venous blood of patients.

Statistical Analysis

Mean, standard deviation, median, minimum, and maximum values were given for continuous variables. The normal distribution of continuous variables was tested by the Kolmogorov-Smirnov test. For dependent variables showing normal distribution, the Dependent t-test was used in the comparison of two groups. Bland Altman analysis was used for comparisons between methods. The Intraclass Correlation Coefficient (ICC) was calculated for the degree of correlation and agreement between measurements of the methods. P values lower than 0.05, with a 95% confidence interval, were considered as statistically significant.

Results

The median age of the 51 patients without hemoglobinopathy (32 women and 19 men, the ages ranged between 20 and 78 years) was 53.27±11.80 years of age. Only one person in this group had chronic renal failure, who did not receive hemodialysis.

The mean HbA1c level of this group analyzed by the HPLC method, by POCT from venous blood and by POCT capillary blood was 8.45 ± 2.30, 8.54±2.18 and 8.50±2.16, respectively.

No statistically significant difference was observed between HPLC - HbA1C and capillary blood POCT HbA1C measurements (p=0.392).

No statistically significant difference was also observed between the HPLC - HbA1C and venous blood POCT HbA1C (p=0.167). POCT - HbA1C measurements from capillary blood and concurrent venous blood did not differ statistically (p=0.288) (Table 1).

ICC of HPLC - HbA1C and POCT - HbA1C capillary measurements, HPLC HbA1C - POCT HbA1C venous blood and POCT - HbA1C capillary - POCT - HbA1C venous blood is 0.988 (0.980-0.993), 0.989 (0.980-0.994) and

Table 1. Comparison of the capillary and venous blood results of point of care testing analysis with high-performance liquid chromatography method

	Mean ± SD	p
HPLC HbA1C	8.45±2.38	0.392
POCT HbA1C capillary	8.5±2.16	
HPLC HbA1C	8.45±2.38	0.167
POCT HbA1C venous (EDTA)	8.54±2.18	
POCT HbA1C capillary	8.5±2.16	0.288
POCT HbA1C venous (EDTA)	8.54±2.18	

SD: standard deviation, POCT: point of care testing, HPLC: high-performance liquid chromatography, HbA1C: hemoglobin A1c, EDTA: ethylenediaminetetraacetic acid

Table 2. Intraclass correlation of the capillary and venous blood results of the point of care testing analysis and high-performance liquid chromatography

	Intraclass correlation coefficient	95% Confidence interval	
		Lower bound	Upper bound
HbA1C / POCT HbA1C capillary	0.988	0.980	0.993
HbA1C / POCT HbA1C venous (EDTA)	0.989	0.980	0.994
POCT HbA1C capillary / POCT HbA1C venous (EDTA)	0.997	0.995	0.998

POCT: point of care testing, HPLC: high-performance liquid chromatography, HbA1C: hemoglobin A1c, EDTA: ethylenediaminetetraacetic acid

0.997 (0.995-0.998), respectively. All of them indicate strong reliability (Table 2) (Figures 1, 2, 3).

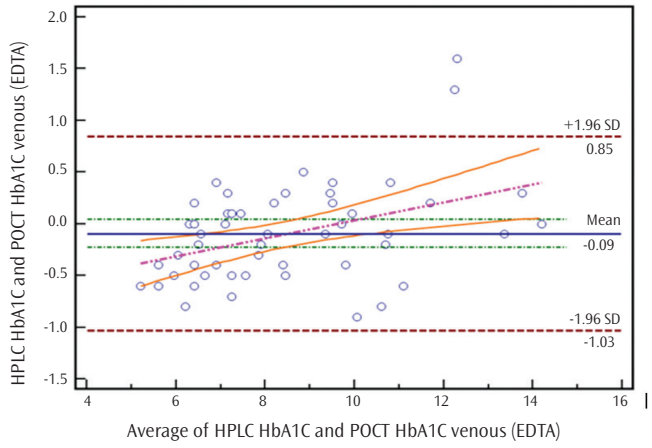

Figure 1. Bland Altman analysis of HPLC HbA1C - POCT HbA1C venous blood measurement

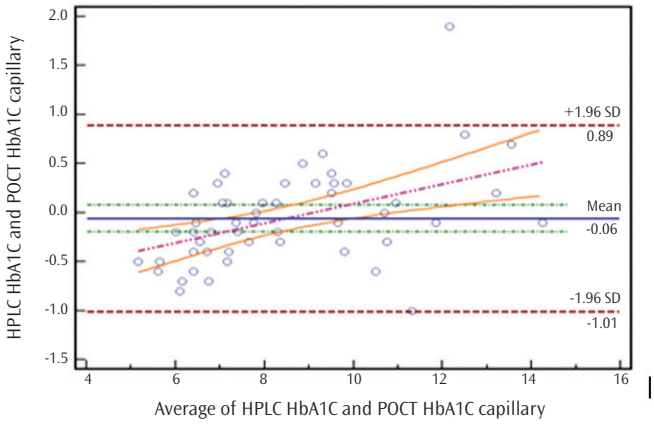

Figure 2. Bland Altman plot of HPLC- HbA1C and POCT- HbA1C capillary blood measurement

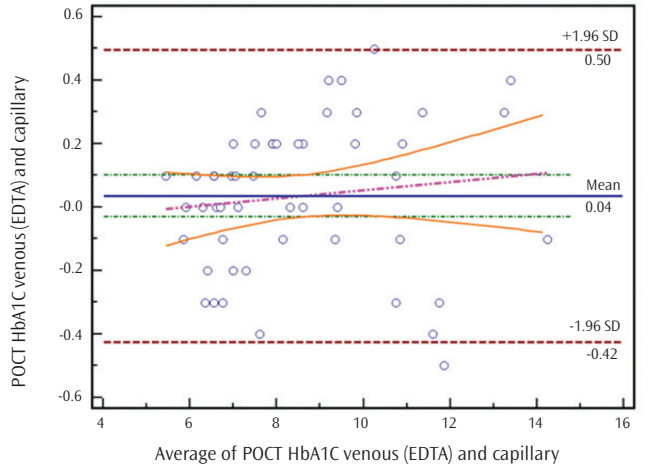

Figure 3. Bland Altman plot of POCT HbA1C capillary - POCT HbA1C venous blood measurement

Mean HbA1C level of 26 patients with hemoglobinopathy and type 2 diabetes analyzed by the HPLC method and by POCT was 6.78±1.45 and 6.45±1.38, respectively. No statistically significant difference was observed (p=0.076) (Table 3).

ICC of HPLC - HbA1C and POCT - HbA1C is 0.994 (0.990-0.997), which indicates strong reliability (Table 4).

Discussion

The ADA guidelines (2010) recommend the use of glycosylated hemoglobin (HbA1c) levels for screening and diagnosis of diabetes (10). Thus, accurate measurement of HbA1c levels is essential for the optimal management of diabetes (11-13).

In recent years, international standardization organizations such as the National Glycohemoglobin Standardization Program and the International Federation of Clinical Chemistry have helped to improve the quality of HbA1c determination (14).

HbA1c measurement is an essential tool in the management of patients with diabetes mellitus. In this context, the possibility of having rapid and accurate methods for HbA1c evaluation is significant.

Several techniques are used for laboratory determination of serum HbA1c levels. The most frequently used form is affinity chromatography, both manual chromatography, and affinity HPLC. As affinity chromatography in principle measures not only the specific glycation on the β-N-terminal, but also on the α-N-terminal and ε-residues of the total hemoglobin molecule, the end-result is total glycated hemoglobin or glycohemoglobin (15).

There are some disadvantages in clinical practice, although the reliability, widespread use, and standardization of laboratory methods are possible.

Physicians in ambulatory settings routinely send blood samples to laboratories for HbA1c testing and wait several days for the HbA1c test results. Thus, patient counseling and treatment adjustments based on HbA1c levels are delayed, and at times follow-up can be lost entirely.

Table 3. Hemoglobin A1c levels of patients with hemoglobinopathy (high-performance liquid chromatography and point of care testing)

	Mean	SD	p
HbA1C (HPLC)	6.78	1.45	0.076
HbA1C (POCT)	6.45	1.38	

SD: standard deviation, HbA1C: hemoglobin A1c, POCT: point of care testing, HPLC: high-performance liquid chromatography

Table 4. Intraclass Correlation Coefficient of high-performance liquid chromatography and point of care testing of patients with hemoglobinopathy

HPLC / POCT	Intraclass correlation coefficient	95% Confidence interval	
		Lower bound	Upper bound
HbA1C	0.994	0.990	0.997

POCT: point of care testing, HPLC: high-performance liquid chromatography

POCT devices can provide excellent convenience for patients receiving home care services, or in places where healthcare facilities are not easy to access. These devices are small, portable, and not expensive. They are easy to use and do not require extensive training.

Several HbA1c POCT devices are currently available for use in clinical practice, including Bayer's A1CNow+® Multi-test A1C System (A1CNow+), the in2it (II) Analyzer (in2it; Bio-Rad Laboratories), the DCA Vantage Analyzer (DCA Vantage; Siemens Healthcare Diagnostics, Inc.), Tri-stat POCT analyzer (Trinity Biotech, Ireland) and the Afinion AS100 Analyzer System (Afinion; Axis-Shield Point-of-Care).

Although new devices are developed every day, the standardization problem and the lack of adequate studies negatively affect the usage of these devices widely.

Our study evaluates a fast and easy way to perform Tri-stat POCT analyzer measurements of glycated hemoglobin HbA1c in comparison with an immunoassay on an automated biochemistry analyzer, Premier Hb 9210 (Trinity Biotech, Ireland), the methods routinely used in our clinical laboratories for the measurement of HbA1c.

In previous studies, POCT analyses were consistent with the HPLC method, but the POCT -HbA1c level was lower than HPLC. In their study, Schwartz et al. (16) compared the performance of the BIO-RAD Micromat II POCT device with a laboratory-based HPLC method (Primus Model 386) for measurement of HbA1c. For each of the laboratory methods, the correlation coefficient was lower than the 0.96 reported by the manufacturer. HbA1c results were also consistently lower than those obtained from laboratory analysis.

Grant et al. (17) compared HPLC and Quo-Test analyzer and obtained similar results. In another study, Arsie et al. (18) reported similar results after comparing HPLC and DCA 2000 POCT analyzer.

In our study, although the results of HPLC and Tri-stat were strongly correlated, however, POCT HbA1c levels were not lower than the results of HPLC, as in the studies mentioned above, even slightly higher. These results did not change in capillary and venous blood samples. These almost identical results may encourage clinicians to rely on this method, which is less invasive for the patient and allows bedside analysis.

Although Tri-stat POCT analyzer results did not differ statistically in comparison to HPLC results in patients with hemoglobinopathy in our study, they were lower than HPLC. This condition may be associated with the shortened lifespan of erythrocytes. Haliassos et al. (19) obtained similar results like ours. In this study, HbA1c levels of patients with hemoglobinopathy were compared using DCA 2000 of Bayer Diagnostics (Tarrytown, NY, USA) and HPLC.

As a result, Tri-stat POCT analyzer results did not differ statistically in comparison to HPLC results in patients. POCT devices approved by the ministry and accepted by the authorities can be used safely in HbA1c analyzes.

Conclusion

Clinicians may prefer both methods in patients with type 2 diabetes who need close follow-up, and whose treatment regimen will be affected by this follow-up. The POCT analyzers should be evaluated for diagnostic purposes by professional organizations developing clinical guidelines.

Ethics Committee Approval: The study protocol was approved by the University of Health Sciences, Bakırköy Dr. Sadi Konuk Training and Research Hospital Local Ethical Committee (decision no: 2014/231).

Informed Consent: All patients signed an informed consent form before being enrolled.

Peer-review: Externally peer-reviewed.

Author Contributions: Surgical and Medical Practices - G.Ş.E., M.K., N.K.; Concept - N.I.; Design - M.K., N.I.; Data Collection and/or Processing - N.I.; Analysis and/or Interpretation - G.Ş.E., N.I.; Literature Search - G.Ş.E.; Writing Manuscript - G.Ş.E.

Conflict of Interest: No conflict of interest was declared by the authors.

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The Value of F-18-fluorodeoxyglucose Positron Emission Tomography/Computed Tomography for the Detection of Residual Breast Tumor or Axillary Metastasis after Neoadjuvant Chemotherapy in Invasive Ductal Carcinoma of the Breast

Memenin İnvazif Ductal Karsinomunda 18F- florodeoksiglukoz Pozitron Emisyon Tomografisi/Bilgisayarlı Tomografi'nin Neoadjuvan Kemoterapiden Sonra Kalan Meme Tümörünü ve Aksilla Metastazını Belirlemedeki Değeri

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ABSTRACT

Introduction: Accurate evaluation of pathological response after neoadjuvant chemotherapy would aid in treatment and surgical planning as well as prediction of outcomes. This study examined the value of F-18-fluorodeoxyglucose positron emission tomography/computed tomography (F-18-FDG PET/CT) in predicting pathologically confirmed residual tumor in breast or presence of axillary metastasis when performed after completion of neoadjuvant therapy in patients with invasive ductal carcinoma (IDC) of the breast cancer.

Methods: This retrospective study included 52 IDC of the breast who received neoadjuvant chemotherapy and underwent F-18-FDG PET/CT between 2015 and 2019 after completion of neoadjuvant chemotherapy. Diagnostic performance parameters of F-18-FDG PET/CT for predicting residual tumor or presence of axillary metastasis were estimated based on histopathological findings.

Results: All patients had IDC. F-18-FDG PET/CT exhibited high specificity for both locations (89.5% and 93.8% and for breast and axilla, respectively). The sensitivity of the method, on the other hand, was low for both locations (66.7% and 30.0% for breast and axilla, respectively), particularly for axilla. False-negative rate (i.e., missing rate) for breast and axilla was 9.1% and 0% for the tumors >8 mm in diameter.

Conclusion: F-18-FDG PET/CT does not seem to provide reliable information on the presence of a residual tumor or node metastasis when performed after the completion of neoadjuvant treatment in IDC of the breast. New diagnostic modalities utilized at different time points or including a combination of different imaging methods are warranted.

Keywords: F-18-fluorodeoxyglucose positron emission tomography/computerized tomography, breast cancer, neoadjuvant chemotherapy, residual tumor, complete response, axillary metastasis, invasive ductal carcinoma

ÖZ

Amaç: Neoadjuvan kemoterapiden sonra patolojik yanıtın doğru değerlendirilmesi hem cerrahi planlanmasına hem de tedavi sonuçların tahmin edilmesine yardımcı olacaktır. Bu çalışma F-18-fluorodeoksiglukoz pozitron emisyon tomografisi/bilgisayarlı tomografi'nin (F-18-FDG PET/BT), memenin invazif duktal karsinomu (İDK) olan hastalarda, neoadjuvan tedavinin tamamlanmasından sonra yapıldığında, cerrahi sonrası patolojik olarak doğrulanmış memede kalan tümörü ve aksillada metastaz varlığını tahmin etmedeki değerini incelemiştir.

Yöntemler: Bu retrospektif çalışmaya 2015 ve 2019 yılları arasında neoadjuvan kemoterapi almış ve kemoterapi sonrasında F-18-FDG PET/BT yapılmış 52 memenin İDK'si dahil edilmiştir. F-18-FDG PET/BT'nin kalan tümörü ya da aksillada metastaz varlığını tahmin etmek açısından, histopatolojik bulgular esas alınarak, diyagnostik performans parametreleri hesaplanmıştır.

Bulgular: Tüm hastalarda İDK tanısı mevcuttu. F-18-FDG PET/BT'nin özgüllüğü her iki lokasyon için de yüksek bulunmuştur (sırasıyla meme ve aksilla için %89,5 ve %93,8). Yöntemin duyarlılığı ise her iki lokasyon için, özellikle de aksilla için düşük idi (sırasıyla, meme ve aksilla için %66,7 ve %30,0). Sekiz milimetreden büyük tümörler incelendiğinde ise yanlış negatif oranı (gözden kaçan tümörler) meme için %9,1, aksilla için %0 idi.

Sonuç: F-18-FDG PET/BT memenin İDK'de neoadjuvan tedavinin tamamlanmasından sonra yapıldığında rezidü tümör ya da nodal metastaz varlığını göstermede güvenilir bilgi veriyor gibi görünmemektedir. Değişik zaman noktalarında kullanılacak veya değişik görüntüleme yöntemlerini kombine edecek yeni tanısal yöntemlere gereksinim vardır.

Anahtar Kelimeler: F-18-fluorodeoksiglukoz pozitron emisyon tomografisi/bilgisayarlı tomografi, meme kanseri, neoadjuvan kemoterapi, rezidü tümör, tam yanıt, aksilla metastazı, invazif duktal karsinom

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Introduction

Breast cancer is the most common malignancy among women in the developed world (1). Advanced diagnostic modalities and new treatment strategies resulted in a decrease in breast cancer-related mortality, although its incidence is on the rise (1). Neoadjuvant therapy is increasingly used for the treatment of patients with breast cancer (2). It helps to downsize the tumor in early breast cancer, thereby increasing chances for breast-conserving surgery (3). Also, it has the potential to convert metastatic lymph nodes to pathologically negative status in a substantial proportion of patients with locally advanced breast cancer (4).

Accurate evaluation of pathological response after neoadjuvant treatment would aid in treatment and surgical planning (5). Correct prediction of the residual tumor site and size would enable successful resection as well as breast tissue preservation as much as possible. Also, it will give an idea of disease prognosis. Several imaging modalities such as magnetic resonance imaging (MRI), positron emission tomography/computerized tomography (PET/CT), ultrasonography, and mammography is currently being used to examine response to neoadjuvant chemotherapy in breast cancer (6). Although widely used, mammography and ultrasonography seems to overestimate tumor volume due to chemotherapy-induced fibrosis and necrosis (7). On the other hand, MRI may overestimate or underestimate the residual tumor in an essential proportion of the patients; thus, it also has some limitations, particularly its inability to discriminate between viable tumor tissue from scar tissue (7). Nevertheless, MRI and F-18-FDG PET/CT are often used to evaluate response after neoadjuvant chemotherapy for breast cancer (8,9).

F-18-FDG PET/CT provides a quantitative estimation of metabolic changes in the tumor tissue; thus, it has the potential to detect such changes occurring early in the course of chemotherapy (10). Several studies demonstrated the advantage of changes in standardized uptake values in predicting pathological response to neoadjuvant chemotherapy (11, 9, 12). On the other hand, FDG-PET has been shown to have low sensitivity for small lesions (13). To date, several studies examined the role of PET/CT in predicting response to neoadjuvant chemotherapy when performed after the completion of neoadjuvant chemotherapy and before surgery, with inconsistent findings, most of them comparing the findings with that of MRI (6,14-19).

This study aimed to examine the value of 18-FDG PET/CT in predicting pathologically confirmed residual tumor in breast and presence of axillary metastasis when performed after completion of neoadjuvant therapy in patients with invasive ductal carcinoma (IDC) of the breast.

Methods

Patients

This retrospective study included 51 female patients (52 tumors) diagnosed with IDC of the breast who received neoadjuvant chemotherapy and underwent F-18-FDG PET/CT between the years 2015 and 2019. Patients were eligible for the study if they fulfill the following criteria: Biopsy-confirmed diagnosis of invasive stage IIA, IIB, or IIIA breast carcinoma with no distant metastasis. Patients with inflammatory breast carcinoma, invasive lobular carcinoma, invasive mucinous carcinoma, and patients with distant metastasis were excluded. F-18-FDG-PET/CT was performed 2-3 weeks after the completion of neoadjuvant chemotherapy. Patients were subjected to either breast-conserving surgery or modified radical mastectomy with sentinel node biopsy and/or axillary lymph node dissection after neoadjuvant chemotherapy. The study protocol of this study was approved by the Anadolu Medical Center Local Ethics Committee (decision no: ASM-EK-19/123, date: 11.12.2019). Informed consent was waived since the trial included retrospective data analysis. Data on patient demographics, tumor histology, assessment of tumor by metabolic response on 18F-FDG-PET/CT imaging was collected.

Neoadjuvant Chemotherapy

Patients received anthracycline-based, taxane-based, or anthracycline and taxane combination neoadjuvant treatment. Selected patients with high hormone receptor positivity and advanced age received only hormone therapy. In patients with HER2 positivity, trastuzumab ± pertuzumab was added.

18-FDG PET/CT Examination after Neoadjuvant Treatment

Patients fasted for at least 6 hours, and the blood glucose level had to be <150 mg/dL. F-18-FDG (3.7 MBq/kg) was administered through the arm opposite to breast tumor using a venous line to prevent extravasation. Imaging started approximately 60 min after injection and was performed from mid-thigh level to the base of the skull with arms raised. An integrated PET/CT scanner (Discovery 690, GE Healthcare, Wisconsin, USA) was used for imaging. CT data were acquired first (120 kV;20-120 mAs, determined automatically based on attenuation). Only an oral contrast agent was used. PET emission data were acquired in a 3-dimensional mode, with 3 min per bed position, and reconstructed using iterative reconstruction algorithm with 5 mm slice thickness. Attenuation-corrected images were normalized for injected dose and body weight, and subsequently converted into SUV, defined as: [tracer concentration (kBq/mL)] / [injected activity (kBq) / patient body weight (g)]. The 3D volume of interest was automatically drawn around the primary tumor and around the axillary lymph nodes, when present.

Postoperative Pathological Examination

All cases were diagnosed by tru-cut biopsy. Estrogen receptor and progesterone receptor positivity, the grade of differentiation, and Ki-67 were determined by immunohistochemical (IHC) staining. HER-2 status was accepted as "positive" if strong (3+) membranous staining was seen on IHC. Fluorescence *in situ* hybridization analysis (FISH) was done in samples with moderate (2+) membranous staining on IHC, and HER-2 status was accepted as "positive" if FISH showed amplification. All postoperative specimens were microscopically evaluated to identify the residual invasive tumor. pCR was defined as no residual invasive tumor cells in the breast or axillary nodes. Pathological responses other than pCR were defined as incomplete response (non-pCR).

Assessment of Metabolic Response

Regions of interest were identified and outlined for both primary tumor and axillary lymph node areas, and SUV values were calculated.

Adjacent breast or axillary tissue without activity was identified, and SUV values were calculated to serve as a background activity. When SUV value for the region of interest is two times or more of background activity, the activity in the region of interest was considered pathological and evidence for residual tumor/metastasis.

Statistical Analysis

Statistical analyses were performed using SPSS software for Windows (Version 21.0; SPSS Inc., New York, New York, USA). Descriptive data were presented as mean ± standard deviation or number (frequency), where appropriate. The sensitivity and specificity of the F-18-FDG PET/CT examination following the completion of neoadjuvant chemotherapy were calculated for the diagnosis of histologically confirmed residual tumor in axilla or breast.

Results

Table 1 shows patient and tumor characteristics. All patients were female and diagnosed with IDC of the breast. Half of the patients received neoadjuvant treatment with anthracycline and taxane combination, whereas 13.5% and 32.7% of them received anthracycline-based and taxane-based neoadjuvant therapy. Only two patients received hormone therapy. Trastuzumab ± pertuzumab was added in 36.5% of patients due to HER2 positivity. Postoperative histopathological examination identified residual invasive tumors in the breast in almost two-thirds of the patients (63.5%). On the other hand, metastasis was present in the axilla in slightly more than one-third of the patients (38.5%).

Table 2 shows diagnostic parameters for F-18-FDG PET/CT for the detection of residual invasive tumor in breast and metastasis in the axilla. F-18-FDG PET/CT exhibited high specificity for primary tumor and axilla (93.8% and 89.5%, respectively). The sensitivity of the method, on the other hand, was low for both locations (66.7% for primary tumor and 30.0% for axilla). Around one-quarter of the residual invasive tumors/metastases were missed at these locations. When only the patients with histologically positive residual invasive tumor/metastasis were analyzed separately (n=20 and 33 for axilla and breast, respectively), a false negative rate (i.e., missing rate) for axilla and breast was 0% and 9.1% for the tumors >8 mm in diameter. Among 33 residual invasive tumors in the breast, 22 of them were >8 mm (66.7%); on the other hand, only 4 of 20 axillary metastases were greater than >8 mm (20.0%).

Discussion

This study examined the diagnostic value of F-18-FDG PET/CT in predicting residual tumor or presence of axillary metastasis after the completion of neoadjuvant treatment in patients with IDC of the breast. F-18-FDG PET/CT exhibited high sensitivity but low specificity in this setting, with a high false-negative rate; however, its diagnostic value seems better for residual tumors or axillary metastasis >8 mm.

To date, several other studies examined the diagnostic value of F-18-FDG PET/CT in predicting residual tumor after the completion of neoadjuvant therapy; and most studies compared F-18-FDG PET/CT with MRI. Choi et al. (20) compared the performances of PET/CT and MRI for response evaluation after neoadjuvant treatment in breast cancer. Imaging studies were performed before and after neoadjuvant treatment, and their diagnostic value in predicting complete/partial response (responders) and stable/progressive disease (non-responders) was evaluated based on postoperative pathological findings. PET/CT exhibited lower specificity and accuracy and higher sensitivity when compared to MRI in response evaluation, although these differences between the two methods did not reach statistical significance (20).

A recent study compared two PET methods [ring-type dedicated breast PET (dbPET) vs whole-body PET-CT (WBPET)] for the assessment of residual tumor after neoadjuvant chemotherapy for breast cancer (14). dbPET was more sensitive than WBPET when quantitative methods

Table 1. Patient and tumor characteristics

Characteristics	n=52
Age, y (mean ± SD)	48.9±10.8
Tumor receptor characteristics	
Estrogen receptor-positive	34 (65.4%)
Progesterone receptor positive	24 (46.2%)
HER2 positive	19 (36.5%)
Triple-negative tumor	12 (23.1%)
Luminal A	7 (13.5%)
Luminal B	15 (28.8%)
Chemotherapy type	
Anthracycline-based	7 (13.5%)
Taxane-based	17 (32.7%)
Anthracycline taxane combined	26 (50.0%)
Hormone	2 (3.8%)
Trastuzumab ± pertuzumab	19 (36.5%)
Histological examination	
Residual invasive tumor in axilla	20 (38.5%)
Residual invasive tumor in the breast	33 (63.5%)
Residual invasive tumor diameter in axilla, mm, (mean ± SD)*	5.8±5.7
Residual invasive tumor diameter in the breast, mm, (mean ± SD)*	17.5±13.0

Unless otherwise stated, data presented in n (%).

HER2: human epidermal growth factor receptor 2, *: for patients with histologically positive residual invasive tumor, SD: standard deviation, Luminal A: hormone-receptor positive (estrogen-receptor and/or progesterone-receptor positive), HER2 negative, and has Ki-67 ≤14, Luminal B: hormone-receptor positive (estrogen-receptor and/or progesterone-receptor positive), and either HER2 positive or HER2 negative, Ki-67 >14

Table 2. Diagnostic value of F-18-fluorodeoxyglucose positron emission tomography/computed tomography for the detection of residual invasive tumor or presence of axillary metastasis after neoadjuvant chemotherapy

	Primary tumor	Axilla
True positive, n (%)	22 (42.3%)	6 (11.5%)
True negative, n (%)	17 (32.7%)	30 (57.7%)
False positive, n (%)	2 (3.8%)	2 (3.8%)
False negative, n (%)	11 (21.2%)	14 (26.9%)
Sensitivity, % (95% CI)	66.7 (48.2-82.0)	30.0 (11.9-54.3)
Specificity, % (95% CI)	89.5 (66.9-98.7)	93.8 (79.2-99.2)

CI: confidence interval

(84% vs 26%) or qualitative methods (58% vs 21%) were used; however, these figures may still be considered relatively low for the detection of a residual tumor (14).

A recent meta-analysis reviewed the findings of 13 studies comparing MRI and PET/CT in predicting response after neoadjuvant treatment (15). The pooled sensitivity for PET/CT and MRI was 77% and 88%, respectively. Corresponding figures for specificity were 88% (PET/CT) and 69% (MRI). The authors recommended that MRI might be a more suitable method for predicting pathologic response to neoadjuvant chemotherapy. Another meta-analysis examining the prediction of pathological complete response to neoadjuvant chemotherapy by these two imaging modalities from six studies found a high sensitivity for PET/CT (86%), and the authors recommended combined use of these modalities for better predicting response to neoadjuvant treatment (16). Another meta-analysis that included ten studies compared the value of MRI and PET/CT in predicting pathological response. During the neoadjuvant treatment, the performance of PET/CT was similar to MRI in terms of sensitivity (91% vs 89%) and better in terms of specificity (69% vs 42%). On the other hand, MRI had better performance when either of the two methods was used after the completion of neoadjuvant treatment, with significantly higher sensitivity (88% vs 57%) (19). There is a wide range of sensitivity and specificity across studies, which may be attributed to the heterogeneity between studies (17).

Studies examining the value of diagnostic methods in predicting complete axillary response after neoadjuvant therapy for breast cancer are relatively few. A study by Vicente et al. (21) examined the predictive value of 18-FDG PET/CT for axillary lymph node response after neoadjuvant chemotherapy performed at different time points: before, during, and after neoadjuvant therapy. Predictive values of axillary response were low for both early and late evaluation (sensitivity, 52% vs 32%), in line with the findings of the present study. A systematic review included four studies with a relatively small sample. The reported positive predictive value (PPV) for complete axillary response ranged between 40% and 100% for different diagnostic methods. Among the studies included in the review, in the study by Hieken et al. (18), post neoadjuvant F-18-FDG PET-CT exhibited sensitivity, specificity, PPV, and negative predictive value of 85%, 63%, 61%, and 86%, respectively. You et al. (6) evaluated the axillary lymph node status after neoadjuvant chemotherapy and compared the diagnostic performances of ultrasound, MRI, and F-18-FDG PET/CT. The sensitivity of ultrasound, MRI, and PET/CT was 50%, 72%, and 22%, respectively. Corresponding figures for specificity were 77%, 54%, and 85%, with PET/CT having the highest specificity. On the other hand, a combination of the three methods had the highest sensitivity.

In this study, 18-FDG PET/CT exhibited relatively better performance for predicting tumors/metastases larger than 8 mm. This is in line with the findings of a study that demonstrated low sensitivity of 18-FDG PET/CT for small lesions. That study by Kumar et al. (13) examined the clinicopathological factors associated with false-negative FDG-PET in primary breast cancer and found that small tumor size (≤10 mm) and low tumor grade were independent predictors of false-negative results. This may also explain the low sensitivity found in our study, particularly for axillary metastasis. The substantial reduction of the tumor size after neoadjuvant treatment seems to be responsible for this finding. Although sensitivity for detecting an invasive tumor in the breast is somewhat acceptable, sensitivity for detecting axillary metastases, in particular, is substantially low; probably, since only one-fifth of axillary metastases were >8 mm whereas, two-thirds of residual invasive tumors in the breast had such a great size.

This study has several limitations. Firstly, the sample size is relatively small for examining clinicopathological factors that may affect the predictive performance of PET/CT. Another limitation is that not all patients were node-positive at baseline before neoadjuvant treatment. Thus, any metastasis in axilla may not necessarily be considered residual after neoadjuvant therapy.

Conclusion

F-18-FDG PET/CT alone does not seem to provide reliable information on the presence of a residual tumor or node metastasis when performed after the completion of neoadjuvant treatment in IDC of the breast. Considering that preoperative restaging is essential in terms of treatment planning and outcome prediction, new diagnostic modalities utilized at different time points or including a combination of different imaging methods are warranted to predict response to neoadjuvant chemotherapy better.

Ethics Committee Approval: The study protocol of this study was approved by the Anadolu Medical Center Local Ethics Committee (decision no: ASM-EK-19/123, date: 11.12.2019).

Informed Consent: Informed consent was waived since the trial included retrospective data analysis. Data on patient demographics, tumor histology, assessment of tumor by metabolic response on 18F-FDG-PET/CT imaging was collected.

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The Effects of Metformin, Ethinyl Estradiol/Cyproterone Acetate, and Metformin Ethinyl Estradiol/Cyproterone Acetate Combination Therapy on Carotid Artery Intima-media Thickness in Patients with Polycystic Ovary Syndrome

Polikistik Over Sendromlu Olgularda Metformin, Etinil Estradiol/Siproteron Asetat ve Metformin-etinil Estradiol/Siproteron Asetat Kombinasyonu Tedavisinin Karotis Arter İntima Media Kalınlığı Üzerine Etkileri

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ABSTRACT

Introduction: Patients with polycystic ovary syndrome (PCOS) are in the risk group for early-onset cardiovascular disease. There are few studies evaluating physiological and inflammatory cardiovascular risk factors in PCOS. Our study aimed to measure carotid intima-media thickness (IMT) in PCOS cases and to assess the effects of metformin, ethinyl estradiol/cyproterone acetate (EE/CA) and metformin + EE/CA combination therapy on carotid IMT, insulin resistance, C-reactive protein (CRP), apelin and adiponectin.

Methods: Basal carotid IMT, insulin resistance [Homeostasis model assessment insulin resistance (HOMA-IR)], apelin, adiponectin, and CRP values were evaluated in 60 women with PCOS and 43 healthy volunteers between the ages of 18 and 45. After baseline evaluation, patients were divided into metformin (n=20), EE/CA (n=20) and metformin + EE/CA (n=20) treatment groups. Treatment regimens were administered for six months. At the end of the treatment, the same parameters were re-evaluated.

Results: Compared with the control group, CRP (p=0.003), HOMA-IR (p=0.004) and IMT (p=0.049) were significantly higher, and adiponectin (p=0.002) and apelin (p=0.031) levels were significantly lower in patients with PCOS. At the end of the six-month treatment, the adiponectin level in the metformin (p=0.012) and metformin + EE/CA groups (p=0.012), and the apelin level in the metformin (p=0.024), EE/CA (p=0.024) and metformin + EE/CA groups (p=0.024) were significantly higher. There was no statistically significant change in CRP level in all

ÖZ

Amaç: Polikistik over sendromu (PKOS) erken dönemde ortaya çıkan kardiyovasküler hastalık için risk grubundadır. PKOS'de, fizyolojik ve enflamatuvar kardiyovasküler risk faktörlerini değerlendiren az sayıda çalışma vardır. Çalışmamızın amacı, PKOS olgularında karotis intima media kalınlığını (İMK) ve ayrıca metformin, etinil estradiol/siproteron asetat (EE/SA) ve metformin + EE/SA kombinasyon tedavisinin karotis İMK, insülin direnci, C-reaktif protein (CRP), apelin ve adiponektin üzerine etkilerini değerlendirmektir.

Yöntemler: Çalışmamıza katılan 18-45 yaş arasındaki 60 PKOS'li kadında ve 43 sağlıklı gönüllü kadında bazal karotis İMK, insülin direnci [Homeostasis model assessment insulin resistance (HOMA-IR)], apelin, adiponektin ve CRP değerlendirmeleri yapıldı. Bazal değerlendirmeden sonra, hastalar metformin (n=20), EE/SA (n=20) ve metformin + EE/SA (n=20) tedavi gruplarına ayrıldı. Tedavi rejimleri 6 ay boyunca uygulandı. Tedavi sonunda aynı parametreler tekrar değerlendirildi.

Bulgular: Kontrol grubuyla karşılaştırıldığında PKOS'li olguların CRP (p=0,003), HOMA-IR (p=0,004), İMK (p=0,049) değerleri istatistiksel olarak anlamlı derecede daha yüksek, adiponektin (p=0,002), apelin (p=0,031) düzeyleri kontrol grubundan istatistiksel olarak daha düşük saptandı. Altı aylık tedavinin sonunda adiponektin seviyesi metformin (p=0,012) ve metformin + EE/SA grubunda (p=0,012), apelin seviyesi ise metformin grubu (p=0,024), EE/SA grubu (p=0,024), ve metformin + EE/SA grubunda (p=0,024) anlamlı olarak yüksek saptandı. Üç tedavi grubunda da CRP seviyesinde istatistiksel olarak anlamlı değişim yoktu (p>0,05). Üç tedavi grubunda

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treatment groups (p>0.05). There was no statistically significant change in carotid IMT value in all treatment groups (p>0.05).

Conclusion: According to these results, we can say that women with PCOS have subclinical atherosclerosis and that metformin treatment has a positive effect on subclinical atherosclerosis.

Keywords: Polycystic ovary syndrome, carotid artery intima-media thickness, insulin resistance, apelin, adiponectin, C-reactive protein

da karotis İMK değerinde istatistiksel olarak anlamlı değişim yoktu (p>0,05).

Sonuç: Bu sonuçlara göre PKOS'de subklinik aterosklerozun varlığını ve metformin tedavisinin subklinik ateroskleroz üzerine olumlu etki gösterdiğini söyleyebiliriz.

Anahtar Kelimeler: Polikistik over sendromu, karotis arter intima media kalınlığı, insülin direnci, apelin, adiponektin, C-reaktif protein

Introduction

Polycystic ovary syndrome (PCOS) is the most common endocrinological disorder (5-10%) in women of reproductive age (1). It was first described by Stein and Leventhal (2) in 1935. The diagnosis of the disease, the symptoms of which spread over a broad spectrum, was based on clinical signs and symptoms and biochemical markers, and today there is no consensus on diagnostic criteria (3).

First, in 1990, the National Institutes of Health Consensus Conference specified the diagnostic criteria for PCOS as the exclusion of other causes of hyperandrogenism, characterized by menstrual irregularity, increased androgen level, and adrenal hyperplasia. Insulin resistance, increased luteinizing hormone/follicle-stimulating hormone ratio, and ultrasonographic symptoms have been identified as possible criteria (4).

In 2003, the European Society for Human Reproduction and Embryology and the American Society for Reproductive Medicine convened in Rotterdam, Netherlands, to rearrange the diagnostic criteria for the diagnosis of PCOS. Since PCOS is a syndrome, it was decided after this meeting that a single criterion was not sufficient for clinical diagnosis and that at least two of the following criteria should be present after the exclusion of androgen excess and other medical causes causing menstrual irregularity:

1. Oligo/anovulation,

2. Clinical or biochemical hyperandrogenemia findings,

3. PCOS appearance in at least one ovary on ultrasound (US) (defined as more than 12 2-9 mm, follicles in the ovary or ovarian volume more than 10 mL) and exclusion of other diseases (5).

In 2009, the diagnostic criteria of PCOS were reorganized by the Androgen Excess Society.

Androgen Excess Society Criteria

1. Hyperandrogenism: Hirsutism and/or hyperandrogenemia,

2. Ovarian dysfunction: Oligo-anovulation and/or polycystic ovaries,

3. Exclusion of other hyperandrogenemia-causing conditions (such as late-onset congenital adrenal hyperplasia, androgen-secreting tumors, Cushing's syndrome, thyroid dysfunction, and hyperprolactinemia) (6).

PCOS progresses with clinical symptoms such as dysfunctional bleeding, infertility, hirsutism, and acne. The most common of these is menstrual irregularity due to anovulation (7). Also, there are women with regular menstrual cycles despite high androgen levels (8). In PCOS, hyperandrogenism is evaluated with clinical (hirsutism, acne, androgenic alopecia) and biochemical (increase in serum total and free testosterone levels) findings (6). There is an 80% PCOS with hyperandrogenemia. Ovaries are enlarged (volume >10 mL), and polycystic ovaries (12 or more follicles with a size of 2-9 mm), can be detected. It is sufficient to see them in a single ovary (9).

Many patients with PCOS have insulin resistance independent of body mass index. The risk of developing type 2 diabetes mellitus (DM) in patients with PCOS has been found to increase 2-5 times (10). Also, the frequency of metabolic syndrome is higher in patients with PCOS than in the normal population. The risk of cardiovascular disease (CVD) increases in patients with DM and metabolic syndrome (11).

Adiponectin is a newly defined adipocytokine expressed in adipocytes. Adiponectin is effective in insulin resistance and in preventing the development of type 2 DM. It plays a vital role in vascular endothelial dysfunction and the pathogenesis of atherosclerosis. In clinical studies, adiponectin has been shown to have a protective effect from atherosclerosis. Serum adiponectin level is low in women with type 2 DM, obesity, and insulin resistance. In studies with PCOS, plasma adiponectin level was also found to be low (12).

Apelin has been shown to have an expression in the central nervous system, lung, adipose tissue, heart, and breast. Apelin is thought to play a role in the regulation of insulin and glucose levels (13).

Many studies have revealed that those with increased C-reactive protein (CRP) levels have a much higher risk of CVD, including stroke, sudden death, and peripheral vascular disease (14,15). The majority of the studies in PCOS have been shown to increase the level of CRP (15).

Carotid intima-media thickness (IMT) measurement is one of the methods that detect the atherosclerotic process in the asymptomatic period and is also a technique showing endothelial dysfunction and early atherosclerotic changes. Studies investigating the subclinical CVD in PCOS by detecting the presence of coronary artery calcification and carotid IMT were performed.

A significant correlation was observed between carotid IMT and testosterone, and androgens were thought to play an essential role in the development of atherosclerosis. It is predicted that atherogenic potential is increased in patients with PCOS in relation to androgen excess, regardless of obesity (16).

Treatment goals in PCOS can be listed as correction of hyperandrogenism, menstrual dysfunction, and providing fertility. Oral contraceptive agents

(OCS), long-acting GnRH analogs, and insulin sensitivity-enhancing agents can be used in androgen-reducing therapy. After understanding that insulin resistance has a significant effect on the development of PCOS in recent years, agents that increase insulin sensitivity have taken their place in the treatment options (17). Insulin sensitivity increasing drugs such as metformin and glitazones have started to be used intensively in the treatment of PCOS (17).

In this study, we aimed to determine whether the carotid IMT, homeostatic model assessment of insulin resistance (HOMA-IR), apelin, adiponectin and CRP levels in PCOS cases differ from the control group, and to evaluate how these parameters respond to three different treatment approaches, including Metformin, Ethinyl estradiol/cyproterone acetate (EE/CA) and metformin + EE/CA.

Methods

Ethics Committee Approval and Project Support

Written approval was obtained for the study from Kırıkkale University Faculty of Medicine Local Ethics Committee on 03.06.2009 with the number of 2009/089.

Selection of the Study Cohort

A total of 60 patients diagnosed with PCOS in accordance with the criteria of Rotterdam 2003, aged between 18 and 45, who applied to the Kırıkkale University Faculty of Medicine Department of Endocrinology and Metabolic Diseases, and 43 age-matched, healthy volunteers were included in our study (5). The patients were randomized to the treatment group according to their order of admission.

Twenty patients received 2x1 g/day metformin, 20 patients received 0.035 mg EE / 2 mg CA / d 1x1 (between 5th-25th day of menstruation, 21 days) and 20 patients received 2x1 g/g metformin + EE/CA (between 5th-25th day of menstruation, 21 days) combined therapy. Baseline examinations were repeated at the 6th month of treatment. These procedures were performed only once in the control group.

Before starting the study, all patients in the PCOS and control groups were informed about the study, and patient consent was obtained. Patients with coronary artery disease, congestive heart failure, late-onset congenital adrenal hyperplasia, Cushing's syndrome, hypothyroidism, hyperthyroidism, type 2 DM, androgen-secreting ovarian or adrenal tumors, and patients receiving antiandrogen treatment and treatment affecting insulin sensitivity were also not included in the study.

Anthropometric Measurements, Collection of Samples, Laboratory Analysis Methods and Vascular Evaluations

In all cases included in the study, fasting blood glucose and fasting serum insulin levels were measured after at least 10 hours of fasting. HOMA-IR was calculated using the formula fasting blood glucose (mmol/L) x fasting insulin (µU/L)/22.5 (18).

CRP was evaluated with the turbidometric method. Serum adiponectin (Human Adiponectin AssayPro ELISA; Biotech Inst Spect) and apelin (Human Apelin Biotech in ELx Autostrip Washer) levels were measured collectively.

The menstrual statuses of the patients were evaluated according to the 6 month menstrual cycles before the study. Menstrual patterns were defined as "regular" (21-35 days), "irregular" (35-180 days), "polymenorrhea" (21 days or less), "oligomenorrhea" (35-180 days) and "amenorrhea" (longer than 180 days) (3). Pelvic US evaluations of all cases were performed on the 3rd-5th days of the menstrual cycle.

For the carotid artery IMT measurement, patients were positioned in the supine position with their heads tilted backward. Measurements were made in the right and left carotid arteries by determining a 1 cm segment within the first 2 cm distal region from the common carotid artery bulb using the General Electric Vivid S5 ultrasonography device and 12L Doppler ultrasonography probe. Based on the far-edge measurement method of the US device, the maximum and mean carotid IMT values of the segment under consideration were determined. The measurement was performed for both common carotid arteries.

Statistical Analysis

All data obtained from the study were analyzed using SPSS 17.0. All data were expressed as mean ± standard deviation (SD). After conducting descriptive statistical analyses (frequency, percentage distribution, mean ± SD), the suitability of variables to normal distribution was evaluated with the Kolmogorov-Smirnov test. Initially, the difference between treatment groups was tested with one-way ANOVA. The levels of biochemical, inflammatory, and carbohydrate metabolism parameters of the groups before and after the treatment were analyzed with the Wilcoxon signed-rank test. $P<0.05$ values were considered statistically significant.

Results

Sixty PCOS patients and 43 volunteers were included in the study. There were 20 patients receiving metformin treatment, 20 patients receiving EE/CA treatment, and 20 patients receiving metformin + EE/CA combination therapy in the PCOS treatment group. Among patients receiving metformin treatment, treatment was discontinued due to B12 deficiency in two patients and severe gastrointestinal complaints in one patient. In one patient receiving EE/CA treatment, treatment was discontinued due to elevated liver enzymes. Also, four patients from the Metformin group, nine patients from the EE/CA group and five patients from the metformin + EE/CA combination group were excluded from the study because they did not come to the 6th month controls and they were not included in the statistical evaluation. The total number of patients who came for control in the 6th month of treatment was 38 (63.3%). The number of patients who completed the study was 13 in the metformin group, 10 in the EE/CA group, and 15 in the metformin + EE/CA combination group.

Comparison of the Pre-treatment Parameters of Individuals in the PCOS and Control Groups

Compared with the control group, CRP ($p=0.003$), HOMA-IR ($p=0.004$) and carotid IMT ($p=0.049$) values of PCOS cases were found to be significantly higher. Adiponectin ($p=0.002$) and apelin ($p=0.031$) levels of PCOS cases were significantly lower than the control group (Table 1).

Comparison of Pre-treatment Parameters of Three Treatment Groups

A statistically significant difference was found between the levels of apelin (p=0.040), adiponectin (p=0.007) and CRP (p=0.019) in the three treatment groups. There was no statistically significant difference between the carotid IMT and HOMA-IR levels of the three groups (p>0.05) (Table 2).

Comparison of Parameters of Three Treatment Groups Measured at Six Months of Treatment

There was a statistically significant difference between IMT (p=0.018), adiponectin (p=0.010) and CRP (p=0.019) levels at the 6th month of treatment of the three treatment groups (Table 3). The significant difference between IMT values was found to be due to the mean value of the metformin group being higher than the mean value of the EE/CA group (p=0.046, respectively). It was found that the significant difference between adiponectin levels was due to the mean level of the metformin group being lower than the mean level of the EE/CA group (p=0.033). It was found that the significant difference between CRP levels was due to the mean level of the metformin group being higher than the mean level of the metformin + EE/CA group (p=0.002). There was no statistically significant difference between HOMA-IR and apelin levels of the three groups (p>0.05).

Comparison of the Parameters Before and After Treatment in Treatment Groups

Metformin treatment group: Significant decrease in HOMA-IR (p=0.048) values and significant increase in apelin (p=0.024) and adiponectin (p=0.012) levels were detected at the end of the 6th month of treatment. There was no significant difference between IMT values and CRP levels before and after treatment (p>0.05) (Table 4).

EE/CA treatment group: There was a significant increase in apelin levels (p=0.024) after six months of treatment. There was no significant difference between the carotid IMT, HOMA-IR, adiponectin, and CRP levels of these cases before and after treatment (p>0.05) (Table 5).

Metformin + EE/CA treatment group: There was a significant increase in apelin (p=0.024) and adiponectin (p=0.012) levels after 6 months of treatment. There was no significant difference between IMT, HOMA-IR, and CRP levels before and after treatment (p>0.05) (Table 6).

Discussion

In patients with PCOS, carotid IMT has been evaluated in many studies. Talari et al. (19) found that the carotid artery IMT increased in patients with PCOS compared to the control group). In the study conducted by Talbott et al. (20), the increase in carotid IMT was shown among women over 45 years of age with PCOS compared to the control group, but not in women with PCOS in the 30-44 years age group. Similar to these studies, we found in our study that the carotid IMT value was higher in PCOS cases than the control group.

Table 1. Comparison of laboratory parameters of individuals in PCOS and control groups

	PCOS (n=60)	Control (n=43)	p
Adiponectin (µg/mL)	10.64±6.76	14.61±5.80	0.002
Apelin (ng/mL)	2.63±2.89	3.78±2.20	0.031
Carotid IMT (mm)	0.42±0.078	0.39±0.06	0.049
CRP (mg/dL)	91.35±27.05	81.79±28.27	0.003
HOMA-IR	3.01±1.78	2.15±0.903	0.004

PCOS: polycystic ovary syndrome, IMT: intima media thickness, CRP: C-reactive protein, HOMA-IR: homeostatic model assessment of insulin resistance

Table 2. Comparison of pre-treatment values of laboratory parameters of the treatment group

Groups				
	Metformin (n=13)	EE/CA (n=10)	Metformin + EE/CA (n=15)	p
	Mean ± SD	Mean ± SD	Mean ± SD	
Carotid IMT (% change)	0.45±0.08	0.40±0.09	0.44±0.05	NS
HOMA-IR	3.60±1.89	2.65±2.00	2.91±1.55	NS
Apelin (ng/mL)	2.69±4.03	3.45±3.49	1.81±0.47	0.040
Adiponectin (µg/mL)	6.37±2.63	12.99±7.67	9.00±5.97	0.007
CRP (mg/dL)	8.94±9.21	2.90±3.62	1.87±1.39	0.019

IMT: intima media thickness, CRP: C-reactive protein, HOMA-IR: homeostatic model assessment of insulin resistance, EE/CA: ethinyl estradiol/cyproterone acetate, SD: standard deviation, NS: not significant

Table 3. Comparison of parameters of three treatment groups measured at six months of treatment

Groups				
	Metformin (n=13)	EE/CA (n=10)	Metformin + EE/CA (n=15)	p
	Mean ± SD	Mean ± SD	Mean ± SD	
IMT	0.45±0.06*	0.39±0.05	0.40±0.05	0.018
HOMA-IR	2.80±1.47	2.63±2.58	2.51±1.66	AD
Apelin (ng/mL)	3.59±2.24	4.71±3.81	4.18±1.88	AD
Adiponectin (µg/mL)	9.53±2.93*	14.91±7.73	13.92±8.86	0.010
CRP (mg/dL)	6.73±6.92**	3.30±2.83	2.43±1.92	0.019

*Significant difference between metformin and EE/CA groups (p<005)

**Significant difference between metformin and metformin + EE/CA groups (p<005).

IMT: intima media thickness, SD: standard deviation, CRP: C-reactive protein, HOMA-IR: homeostatic model assessment of insulin resistance, EE/CA: ethinyl estradiol/cyproterone acetate

Table 4. Comparison of pre-treatment and post-treatment levels of parameters in the group receiving metformin treatment

Metformin			
	Pre-treatment (n=13)	Post-treatment (n=13)	p
	Mean ± SD	Mean ± SD	
IMT (% change)	0.45±0.08	0.45±0.06	NS
HOMA-IR	3.60±1.89	2.80±1.47	0.048
Apelin (ng/mL)	2.69±4.03	3.59±2.24	0.024
Adiponectin (µg/mL)	6.37±2.63	9.53±2.93	0.012
CRP (mg/dL)	8.94±9.21	6.73±6.92	NS

SD: standard deviation, IMT: intima media thickness, CRP: C-reactive protein, HOMA-IR: homeostatic model assessment of insulin resistance, NS: not significant

In their study, Song et al. (21) found that PCOS cases had high insulin resistance. In our study, we found that HOMA-IR values in PCOS cases were higher than the control group.

The presence of a correlation between serum apelin levels and insulin resistance in PCOS is still controversial (22). Chang et al. (23) evaluated both obese and non-obese cases in their study and found that the level of apelin in PCOS patients was lower than the control group. We found lower levels of apelin in PCOS cases than the control group. To date, no study investigating the effect of metformin and EE/CA treatments on apelin in patients with PCOS has been conducted. On the other hand, there was a significant increase in serum apelin levels in all treatment groups (metformin, EE/CA, and metformin + EE/CA) in PCOS at the end of the 6th month of treatment. We think that this result is vital since it is the first and significant result related to the subject in the literature.

Serum adiponectin levels in PCOS patients have been investigated in many studies. In the study conducted by Demirci et al. (24), plasma adiponectin level was found to be low in patients with PCOS. In our study, adiponectin level (p=0.002) of patients with PCOS was found statistically lower than the control group.

The majority of the studies in PCOS have shown increased levels of CRP (19,25). In a meta-analysis, it was found that increased CRP levels decreased in patients with PCOS after metformin treatment (25). In our study, we found that CRP level was significantly higher in patients with PCOS compared to the control group.

In a study by Sahin et al. (26), 20 women with PCOS were compared with 20 age-matched healthy women, and it was shown that six months of metformin 2550 mg/day treatment did not cause a significant change in the carotid IMT value in women with PCOS. Similar to the study of Sahin et al. (26), we determined that six months of metformin 2000 mg/day treatment did not cause a significant change in the carotid IMT value. However, we found that six months of metformin 2000 mg/day treatment decreased the HOMA-IR value and increased the levels of adiponectin and apelin.

A combination of OCS and metformin is a crucial treatment option in obese and non-obese PCOS patients. In a study conducted by Mitkov et al. (27), it was shown that combined EE/CA treatment with metformin did not impair insulin sensitivity. In our study, we found that six months of treatment with metformin and EE/CA did not change the HOMA-IR value. We found that while PCOS cases treated with metformin + EE/CA did not change carotid IMT, HOMA-IR and CRP levels, apelin, and adiponectin levels increased significantly.

Table 5. Comparison of pre-treatment and post-treatment levels of parameters in the group receiving ethinyl estradiol/cyproterone acetate treatment

EE/CA			
	Pre-treatment (n=10)	Post-treatment (n=10)	p
	Mean ± SD	Mean ± SD	
IMT (% change)	0.40±0.09	0.39±0.05	NS
HOMA-IR	2.65±2.00	3.63±3.58	NS
Apelin (ng/mL)	3.45±3.49	4.72±3.81	0.024
Adiponectin (µg/mL)	12.99±7.67	14.91±7.73	NS
CRP (mg/dL)	2.90±3.62	3.30±2.83	NS

SD: standard deviation, IMT: intima media thickness, CRP: C-reactive protein, HOMA-IR: homeostatic model assessment of insulin resistance, EE/CA: ethinyl estradiol/cyproterone acetate, NS: not significant

Table 6. Comparison of pre-treatment and post-treatment levels of parameters in the group receiving metformin + EE/CA treatment

Metformin + EE/CA			
	Pre-treatment (n=15)	Post-treatment (n=15)	p
	Mean ± SD	Mean ± SD	
IMT (% change)	0.44±0.05	0.40±0.05	NS
HOMA-IR	2.91±1.55	2.51±1.66	NS
Apelin (ng/mL)	1.81±0.47	4.18±1.88	0.024
Adiponectin (µg/mL)	9.00±5.97	13.91±8.86	0.012
CRP (mg/dL)	1.87±1.39	2.43±1.92	NS

SD: standard deviation, IMT: intima media thickness, CRP: C-reactive protein, HOMA-IR: homeostatic model assessment of insulin resistance, EE/CA: ethinyl estradiol/cyproterone acetate, NS: not significant

Conclusion

We detected increased carotid IMT in PCOS cases, which is an early symptom of atherosclerosis. However, in patients with PCOS, serum levels of adiponectin and apelin were low, and CRP levels were high. We did not find a significant decrease in carotid IMT in all three treatment groups after treatment. We found an increase in serum apelin levels in all three treatment groups, and we found that this is the first and significant result in the literature. While adiponectin level increased in metformin and metformin + EE/CA group, it did not change in the EE/CA group. According to these results, we think that new, large-scale, prospective, and randomized studies are needed to evaluate subclinical and clinical atherosclerosis in PCOS cases.

Ethics Committee Approval: Written approval was obtained for the study from Kırıkkale University Faculty of Medicine Local Ethics Committee on 03.06.2009 with the number of 2009/089.

Informed Consent: Patient consent was obtained.

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Are ABO Blood Groups and Rh Factor Risk Factors for Hypertensive Diseases of Pregnancy?

ABO Kan Grupları ve Rh Faktörü Gebeliğin Hipertansif Hastalıkları için Risk Faktörü mü?

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ABSTRACT

Introduction: The primary aim of our study was to investigate the relationship between hypertensive diseases of pregnancy (HDP) and blood groups. Our secondary aim is to determine the risk factors that play a role in the development of HDP.

Methods: Pregnant women aged 15-49 years were included in this cross-sectional, observational, and prospective study. Pregnant women were divided into two groups. Group 1 (n=266) included patients admitted to the intensive care unit due to HDP. Group 2 (n=402) included normotensive patients without any complications during pregnancy. The groups were compared in terms of age, blood groups, Rh factor, gravida, parity, gestational week, and primiparity-multiparity.

Results: There was no statistically significant difference between the groups in terms of ABO blood group and Rh factor. The mean age, gravida, and parity were significantly higher in Group 1 than in Group 2 (p=0.028; 0.001; 0.004, respectively), while mean gestational age was lower than Group 2 (p<0.001). We also found that high gravida, low gestational age, presence of comorbidity, and primiparity were risk factors for HDP.

Conclusion: We did not find any relationship between HDP and blood groups. We found that high gravida, low gestational age, presence of comorbidity, and primiparity are risk factors for HDP.

Keywords: ABO blood group, Rh factor, hypertensive diseases of pregnancy, risk factors

ÖZ

Amaç: Çalışmamızın öncelikli amacı gebeliğin hipertansif hastalıkları (GHH) ile kan grupları arasındaki ilişkiyi incelemektir. İkincil amacımız ise GHH'lerin gelişiminde rol oynayan risk faktörlerini belirlemektir.

Yöntemler: Kesitsel, gözlemsel ve prospektif olarak planlanan çalışmamıza 15-49 yaş arası gebeler dahil edildi. Gebeler iki gruba ayrıldı. Grup 1 (n=266), GHH nedeniyle yoğun bakım ünitesine kabul ettiğimiz hastalar; grup 2 (n=402), gebelik süresince herhangi bir komplikasyon gelişmeyen ve normotansif olan hastalardan oluştu. Gruplar yaş, kan grupları, Rh faktörü, gravida, parite, gebelik haftası ve primiparite-multiparite açısından karşılaştırıldı.

Bulgular: Gruplar arasında ABO kan grubu ve Rh faktörü açısından istatistiksel olarak anlamlı bir fark saptanmadı. Grup 1'in yaş, gravida ve parite ortalaması grup 2'ye göre istatistiksel olarak anlamlı bir şekilde daha yüksek saptanırken (sırasıyla, p=0,028; 0,001; 0,004), gebelik haftası ortalaması grup 2'ye göre daha düşük idi (p<0,001). Ayrıca yüksek gravida, düşük gebelik haftası, komorbidite varlığı ve primiparitenin GHH için risk faktörleri olduğunu saptadık.

Sonuç: Çalışmamızda GHH ile kan grupları arasında herhangi bir ilişki saptamadık. Yüksek gravida, düşük gebelik haftası, komorbidite varlığı ve primiparitenin GHH için risk faktörleri olduğunu gösterdik.

Anahtar Kelimeler: ABO kan grubu, Rh faktörü, gebeliğin hipertansif hastalıkları, risk faktörleri

Introduction

Many risk factors such as age, parity, history of preeclampsia, multiple pregnancy, comorbidities [Diabetes Mellitus (DM), chronic hypertension, etc.], history of thrombophilia and obesity have been defined related to hypertensive diseases of pregnancy (HDP), which is the cause of approximately 18% of maternal deaths in the world (Table 1) (1,2). Despite this, the pathogenesis of preeclampsia and other HDP is not fully understood (3). Although it is not known, oxidative stress in the spiral arterioles in the placenta and endothelial dysfunction developed by various mediators may be responsible for the pathogenesis of preeclampsia (3). One of the risk factors identified for preeclampsia is the history of thrombophilia (4). Due to being a risk factor for preeclampsia, thrombophilia increased the interest in ABO blood groups that play an essential role in coagulation by interacting with factor VIII and von

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Willebrand factor (5). Studies have shown that bleeding is more common in people with blood group O and that thromboembolic events and ischemic heart diseases are more common in people with other blood types (6-8).

Different results have been found in studies investigating the relationship between preeclampsia and ABO in different parts of the world. While no relationship has been reported between preeclampsia and blood groups in some studies (9-12), other studies have reported an association between preeclampsia and blood groups A, AB, or O (13-17). These conflicting results also were observed in our country (11,17). We aimed to investigate the relationship between HDP and ABO blood group-Rh factor and contribute to the literature by examining the factors that play a role in the development of HDP in Diyarbakır Obstetrics and Gynecology Hospital, where the patient density is much higher than most hospitals in our country.

Methods

After the approval of the University of Health Sciences, Gazi Yaşargil Training and Research Hospital Ethics Committee (decision no: 27, date: February 16, 2018), our study was carried out between February 20, 2018, and February 01, 2019, in Diyarbakır Obstetrics and Gynecology Hospital, where there are approximately 24.000 births annually. Our study was planned as a cross-sectional, prospective, and observational study. Pregnant women aged 15-49 years were included in the study. Pregnant women were divided into two groups. Group 1 (n=266) included the patients who were hospitalized and followed up in the intensive care unit (ICU) due to HDP. Group 2 (n=402) was randomly selected among normotensive and follow-up patients who did not develop any complications during pregnancy. Patients under the age of 15, patients older than 49 years, patients who were pregnant and hospitalized in the ICU for non-HDP reasons, and those hospitalized in the ICU after gynecological surgeries were not included in the study. This study was conducted in accordance with the Helsinki Declaration 2008 criteria.

Age, blood group, Rh factor, gravida, parity, gestational week, comorbidities, and primiparity-multiparity status of the patients were recorded. Also, the length of ICU stay of group 1 patients was recorded. The groups were firstly compared in terms of blood groups and Rh factor, and then in terms of other factors. Then, statistically significant values were considered as independent risk factors, and estimated relative risk and 95% confidence interval (CI) were calculated by logistic regression analysis.

Table 1. Risk factors for preeclampsia*

Primiparity
History of preeclamptic pregnancy
Chronic hypertension, chronic kidney disease or both
History of thrombophilia
Multiple pregnancies
In vitro fertilization
Family history for preeclampsia
Type 1 or type 2 Diabetes Mellitus
Obesity
Systemic lupus erythematosus
Advanced maternal age (>40)

*: Hypertension in pregnancy. American College of Obstetricians and Gynecologists, 2013

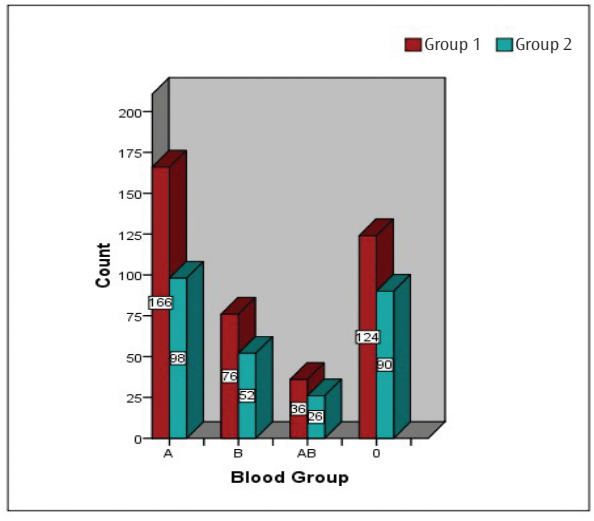
Statistical Analysis

SPSS 16.0 for Windows program was used for statistical analysis. Statistical data were expressed as mean and standard deviation (SD), and categorical data were expressed as number and percentage. The comparison of the categorical data was made with a chi-square test, and the results were given as n (%). The Kolmogorov-Smirnov test was used to determine whether the non-categorical data showed normal distribution. Mann-Whitney U test was used because the data did not show normal distribution. Results regarding numerical data were given as mean ± SD. Age, gravida, parity, gestational age, comorbidity, and primiparity-multiparity status were accepted as independent risk factors for HDP and evaluated by logistic regression. $P<0.05$ was accepted as statistically significant.

Results

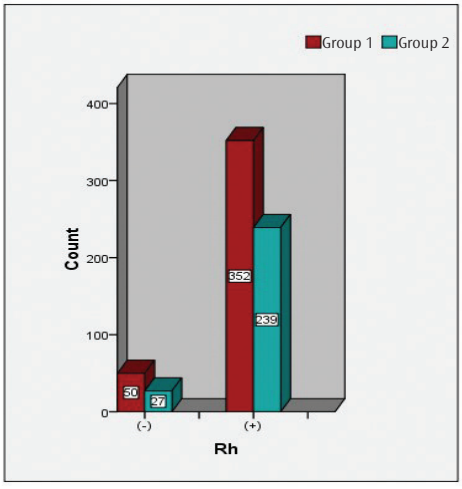
A total of 668 patients were included in the study. The mean age of the patients included in the study was 30.30±6.73 years, gravida was 3.22±2.1, parity was 2.81±1.75, and the gestational week was 36.73±2.98. One hundred and twenty-nine patients (19.3%) were primiparous, and 539 patients (81.7%) were multiparous. Thirty-one patients (4.6%) had comorbidity. The most frequent comorbidities were hypertension, anemia, and DM. The most frequently observed blood group was A blood group (264 patients, 39.5%), and 591 patients (88.5%) were Rh (+). Blood group and Rh types according to groups are shown in Graphs 1, 2.

Of all patients admitted to the ICU due to HDP (group 1), 23 (8.64%) had pregnancy-related hypertension, 229 had (86.09%) preeclampsia, 10 (3.75%) had eclampsia, and four (1.5%) had HELLP syndrome. The mean length of ICU stay was 2.25±0.79 days.



Graphic 1. Blood group frequency in groups

When group 1 and group 2 were compared in terms of age, gravida, parity, and gestational week, the following results were observed: The mean age of group 1 was higher than group 2, and this difference was statistically significant (p=0.028). The mean gravida and parity of group 1 were also significantly higher than group 2 (p=0.001 and 0.004, respectively). The mean gestational age of the patients in group 1 was found to be significantly lower than group 2 (p<0.001) (Table 2).



Graphic 2. Distribution of groups according to Rh factor

When group 1 and group 2 were evaluated in terms of primiparity-multiparity, it was found that HDP developed more than expected in the primiparous, and this difference was statistically significant (p=0.001). When group 1 and group 2 were evaluated in terms of comorbidity, it was found that HDP developed more than expected in patients with comorbidity, and this difference was statistically significant (p<0.001). When group 1 and group 2 were compared in terms of blood groups and Rh factor, no statistically significant difference was found between the groups (p=0.706 and 0.365, respectively) (Table 3).

The logistic regression analysis performed by accepting age, gravida, parity, gestational week, comorbidity and primiparity-multiparity status as independent risk factors for HDP revealed that high gravida [odds ratio (OR): 1.43, 95% CI: 1.142-1.811, p=0.02], low gestational week (OR: 0.59, 95% CI: 0.527-0.666, p<0.001), the presence of comorbidity (OR: 4.64, 95% CI: 1.817-11.662, p=0.001) and primiparity (OR: 0.152, 95% CI: 0.084-0.274, p<0.001) were found to be independent risk factors for HDP (Table 4).

Discussion

In studies examining the relationship between preeclampsia and ABO blood groups in HDP, it has been reported that AB blood group is mostly associated with preeclampsia, and blood group A was also reported in some studies (14-18). Compared to other blood groups, Placental protein 13 was reported to bind more strongly to AB blood group (15). Also, high

Table 2. Comparison of groups in terms of age, gravida, parity and gestational age

	Group 1 (Mean ± SD)[1]	Group 2 (Mean ± SD)	p*
Age	31.04±7.37	29.81±6.21	0.028
Gravida	3.86±2.64	2.81±1.52	0.001
Parity	3.26±2.28	2.51±1.20	0.004
Gestational age	35.09±3.92	37.80±1.27	<0.001

[1]: standard deviation, *Mann-Whitney U test result p-value

Table 3. Comparison of groups in terms of blood group, Rh factor, primiparity-multiparity and comorbidity

Blood group	Group 1 (n) (%)	Group 2 (n) (%)	p#
A	98 (37.12)	166 (62.88)	0.706
B	52 (40.62)	76 (59.38)	
AB	26 (41.93)	36 (58.07)	
0	90 (42.05)	124 (57.95)	
Rh			
(+)	239 (40.45)	352 (59.55)	0.365
(-)	27 (35.06)	50 (64.94)	
Primiparity-multiparity			
Primiparous	68 (52.72)	61 (47.28)	0.001
Multiparous	198 (36.73)	341 (63.27)	
Total	266	402	-
Comorbidity			
(+)	22 (8.3)	9 (2.2)	<0.001
(-)	244 (91.7)	393 (97.8)	
Total	266 (100%)	402 (100%)	-

#: Chi-square test result p-value

Table 4. Risk factors for the development of hypertensive diseases of pregnancy

Risk Factor	RR (95% CI)*	p**
Age	0.99 (0.965-1.034)	0.94
Gravida	1.43 (1.142-1.811)	0.02
Parity	1.204 (0.917-1.581)	0.182
Gestational age	0.59 (0.527-0.661)	<0.001
Comorbidity	4.64 (1.817-11.662)	0.001
Primiparity-multiparity	0.152 (0.084-0.274)	<0.001

*RR: estimated relative risk and 95% confidence interval indicated by odds ratio, **: p value after logistic regression analysis, CI: confidence interval

levels of coagulation factors (including factor VIII and von Willebrand factor) leading to preeclampsia and triggering pathophysiological events were reported in women with AB blood group (11). The fact that thromboembolic events are more common in other blood groups compared to the O blood group is another evidence supporting the association between the AB blood group and preeclampsia (15,18). However, there are studies in the literature indicating that there is no relationship between preeclampsia and the ABO blood group (9-12). In these studies, other factors (such as obesity, cardiovascular disease, and hypertensive diseases) were suggested to play a role in the development of preeclampsia except for the ABO blood group (9). In our study, no relationship was found between preeclampsia and ABO blood groups. However, in accordance with some publications in the literature, we found that the presence of comorbidity in pregnant patients (hypertension, DM) is a risk factor for the development of HDP.

There are some studies in the literature examining the relationship between the Rh factor and preeclampsia. Sharami et al. (12) stated that Rh (-) is a significant risk factor for moderate and severe preeclampsia and that the reason for this may be an immunological incompatibility between mother and fetus. Lee et al. (15) reported that women with Rh (+) had a slightly increased risk for HDP and preeclampsia. Avci et al. (17) also reported that Rh (+) was a risk factor in the development of preeclampsia and postpartum hypertension. The ratio of people with Rh (+) factor in our country is between 83.7-90.83%, and the ratio of Rh (-) is between 9.17-16.30% (19). In our study, 88.5% of the patients were Rh (+), and 11.5% were Rh (-), reflecting the general population. However, we could not find any relationship between the Rh factor and HDP in our study.

In the literature, various risk factors for HDP have been described in various publications. One of these publications is the guideline for hypertension in pregnancy, published in 2013 by The American College of Obstetricians and Gynecologists (4). In addition to the risk factors described in this guideline, in 2016, Rezk et al. (20) stated that having a history of miscarriage might be a risk factor for the development of preeclampsia. In our study, in accordance with the literature, we detected the presence of comorbidity and primiparity as risk factors for HDP development. Another result of our study, which was consistent with the literature, was that the mean age of patients with HDP was statistically higher than the control group. We think that increased frequency of preeclampsia with increasing age may be related to the increase in the incidence of chronic hypertension and other comorbid diseases with advanced age. Oxidative stress and endothelial dysfunction due to hypertension, DM, and many other chronic diseases may increase the risk of developing preeclampsia. In addition to these risk factors, we found that high gravida and low gestational age were also risk factors for preeclampsia. We could not find any literature examining the relationship between gravida and preeclampsia. In most of the studies in the literature, parity and primiparity-multiparity status were examined for preeclampsia (1,10,13,21,22). The fact that patients with high gravida are older, and the possibility of comorbidity increases with older age may cause preeclampsia to be seen more frequently in this patient group.

In their study, Khader et al. (22) reported that the incidence of preeclampsia was high in pregnant women with a gestational age of ≤31 weeks. In our study, we found that the gestational week was lower in the HDP group than the control group and that the low gestational week was a risk factor for preeclampsia. We believe that this result was due to the control group, including full-term pregnancies without any problems (mean gestational age: 37.8±1.27) and HDP group, including mostly preterm pregnant women (mean gestational age: 35.09±3.92). Pregnancy was terminated by cesarean section in most cases in order to maintain the well being of mother and fetus in HDP group.

Conclusion

No significant relationship was found between HDP and ABO blood groups and Rh factor. We found that high gravida, low gestational age, presence of comorbidity, and primiparity were risk factors for HDP. We think that more comprehensive studies on HDP, which is one of the most important diseases of the pregnancy that has many unclarified issues related to its pathophysiology, will contribute to understanding the pathophysiology of the disease and reducing maternal morbidity and mortality.

Ethics Committee Approval: After the approval of the University of Health Sciences, Gazi Yaşargil Training and Research Hospital Ethics Committee (decision no: 27, date: 16/02/2018).

Informed Consent: Informed consent was obtained from the parents of the children.

Peer-review: Externally peer-reviewed.

Author Contributions: Concept - O.U., A.Y.; Design - O.U., C.K.K., C.A., A.Y.; Data Collection and/or Processing - O.U., C.K.K., C.A.; Analysis and/or Interpretation - O.U., A.Y.; Literature Search - O.U., C.K.K., C.A., A.Y.; Writing Manuscript - O.U.

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Systemic Inflammatory Response in Unilateral Sinonasal Polyps

Tek Taraflı Sinonazal Poliplerde Sistemik Enflamatuvar Yanıt

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ABSTRACT

Introduction: Sinonasal polypoid disease is an inflammatory condition that can be unilateral. Various inflammatory markers have been used for evaluating systemic inflammatory response to diseases. This study aimed to evaluate systemic inflammatory markers in unilateral sinonasal polyps, to compare antrochoanal polyps and unilateral sinonasal polyposis in means of a systemic inflammatory reaction, and to assess systemic inflammatory reflection of involvement grade of paranasal sinuses.

Methods: Eighty-five patients were retrospectively analyzed and were divided into two groups as antrochoanal polyp group (APG) and unilateral polyposis group (UPG). Twenty patients who underwent septoplasty operation were taken as control septoplasty group (CSG). Preoperative neutrophil (Neu %), lymphocyte (Lym %) and eosinophil percentages (Eos %), thrombocyte counts (Plt), red cell distribution width (RDW), neutrophil to lymphocyte ratio, eosinophil to lymphocyte ratio (ELR), and extent of polyps in paranasal sinus computed tomography (PNS-CT) were recorded.

Results: Total polyp grade in PNS-CT of UPG was higher than APG ($p<0.05$). There was no significant difference in Plt, Neu %, Lym %, Eos %, ELR of APG, UPG, and CSG ($p>0.05$). RDW in CSG was lower than APG and UPG ($p<0.05$), and it showed no significant difference between APG and UPG ($p>0.05$).

Conclusion: Extent of unilateral sinonasal polypoid disease does not affect the severity of systemic inflammation. The effects of antrochoanal polyps on systemic inflammation are similar to the effects of unilateral sinonasal polyps.

Keywords: Chronic rhinosinusitis with nasal polyp, systemic inflammation, antrochoanal polyp, nasal polyp, neutrophil to eosinophil ratio, red cell distribution width

ÖZ

Amaç: Sinonazal polipler kronik sinonasal enflamasyon sonrasında gelişen tek taraflı da olabilen patolojilerdir. Çeşitli kan parametreleri sistemik enflamasyon cevabını değerlendirmede kullanılmaktadır. Bu çalışmada amaçlarımız; tek taraflı nazal poliplerde sistemik enflamasyon işaretçilerini incelenmesi, tek taraflı sinonazal hastalık ve antrokoanal polipler arasında sistemik enflamatuar cevap açısından fark olup olmadığının görülmesi ve paranazal sinüslerdeki polipoid hastalığın sistemik enflamasyona yansımasının araştırılmasıdır.

Yöntemler: Retrospektif olarak 85 hasta iki gruba ayrılarak incelendi: Antrokoanal polip grubu (APG), unilateral sinonazal polip grubu (UPG). Septoplasti operasyonu yapılan 20 hasta da kontrol grubu (CSG) olarak alındı. Preoperatif nötrofil, lenfosit ve eozinofil yüzdeleri, trombosit sayısı (Plt), eritrosit dağılım genişliği (RDW), nötrofil lenfosit oranı, eozinofil lenfosit oranı (ELR), paranazal sinüs bilgisayarlı tomografisindeki (PNS-BT) polip yaygınlığı kaydedildi.

Bulgular: UPG'deki polip yaygınlığı, APG'ye göre daha fazlaydı ($p<0.05$). Plt, nötrofil, eozinofil, lenfosit yüzdeleri, ELR; APG, UPG ve CSG arasında anlamlı fark göstermedi ($p>0,05$). APG ve UPG'de RDW değeri CSG'ye göre yüksekti ($p<0,05$) ancak APG ve UPG arasında fark göstermiyordu ($p>0,05$).

Sonuç: Tek taraflı sinonazal poliplerin yaygınlığı sistemik enflamasyon yanıtının şiddetini etkilemez. Antrokoanal polipler ve tek taraflı sinonazal poliplerin sistemik enflamasyona etkileri benzerdir.

Anahtar Kelimeler: Nazal polipli kronik rinosinüzit, sistemik enflamasyon, antrokoanal polip, nazal polip, nötrofil iyozinofil oranı, eritrosit dağılım genişliği

Introduction

Chronic rhinosinusitis that affects 4-10% of people worldwide is defined as the inflammatory condition of the paranasal sinuses and nasal passages, lasting for at least 12 weeks. The inflammation is named as chronic rhinosinusitis with nasal polyps when there is visible polyp tissue in the middle nasal meatus (1).

Nasal polyps are diagnosed by anterior rhinoscopy and nasal endoscopy. Paranasal sinus computed tomography (PNS-CT) shows the disease

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extent. Generally, polyps are multiple, bilateral, and seen in 1-4% of the population. They mostly accompany chronic rhinosinusitis. A nasal polyp is characterized by progressive inflammation of sinonasal mucosa in which eosinophils, T-cells, neutrophils, and plasma cells are present (2).

They result from edematous changes in inflamed sinonasal mucous membranes, and cause nasal congestion and discharge, headache, and olfactory dysfunction. They are seen mostly in patients of 40-70 years old and they are rarely seen under the age of 10 (3).

Histopathologically, nasal polyps are characterized by thickening of the basement membrane, formation of the atypic glands, goblet cell hyperplasia, infiltration of the inflammatory cells, and subepithelial edema. Eosinophils are the most common inflammatory cells. Inflammatory mediators, cytokines, adhesion molecules, and endothelial receptors involve in (4).

Multiple hypotheses for their unknown ethiopathogenesis were suggested in the literature, such as chronic inflammation, environmental pollution, aspirin intolerance, allergy, genetic, hereditary, and environmental problems. They have a multifactorial pathology (5).

The solitary, benign soft-tissue masses extending into the nasopharynx that originate from the nasal cavity are called choanal polyps. Their common origin is maxillary sinus mucosa, and the name is antrochoanal polyp. They constitute 3-6% of nasal polyps in all population and 35% of pediatric nasal polyps. The medical treatment is useless and the treatment is surgery (6).

Ethiopathogenesis is still not known. Although, they were suggested to be due to complication of chronic antral inflammation, allergic polyps (more eosinophils) are found more frequently than the inflammatory polyps (more neutrophils) (7).

In literature, the ratios of neutrophil to lymphocyte (NLR), platelet to lymphocyte (PLR), eosinophil to lymphocyte ratio (ELR), and eosinophil count and red blood cell distribution width (RDW) value that can be simply obtained from complete blood count have been studied for a correlation with nasal polyp severity and recurrence. In sinonasal polyposis, the correlation was explained by the eosinophil-rich inflammation and chronic inflammation that causes the body to produce lymphopenia, thrombocytosis, and neutrophilia (8-10).

The aim of this study was to evaluate the systemic inflammatory markers in unilateral sinonasal polypoid disease, to show if antrochoanal polyps and unilateral sinonasal polyposis results in different systemic inflammatory reactions and whether these possible changes in inflammatory status is reflected by extent of disease seen on PNS-CT and endoscopic nasal examination.

Methods

Our study was approved by the University of Health Sciences, İstanbul Training and Research Hospital Local Clinical Research Ethics Committee on August 29th, 2019, with the decision number of 1958 (decision no: 2011-KAEK-50).

The patients, who underwent unilateral functional endoscopic sinus surgery and antrochoanal polyp excision surgery with indications of unilateral sinonasal polyposis or antrochoanal polyp, respectively, in our otolaryngology clinic between January 2014 and August 2019, were analyzed. Informed consent was taken from all patients for their surgeries and the use of their medical data in academic medical research. A total of 85 patients (55 male, 30 female) were included in our study. Twenty patients who were operated for nasal septum deviation (septoplasty operation) in the same period (with no acute or chronic inflammation, nasal or systemic disease) were taken as control septoplasty group (CSG).

The patients with chronic diseases, malignancies, chronic sinusitis without nasal polyposis, hypothyroidism, malnutrition, obstructive sleep apnea, allergic rhinitis, acute or chronic infections, parasitic infections, atopic conditions, asthma, familial history of atopy or allergy, patients who used systemic medications or steroid recently or used preoperative oral steroids were excluded from the study. Bilateral sinonasal disease and recurrent sinonasal disease were also excluded from the study. The patients, whose surgical specimen was analyzed pathologically and diagnosed as other than inflammatory polyp, were also excluded.

The patients were divided into two groups, namely antrochoanal polyp group (APG) and unilateral polyposis group (UPG). Preoperative records of laboratory tests and PNS-CT scans of all patients were analyzed. PNS-CT scans were screened for the involvement of the sinuses, burden of disease, and scoring was done according to the modified Lund-Mackay system (11) [no polyp: 0 point, partial sinus involvement: 1 point, complete involvement in sinüs: 2 points; osteomeatal complex disease: 2 points, no disease in osteomeatal complex: 0 points for every unilateral paranasal sinus (maxillary, ethmoid, frontal and sphenoid sinuses)]. A total unilateral involvement score is obtained from the summation of these points.

At the endoscopic nasal examination, the size of the polyps was scored according to extension volume in nasal cavity (12) (polyp grading system: 0, no visible nasal polyp; 1, small amount of polypoid disease confined within the middle meatus; 2, multiple polyps occupying the middle meatus; 3, polyps extending beyond the middle meatus, within the sphenoethmoid recess but not totally obstructing, or both; 4, massive polyps completely obstructing the nasal cavity).

For every patient; neutrophil (Neu %), lymphocyte (Lym %) and eosinophil percentages (Eos %), thrombocyte counts (Plt), RDW values were recorded, NLR, ELR were calculated from their preoperative complete blood counts. These values were compared between the groups.

Associations between complete blood count parameters and PNS-CT scores, the extent of polypoid disease, size of the polyps at endoscopic nasal examinations were analyzed, and both groups (APG and UPG) were compared in means of systemic inflammatory response markers.

Statistical Analysis

SPSS 22.0 program was used to evaluate all data obtained in the study. Descriptive statistics (mean and standard deviation, median, lowest and highest frequencies, and ratio values) were used to evaluate the data. Kolmogorov-Smirnov test was used for analyzing the distribution of the variables. ANOVA, Kruskal-Wallis, and Mann-Whitney U tests were used for the analysis of the independent quantitative data. A chi-square test was used for the analysis of independent data.

Results

A total of 85 patients (55 male, 30 female, mean age: 22.63 years and age range: 5-56 years) were included (APG included 31 male and 20 female patients with a mean age of 22.63 years age range of 5-52 years; UPG included 24 male and 10 female patients with a mean age of 36.12 years and age range of 12-56 years). Ranges and mean values of measured parameters are shown in Table 1.

The mean age of the patients in APG and CSG was significantly lower than in UPG ($p<0.05$). The mean age of the patients in CSG was significantly higher than that of APG ($p<0.05$). Total polypoid disease grade in PNS-CT of UPG was higher than that of APG ($p<0.05$). The endoscopic examination of polyp grade in APG was higher than in UPG ($p<0.05$). There was no significant difference in Plt, Neu %, Lym %, Eos %, ELR values of APG, UPG, and CSG ($p>0.05$). RDW value in CSG was lower than that of APG and UPG ($p<0.05$). RDW value showed no significant difference between APG and UPG ($p>0.05$). NLR value was higher in CSG than in APG and UPG ($p<0.05$). NLR value showed no significant difference between APG and UPG ($p>0.05$) (Table 2).

Discussion

The number of biochemistry, microbiology, and immunology studies regarding nasal polyps is growing in the last years.

Sinonasal polyps are characterized by chronic inflammation causing stromal edema. Nasal polyp epithelial cells produce many inflammatory cytokines, including interleukin-8 (IL-8), granulocyte-macrophage colony-stimulating factor, IL-6, IL-1B, Tumor necrosis factor-alpha, and vascular endothelial growth factor. These cytokines cause eosinophilia because they increase the peripheral circulation of eosinophils (13).

There are few studies for antrochoanal polyps and inflammation. In the literature, the relationship between antrochoanal polyps and allergy was examined, and different results were obtained (14,15).

Neutrophils, lymphocytes, and thrombocytes are functionary blood cells in inflammatory processes. Thrombocytosis, neutrophilia, and peripheral lymphopenia reflect the inflammatory status of the whole body, according to previous studies in the literature. An increased neutrophil count is a reflection of ongoing inflammation, and low lymphocyte count reflects malnutrition and inflammatory status (16-18).

It was reported that there is a correlated increase of NLR with poor clinical progress in cardiac disorders and malignancies (19,20). Like NLR, the increased RDW was also shown as an indicator of systemic inflammation (21).

Thrombocyte to lymphocyte ratio (TLR) was found high and was suggested as a poor prognostic factor in various peripheral vascular and coronary artery diseases, some gynecologic, and hepatobiliary malignancies. In the terminal stage of renal failure, TLR was reported to be more valuable than NLR value in means of indicating the systemic inflammation (22).

In a study, Ulu et al. (23) found that NLR value was significantly higher than the control group in idiopathic sudden sensorineural hearing loss. In patients whose NLR value was higher, the positive response to the treatment was lower. According to these findings, they suggested it as a poor prognostic factor. In Bucak et al. (24) study on Bell's palsy, they found neutrophil and NLR value to be significantly higher than the healthy control group.

The sinonasal polyps and inflammation were examined in various studies. Total serum immunoglobulin E and eosinophil values were shown to be significantly higher in sinonasal polyposis than in the

Table 1. Ranges and mean values of measured parameters

		Minimum-maximum			Median	Mean ± SD		
Age		5.0	-	56.0	27.0	28.1	±	12.8
Gender	Female	-	-	-	-	33	-	31.4%
	Male	-	-	-	-	72	-	68.6%
PLT		131.0	-	405.0	252.0	254.5	±	56.2
RDW		11.2	-	34.2	13.1	13.8	±	3.2
Neu %		33.2	-	84.8	56.3	56.5	±	9.9
Lym %		8.5	-	58.9	32.2	32.9	±	8.8
NLR		0.2	-	10.0	1.8	2.3	±	1.7
Eo %		0.4	-	13.0	2.0	2.7	±	2.3
ELR		0.0	-	0.6	0.1	0.1	±	0.1
Ethmoid		0.0	-	2.0	1.0	0.8	±	0.7
Frontal		0.0	-	2.0	0.0	0.4	±	0.7
Sphenoid		0.0	-	2.0	0.0	0.3	±	0.6
Maxillary		0.0	-	3.0	2.0	1.7	±	0.5
OMC		0.0	-	2.0	2.0	1.8	±	0.6
Total PNS-CT Score		1.0	-	10.0	5.0	5.0	±	1.9
Endoscopic polyp grade		1.0	-	4.0	3.0	3.0	±	0.8

SD: standard deviation, Plt: thrombocyte count, RDW: red cell distribution width, Neu: neutrophil, Lym: lymphocyte, NLR: neutrophil to lymphocyte ratio, Eo: eosinophil, ELR: eosinophil to lymphocyte ratio, OMC: osteomeatal complex

healthy control group (25). Peripheral blood eosinophil count and ELR were reported as relevant with recurrences of sinonasal polyposis in chronic rhinosinusitis (10,26).

In the study of Atan et al. (9), they found significantly higher leucocyte and thrombocyte counts in bilateral sinonasal polyposis than in the healthy control group. They also found higher TLR, but that was not significant. In this study, NLR and TLR were shown to be not associated with the extent of polyp disease in PNS-CT.

In the study of Tecimer et al. (27), unlike other studies in literature, no association was found between neutrophilic or eosinophilic nature of sinonasal polyposis and polyp recurrences. In Boztepe et al.'s (28) study, they suggested that NLR could be used for the prediction of recurrences in sinonasal polyposis before surgery.

Chronic rhinosinusitis with sinonasal polyposis is mostly bilateral, but it can also be unilateral. In literature, the studies concerning nasal polyps and inflammation are mostly performed in bilateral disease. For this reason, unilateral polyposis and its effects on systemic inflammatory responses were preferred for this study.

Comparing antrochoanal polyps with bilateral sinonasal polyposis would be an imbalanced challenge due to the extent and burden of polypoid disease; so unilateral polyps were preferred for study. Systemic inflammation burden, type, and the differences between the groups were studied.

Steroids are effective in the treatment of sinonasal polyposis. The topical or systemic steroids block the inflammatory signal that is activated by vasoactive mediators (29). They reduce eosinophilia by lowering the granulocyte-macrophage colony-stimulating factor synthesis and increasing eosinophil apoptosis (30). For all bilateral sinonasal polyp patients, preoperative systemic steroid use is routinely preferred in our clinic. Steroids have effects on complete blood count parameters and inflammation (29). In unilateral sinonasal polyps and antrochoanal polyps, preoperative use of steroids is not preferred in our clinic. This is another reason for taking unilateral disease into this study.

Like neutrophil lymphocyte ratio, increased RDW has also been reported as an inflammatory marker in the literature (21). In our study, Plt, Neu %, Lym %, Eos %, ELR values showed no statistically significant differences between the groups. Only the RDW value was significantly higher in UPG and APG than CSG. This value had no significant difference between the UPG and APG.

Although PNS-CT polypoid disease involvement grade score was significantly higher than antrochoanal polyp disease in unilateral sinonasal polyposis disease, this extension grade or polyp volume burden could not create a difference in systemic inflammation. Antrochoanal polyps and unilateral sinonasal polypoid disease increase systemic inflammation; however, they do not have significant differences in systemic inflammation response relative to each other.

The main limitation of our study was that previously established inflammatory indicators like C-reactive protein were not analyzed. These are not used in routine preoperative patient preparation. The large-scaled prospective studies measuring multiple inflammatory parameters may help better explain the relation between sinonasal polyposis and systemic inflammation.

Table 2. Comparison of blood and computed tomography parameters between groups

		Antrochoanal polyp				Unilateral polyps				Septum deviation				p	
		Mean ± SD			Median	Mean ± SD			Median	Mean ± SD			Median		
Age		22.6	±	11.8	19.0	36.1	±	11.8	37.5	28.5	±	9.6	25.0	0.000	K
Gender	Female	20	-	39.2%	-	10	-	29.4%	-	3	-	15.0%	-	0.135	X^2
	Male	31	-	60.8%	-	24	-	70.6%	-	17	-	85.0%	-		
PLT		262.9	±	53.9	254.0	244.0	±	64.0	239.0	251.2	±	45.9	247.5	0.420	A
RDW		14.5	±	4.5	13.3	13.2	±	0.6	13.1	12.7	±	0.8	12.6	0.007	K
Neu%		57.0	±	11.6	57.4	56.9	±	8.6	56.2	54.7	±	7.1	55.0	0.626	K
Lym%		32.6	±	10.0	31.5	32.4	±	7.5	32.5	34.8	±	7.8	32.8	0.654	K
NLR		2.2	±	1.7	1.8	2.0	±	1.3	1.7	3.0	±	2.1	2.6	0.040	K
Eo%		3.3	±	2.8	2.5	2.5	±	1.9	2.1	1.7	±	0.5	1.7	0.060	K
ELR		0.1	±	0.1	0.1	0.1	±	0.1	0.1	0.1	±	0.1	0.1	0.269	K
Ethmoid		0.5	±	0.7	0.0	1.1	±	0.6	1.0	-	-	-	-	0.000	m
Frontal		0.2	±	0.6	0.0	0.7	±	0.9	0.0	-	-	-	-	0.010	m
Sphenoid		0.3	±	0.5	0.0	0.5	±	0.7	0.0	-	-	-	-	0.244	m
Maxillary		1.8	±	0.5	2.0	1.5	±	0.6	1.5	-	-	-	-	0.003	m
OMC		1.8	±	0.6	2.0	1.8	±	0.5	2.0	-	-	-	-	0.840	m
Total CT score		4.6	±	1.8	4.0	5.6	±	1.9	5.0	-	-	-	-	0.033	m
Endoscopic polyp grade		3.4	±	0.7	3.0	2.5	±	0.7	2.5	-	-	-	-	0.000	m

K: Kruskal-Wallis (Mann-Whitney U test), A: ANOVA / X^2: chi-square test

Plt: thrombocyte count, RDW: red cell distribution width, Neu: neutrophil, Lym: lymphocyte, NLR: neutrophil to lymphocyte ratio, Eo: eosinophil, ELR: eosinophil to lymphocyte ratio, OMC: osteomeatal complex

Changes in systemic inflammation parameters in preoperative and postoperative measures may explain the effects of unilateral polyps and antrochoanal polyps on the systemic inflammatory process. Changes in systemic inflammatory parameters may help our timing of the medical or surgical therapy or evaluating the response to the treatment.

Conclusion

The extent of unilateral sinonasal polypoid disease does not affect the severity of systemic inflammation. The effects of antrochoanal polyps on systemic inflammation are similar to the effects of unilateral sinonasal polyps.

Ethics Committee Approval: Our study was approved by the University of Health Sciences, İstanbul Training and Research Hospital Local Clinical Research Ethics Committee on August 29th, 2019, with the decision number of 1958 (decision no: 2011-KAEK-50).

Informed Consent: Informed consent was taken from all patients for their surgeries and the use of their medical data in academic medical research.

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Effect of Concha Bullosa on Skull Base

Konka Büllozanın Kafatası Tabanı Üzerine Etkisi

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ABSTRACT

Introduction: To demonstrate the effect of concha bullosa (CB) on the height of the skull base.

Methods: We retrospectively scanned the files of 1022 patients who had undergone paranasal sinus tomography for sinusitis in our hospital. Three hundred thirty patients had bilateral, and 330 had unilateral CB, but 330 patients did not have CB. Patients with inappropriate sections and positions were excluded from the study. A total of 990 patients aged between 18 and 72 years were included in the study. Five hundred sixty-seven of the patients were female, and 423 were male. In each group, the heights of the right and left skull base of male and female patients were compared within themselves and between each other. The height of the skull base was measured in the coronal plane along the lateral lamella between the cribriform plate and the fovea ethmoidalis, and these measurements were classified as Keros classification. (Keros type 1: 1-3 mm, Keros type 2: 4-7 mm, Keros type 3: 8-16 mm).

Results: There was no statistically significant difference in the comparison between right and left skull base heights of female and male patients in bilateral CB group. Also, there was no statistically significant difference between the comparison of women and men within themselves. There was a statistically significant difference between CB side and non-CB side skull base heights of female and male patients in unilateral CB group, although there was no statistically significant difference between the comparison of women and men within themselves.

Conclusion: In patients with unilateral CB, there is skull base asymmetry between the sides of CB and non-CB, and this should be taken into consideration to prevent complications.

Keywords: Concha bullosa, skull base, paranasal sinus tomography

ÖZ

Amaç: Konka büllozanın (KB) kafa tabanı yüksekliği üzerindeki etkisini göstermektir.

Yöntemler: Hastanemizde sinüzit nedeniyle paranazal sinüs tomografisi çekilen 1022 hastanın dosyaları retrospektif olarak tarandı. Üç yüz otuz hastada bilateral, 330 hastada unilateral KB vardı, ancak 330 hastada KB yoktu. Kesitleri ve pozisyonları uygun olmayan hastalar çalışma dışı bırakıldı. Çalışmaya 18-72 yaş arası toplam 990 hasta dahil edildi. Hastaların beşyüz altmış yedisi kadın, 423'ü erkekti. Her grupta erkek ve kadın hastaların sağ ve sol kafatası tabanının yükseklikleri kendi içlerinde ve birbirleri arasında karşılaştırıldı. Kafatası tabanının yüksekliği, kribriform plaka ile fovea etmoidalis arasındaki lateral lamel boyunca koronal düzlemde ölçülmüş ve bu ölçümler Keros sınıflandırması kullanılarak sınıflandırılmıştır. (Keros tip 1: 1-3 mm, Keros tip 2: 4-7 mm, Keros tip 3: 8-16 mm).

Bulgular: Bilateral KB grubunda kadın ve erkek hastaların sağ ve sol kafatası tabanı yükseklikleri arasında istatistiksel olarak anlamlı bir fark saptanmadı. Ayrıca, kadın ve erkeklerin kendi içlerinde yapılan karşılaştırmalarında da istatistiksel anlamlı bir fark saptanmadı. Unilateral KB grubunda kadın ve erkek hastaların KB'si olan ve KB'si olmayan taraf kafatası tabanı yükseklikleri arasında istatistiksel olarak anlamlı bir fark saptanmasına rağmen, kadın ve erkeklerin kendi içlerinde yapılan karşılaştırmalarında istatistiksel anlamlı bir fark saptanmadı.

Sonuç: Unilateral KB'li hastalarda, KB olan ve olmayan taraflar arasında kafatası tabanı asimetrisi vardır ve komplikasyonları önlemek için bu dikkate alınmalıdır.

Anahtar Kelimeler: Konka bülloza, kafatası tabanı, paranazal sinüs tomografisi

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Introduction

The roof of the ethmoidal labyrinth is formed by the fovea ethmoidalis, which is an extension of the frontal bone, primarily separating the ethmoidal cells from the anterior cranial fossa. Fovea ethmoidalis is part of the ethmoid bone that attaches to the lateral lamella of the cribriform plate medially. The depth of the olfactory fossa is determined by the height of the lateral lamella of the cribriform plate (1,2). According to the literature, in the skull base, iatrogenic lesions occur predominantly in the lateral lamella of the cribriform plate. The site where the anterior ethmoidal artery penetrates the cranial fossa is particularly interesting, considering that this is the thinnest and less resistant region of the whole skull base (1,2).

Depending on the Keros type, a variable segment of the lateral wall of the olfactory fossa will be exposed during the dissection of the frontoethmoidal region. Keros divided the roof of the ethmoid into three categories according to the depth of the cribriform plate (Keros type 1: 1-3 mm, Keros type 2: 4-7 mm, Keros type 3: 8-16 mm) (3). The Keros type 3 is the most vulnerable one, considering the major risk for an iatrogenic lesion of the lateral lamella of the cribriform plate (2,4).

The knowledge about the complex skull base anatomy and anatomical relations, including the fovea ethmoidalis and lateral lamella of the cribriform plate, is essential in the prevention of complications in endoscopic nasal surgeries (5,6). Computed tomography (CT) is considered to be a radiological method that can evaluate the anatomy and anatomical variants of the paranasal sinuses in the right way and is extremely useful in the planning of the preoperative endonasal surgery (7).

Concha bullosa (CB) represent the entity of an air cell in the turbinates. Middle turbinate (MT) pneumatization results from changes in the development of the ethmoid air cell system. The incidence of pneumatization of the MT is between 13 and 53.6% (8). CB is usually asymptomatic and diagnosed incidentally by CT. From time to time, an over-pneumatized MT can lead to deviated nasal septum, contact headache, nasal obstruction, and sinusitis (9).

Evaluation of anatomical findings may determine a higher intraoperative safety during endonasal surgeries in the frontoethmoidal region, giving the surgeon previous knowledge about the configuration of the ethmoidal roof and depth of olfactory fossae, consequently reducing the patient exposure to potential complications. There are no studies investigating the relationship between different CB types and skull base configurations in the literature. This study aimed to determine the effect of CB on the skull base.

Methods

We retrospectively scanned the files of 1022 patients who had undergone paranasal sinus CT for sinusitis in our hospital between 2014 and 2018. Patients were divided into three groups as unilateral CB, bilateral CB, and non-CB. In our study, the sample size was accepted as 330 for each group. When 330 patients were selected for each group, the retrospective scan was completed. Patients with inappropriate sections and positions were excluded from the study. Each group of patients was divided into two groups as men and women. The right and left heights of the skull base of the groups were compared between male and female patients as well as within itself, both male and female patients.

Examinations were performed using CT equipment (GE Optima CT660, General Electric, Waukesha, Wisconsin, USA) with 64 detectors-128 Slices. For the scanning parameters, 120 kVp; 120-150 mA; a spacing of 200 mm field of view and 1.75 was used. The scans were performed in a coronal plane with a cross-section thickness of 2.5 mm and a cross-section of 3 mm. CT images were analyzed using GE Volume Viewer SW. The images were examined in the bone window on a digital screen. The same otolaryngologist evaluated all of the cases included in the study. The ethmoid roof measurements were performed manually using a digital screen-standard anatomic points (Figure 1).

The study was approved by University of Health Sciences, Ümraniye Training and Research Hospital Ethics Committee (B.10.1.TKH.4.34.H.GP.0.01/132, date: 21.12.2017).

Statistical Analysis

IBM SPSS Statistics Version 22 (IBM Turkish limited company, İstanbul, Turkey) program was used for statistical analysis. The normality of the parameters was evaluated by the Shapiro-Wilks test. Descriptive statistical methods (mean, standard deviation, and median) were calculated. When the groups were evaluated together, nonparametric data were assessed by Kruskal-Wallis test. P values were confirmed by the Bonferroni test. Mann-Whitney U test was used in the comparison of nonparametric data between groups. Significance was assessed at $p<0.05$ level.

Results

Three hundred thirty patients were included in each group. Of the patients included in the study, 567 were female, and 423 were male. Of the patients in the bilateral CB group, 231 were female, and 99 were male. The ages of the women ranged from 20 to 68 years, and the mean age was 35.27±13.9 years, while the ages of the males ranged from 18 to 72 years, and the mean age was 36.67±14.21 years. In the unilateral CB group, 193 patients were female, and 137 were male. The ages of the women ranged from 21 to 61 years, and the mean age was 38.86±14.21

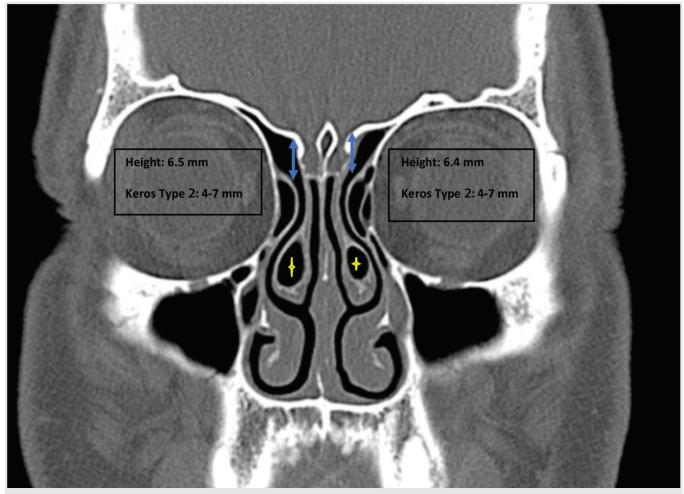

Figure 1. Skull base measurement in a patient with bilateral concha bullosa

years, while the ages of the males ranged from 25 to 57 years, and the mean age was 34.39±10.16 years. In the non CB group, 142 patients were female, and 188 were male. The ages of the women ranged from 19 to 65 years, and the mean age was 35.19±13.07 years, while the ages of the men ranged from 23 to 70 years, and the mean age was 35.28±11.86 years.

The right and left skull base heights of females and males in bilateral CB group, there were 3.9% Keros type 1, 69.7% Keros type 2, 26.4% Keros type 3; 3.03% Keros type 1, 79.8% Keros type 2, 17.17% Keros type 3; 6.06% Keros type 1, 76.19% Keros type 2, 17.75% Keros type 3; and 6% Keros type 1, 81% Keros type 2 and 13% Keros type 3, respectively (Table 1).

The right and left skull base heights of females and males without CB, there were 1.41% Keros type 1, 84.51% Keros type 2, 14.08% Keros type 3; 1.06% Keros type 1, 73.4% Keros type 2, 25.54% Keros type 3; 3.52% Keros type 1, 85.21% Keros type 2, 11.27% Keros type 3; and 0.53% Keros type 1, 76.07% Keros type 2 and 23.4% Keros type 3, respectively (Table 2).

Skull base values of females and males in the sides with and without CB in the group with unilateral CB, there were 3.11% Keros type 1, 70.98% Keros type 2, 25.91% Keros type 3; 0.73% Keros type 1, 70.8% Keros type 2, 28.47% Keros type 3; 8.29% Keros type 1, 80.31% Keros type 2, 11.4% Keros type 3; and 7.3% Keros type 1, 83.21% Keros type 2 and 9.49% Keros type 3, respectively (Table 3).

In the evaluation of the bilateral CB group, the right and left skull base heights of females and males were 5.91±1.71 mm, 5.74±1.68 mm, 5.66±1.67 mm, and 5.47±1.64 mm, respectively. There was no statistically significant difference between women and men both in their own and in comparison with each other ($p<0.144$, $p=0.323$, $p=0.25$, $p=0.27$) (Table 4).

In the group without CB, the right and left skull base values of females and males were 5.61±1.39 mm, 6.03±1.57 mm, 5.46±1.52 mm and 5.82±1.51 mm, respectively. There was no statistically significant difference between women and men both in their own and in comparison with each other (except female-male left comparison) ($p=0.318$, $p=0.949$, $p=0.06$, $p<0.001$) (Table 5).

Skull base values of females and males in the group with unilateral CB in the sides with and without the CB were 5.95±1.64 mm, 6.13±1.55 mm,

Table 1. Bilateral concha bullosa group (female right/left keros, male, right/left keros)

Right female	**n**	**%**	**Mean ± SD**
Keros 1	9	3.9	2.67±0.23
Keros 2	160	69.7	5.26±0.98
Keros 3	61	26.4	8.11±1.02
Left female	**n**	**%**	**Mean ± SD**
Keros 1	14	6.06	2.65±0.39
Keros 2	176	76.19	5.3±1.04
Keros 3	40	17.25	8.23±0.89
Right male	**n**	**%**	**Mean ± SD**
Keros 1	3	3.03	2.73±0.3
Keros 2	79	79.8	5.25±0.96
Keros 3	18	17.17	8.57±1.21
Left male	**n**	**%**	**Mean ± SD**
Keros 1	6	6	2.58±0.37
Keros 2	81	81	5.21±1.02
Keros 3	13	13	8.37±1.15

n: number of patients, %: patient percentage, SD: standard deviation, Keros 1: 1-3 mm, Keros 2: 4-7 mm, Keros 3: 8-16 mm

Table 2. Group without concha bullosa (female right/left keros, male, right/left keros)

Right female	**n**	**%**	**Mean ± SD**
Keros 1	2	1.41	2.65±0.07
Keros 2	120	84.51	5.24±0.85
Keros 3	20	14.08	8.15±1.1
Left female	**n**	**%**	**Mean ± SD**
Keros 1	5	3.52	2.66±0.28
Keros 2	121	85.21	5.18±0.94
Keros 3	16	11.27	8.49±1.34
Right male	**n**	**%**	**Mean ± SD**
Keros 1	2	1.06	2.6±0.28
Keros 2	138	73.4	5.37±0.99
Keros 3	48	25.54	8.07±0.98
Left male	**n**	**%**	**Mean ± SD**
Keros 1	1	0.53	2.3
Keros 2	143	76.07	5.43±1.03
Keros 3	44	23.4	8.03±0.92

n: number of patients, %: patient percentage, SD: standard deviation, Keros 1: 1-3 mm, Keros 2: 4-7 mm, Keros 3: 8-16 mm

Table 3. Unilateral concha bullosa group (female concha bullosa side/non-concha bullosa side, male concha bullosa side/non-concha bullosa side)

The CB side			
Female	**n**	**%**	**Mean ± SD**
Keros 1	6	3.11	2.38±0.22
Keros 2	137	70.98	5.34±0.97
Keros 3	50	25.91	8.05±0.92
The non-CB side			
Female	**n**	**%**	**Mean ± SD**
Keros 1	16	8.29	2.47±0.48
Keros 2	155	80.31	4.86±0.97
Keros 3	22	11.4	7.92±0.94
The CB side			
Male	**n**	**%**	**Mean ± SD**
Keros 1	1	0.73	2.3
Keros 2	97	70.8	5.38±0.94
Keros 3	39	28.47	8.10±0.75
The non-CB side			
Male	**n**	**%**	**Mean ± SD**
Keros 1	10	7.3	2.73±0.23
Keros 2	114	83.21	5.18±0.94
Keros 3	13	9.49	7.86±1.02

CB: concha bullosa, n: number of patients, %: patient percentage, SD: standard deviation, Keros 1: 1-3 mm, Keros 2: 4-7 mm, Keros 3: 8-16 mm

5.01±1.55 mm and 5.26±1.4 mm, respectively. There was a statistically significant difference between right and left skull base heights in male and female patients, but there was no statistically significant difference between them (p<0.001, p<0.001, p=0.348, p=0.74) (Tables 6,7).

In the comparison of the right and left levels of the groups, a statistically significant difference was not found between the bilateral CB group and the non-CB group (p=0.998, p=0.171) (Table 8).

In the comparison of the right and left levels of the groups, a statistically significant difference was found between the bilateral CB group right and the unilateral CB group non-CB side (p<0.001) (Table 9).

Table 4. Bilateral concha bullosa group (female right/left, male right/left comparison) and (female-male right/left comparison)

Female	**n**	**Mean ± SD**	**p**
Right	231	5.91±1.71	0.144
Left	231	5.66±1.67	
Male	**n**	**Mean ± SD**	**p**
Right	99	5.74±1.68	0.323
Left	99	5.47±1.64	
Female-male	**n**	**Mean ± SD**	**p**
Female right	231	5.91±1.71	0.25
Male right	99	5.74±1.68	
Female-male	**n**	**Mean ± SD**	**p**
Female left	231	5.66±1.67	0.27
Male left	99	5.47±1.64	

Mann-Whitney U test p<0.05, n: number of patients, SD: standart deviation

Table 5. Group without concha bullosa (female right/left, male right/left and female-male right/left comparison

Female	**n**	**Mean ± SD**	**p**
Right	142	5.61±1.39	0.318
Left	142	5.46±1.52	
Male	**n**	**Mean ± SD**	**p**
Right	188	6.03±1.57	0.949
Left	188	5.82±1.51	
Female-male right	**n**	**Mean ± SD**	**p**
Female right	142	5.61±1.39	0,06
Male right	188	6.03±1.57	
Female-male left	**n**	**Mean ± SD**	**p**
Female left	142	5.46±1.52	0,001
Male left	188	5.82±1.51	

Mann-Whitney U test, p<0.05, n: number of patients, SD: standart deviation

Table 6. Unilateral concha bullosa group female (+)/(-) and male (+)/(-) comparison

Female	**n**	**Mean ± SD**	**p**
CB side	193	5.95±1.64	0.001
Non-CB side	193	5.01±1.55	
Male	**n**	**Mean ± SD**	**p**
CB side	137	6.13±1.55	0.001
Non-CB side	137	5.26±1.4	

Mann-Whitney U test, p<0.05, (+): CB side, (-): Non-CB side, n: number of patients, SD: standart deviation, CB: concha bullosa

A statistically significant difference was found between the left side of the bilateral CB group and the CB side of the unilateral CB group as well as the non-CB side of the unilateral CB group (p<0.001) (Table 10).

A statistically significant difference was found between the right side of the non-CB group and the non-CB side of the unilateral CB group (p<0.001) (Table 11).

A statistically significant difference was found between the left side of the non-CB group and the non-CB side of the unilateral CB group (p<0.001) (Table 12).

There was no statistically significant difference in the other comparisons of the groups.

Table 7. Unilateral concha bullosa group (female-male concha bullosa/non-concha bullosa side comparison)

Female-male	**n**	**Mean ± SD**	**p**
Female CB side	193	5.95±1.64	0.348
Male CB side	137	6.13±1.55	
Female-male	**n**	**Mean ± SD**	**p**
Female non-CB side	193	5.01±1.55	0.74
Male non-CB side	137	5.26±1.4	

Mann-Whitney U test p<0.05, n: number of patients, SD: standart deviation, CB: concha bullosa

Table 8. Comparison of bilateral concha bullosa and non-concha bullosa group

	n	**Min-max**	**Mean ± SD**	**p**
BCB group right	330	2.4-11.8	5.86±1.705	0.998
Non-CB group right	330	2.4-11.4	5.85±1.51	
BCB group left	330	1.7-10.2	5.6±1.66	0.171
Non-CB left	330	2.3-11.5	5.78±1.54	

Mann-Whitney U test, p<0.05, BCB: bilateral concha bullosa, CB: concha bullosa, SD: standard deviation, min: minimum, max: maximum

Table 9. Comparison of bilateral concha bullosa and unilateral concha bullosa group

	n	**Min-max**	**Mean ± SD**	**p**
BCB group right	330	2.4-11.8	5.86±1.705	0.115
UCB group CB side	330	2.2-11.4	6.03±1.607	
BCB group right	330	2.4-11.8	5.86±1.705	0.001
UCB group non-CB side	330	1.1-11	5.11±1.49	

Mann-Whitney U test, p<0.05, BCB: bilateral concha bullosa, CB: concha bullosa, min: minimum, max: maximum, SD: standard deviation, UCB: unilateral concha bullosa

Table 10. Comparison of bilateral concha bullosa and unilateral concha bullosa group

	n	**Min-max**	**Mean ± SD**	**p**
BCB group left	330	1.7-10.20	5.60±1.66	0.001
UCB group CB side	330	2.2-11.4	6.03±1.607	
BCB group left	330	1.7-10.20	5.60±1.66	0.001
UCB group non-CB side	330	1.1-11	5.11±1.49	

Mann-Whitney U test, p<0.05, BCB: bilateral concha bullosa, CB: concha bullosa, min: minimum, max: maximum, SD: standard deviation, UCB: unilateral concha bullosa

Also, no statistically significant difference was found in the comparison of all groups (p=0.22) (Table 13).

Discussion

The anatomy of the paranasal sinuses and skull base are highly complex, with many anatomic variations. Detailed knowledge of anatomic variations in the paranasal sinus and skull base are essential to understand sinonasal pathology and avoid complications if surgery is indicated. Variations of the paranasal anatomic structures can be detected easily with paranasal CT (10). CB is the pneumatization of the concha and is a frequent variation of the sinonasal anatomy. CB is most commonly seen in MTs (11). It is rarely found in upper and lower turbinates (12,13). Because we performed only MT analysis in our study, anatomical variations other than the MT were excluded from the study.

The frontal bone of the ethmoid roof area is dense and thick. The thicker frontal bone is medially located near the thin lateral lamellae of the ethmoid bone (14). It is already well established that the area at risk is not in the highest point of the ethmoid sinus formed by the fovea ethmoidalis, but in the lateral lamella of the cribriform plate in the region of the ethmoidal sulcus. This is the most vulnerable site in the whole skull base where the anterior ethmoidal artery leaves the ethmoid sinus and courses anteriorly in the ethmoidal sulcus of the olfactory fossa (1,2).

The lateral lamella, a thin bone component of the lamina cribrosa, forms the medial wall of the ethmoid roof. Determining the length and width of the olfactory fossa and the depth of the ethmoid roof by radiological methods is significant for determining the upper limit of the dissection. Radiologic examinations can help us to prevent complications such as anterior cranial fossa penetration, cerebral damage, bleeding, and cerebrospinal fluid fistulae (15).

CT has contributed not only to the evaluation of sinonasal diseases but also to the characterization of the paranasal sinuses anatomy. Paranasal sinus CT studies on the coronal plane may provide sufficient information about individual variations of patients and ethmoid roof depths (14). Coronal images can particularly be considered as maps in the evaluation of the anatomy that is highly variable even between the two sides of the same individual, demonstrating areas potentially at risk for complications in the planning of endoscopic nasal surgeries (16-18). The ethmoid roof height difference was shown to be a risk factor regarding complications, and therefore continuous analysis of the ethmoid roof by CT during the intraoperative period was fundamental regarding surgical safety (19). Disregard to consider asymmetry in the skull base during the intraoperatively or preoperative period may result in significant complications (20).

Ethmoid roof configuration may vary in different societies (21,22). In their ethmoid roof analysis of 136 cases, Erdem et al. (23) found that 8.1% of the cases were Keros type 1, 59.6% were Keros type 2, and 32.3% were Keros type 3. Şahin et al. (24) examined 100 paranasal sinus CT scans in their study and reported that 10% of the cases were Keros type 1, 61% were Keros type 2, and 29% were Keros type 3. Kaplanoglu et al. (25) stated that, of the 1.000 total examinations (two sides in each patient), 13.4% were Keros type 1, 76.1% were Keros type 2, and 10.5% were Keros type 3. Type 1 was more prevalent in women than men (31.6% vs 21.4%), and type 3 was more prevalent in men than women (24.4% vs 17.9%). These studies support and make strong the hypothesis that the ethmoid roof configuration varies in the Turkish population. In our study, it was found that Keros type 2 in both men and women was seen more frequently than Keros type 1 and 3. In some of the other studies, although gender difference was found to affect the height of the skull base, no such condition was found in our study.

The MT is one of the essential marking places in endoscopic sinus surgery. In a study comparing the height of the MT to the depth of the cribriform plate, it was determined that the olfactory fossa was less deep and that the MT was longer in Keros type 1 cases, whereas in the Keros type 3 cases, where the olfactory fossa was deeper, and the MT length was detected shorter (26). During endoscopic sinus surgery, it is advisable that it should not move beyond the MT attachment site to prevent trauma to the skull base (26).

Determining the relation of the middle concha to the surrounding anatomic formations can provide important benefits in endoscopic sinus surgery. In a study comparing the total height of the nasal cavity with the depth of the olfactory fossa, it was determined that the depth of the olfactory fossa was parallel to the depth of the nasal cavity. In the same study, the height of the orbit was compared with the depth of the olfactory fossa, and it was determined that this height of the most stable formation (23). Knowing the average lengths of the skull base composition and surrounding anatomical structures in patients

Table 11. Comparison of non-concha bullosa group and unilateral concha bullosa group

	n	Min-max	Mean ± SD	p
Non-CB group right	330	2.4-11.4	5.85±1.51	0.09
UCB group CB side	330	2.2-11.4	6.03±1.60	
Non-CB group right	330	2.4-11.4	5.85±1.51	0.001
UCB group non-CB side	330	1.1-11	5.11±1.49	

Mann-Whitney U test, p<0.05, BCB: bilateral concha bullosa, CB: concha bullosa, min: minimum, max: maximum, SD: standard deviation, UCB: unilateral concha bullosa

Table 12. Comparison of non-concha bullosa and unilateral concha bullosa group

	n	Min-max	Mean ± SD	p
Non-CB group left	330	2.3-11.5	5.78±1.54	0.3
UCB group CB side	330	2.2-11.4	6.03±1.60	
Non-CB group left	330	2.3-11.5	5.78±1.54	0.001
UCB group non-CB side	330	1.1-11	5.11±1.49	

Mann-Whitney U test, p<0.05, BCB: bilateral concha bullosa, CB: concha bullosa, min: minimum, max: maximum, SD: standard deviation, UCB: unilateral concha bullosa

Table 13. Comparison of all groups

	n	Min-max	Mean ± SD	p
BCB group	330	1.7-11.8	5.73±1.69	0.22
UCB group	330	1.1-11.4	5.57±1.61	
Non-CB group	330	2.3-11.5	5.82±1.52	

Kruskall-Wallis test p<0.05, BCB: bilateral concha bullosa, CB: concha bullosa, min: minimum, max: maximum, SD: standard deviation UCB: unilateral concha bullosa

during endoscopic sinus surgery may prevent serious complications that may occur during the operation. Therefore, careful evaluation of preoperative CT for safer surgery is one of the most critical steps.

The MT is formed by the medial part of the ethmoid bone. As it elongates in the nasal cavity, anterosuperior stabilization is achieved by the cribriform plate, and posterolateral stabilization is achieved by the lamina papyricea. CB is the pneumatization of the MT and is one of the most common variations of the sinonasal anatomy. Pneumatization of the MT happens due to variation in the ethmoidal air cell system development (27-29). The CB turns into apparent after 7-8 years of age and continues its development even after the period of adolescence. The mean age (30.3 years) of CB was consistent with other studies on the same topic (30). CB can be unilateral or bilateral and can be classified into three types according to the site of pneumatization. They are lamellar-type (vertical lamella of MT pneumatization), bulbous-type (the inferior portion of MT pneumatization), and extensive/large type (vertical lamella and inferior portion of the MT pneumatization) (13). The rate of pneumatization and the inflammatory changes that take place within the CB may correlate with the presentation, and the severity of symptoms (31).

In a study conducted by Bolger et al. (13), Paranasal sinus CT of 207 patients was investigated, and lamellar type CB was found in 46.2%, bullous type CB in 31.2% and extant type CB in 15.7%. Similar to this study, we found 43.1% lamellar type CB, 33.5% bullous type CB and 23.4% extensive type CB. In our study, we evaluated all types of CB, and we did not distinguish between the types of CB in the evaluation of the skull base. In 85.3% of the patients from mild to advanced nasal septal deviation, 14% were nasal polyps, and 56% had sinusitis. CB may cause nasal septal deviation and sinusitis findings in these patients, but may also be effective on skull base height. In this study, we evaluated the effect of CB on the skull base height. We found that unilateral CB effects the height of the skull base.

The fact that it is different only in the unilateral CB group in the evaluation of skull base height may be caused by embryological development. There may be a need for more studies to make this clear.

Conclusion

Because unilateral CB causes changes in the skull base height, it may be useful to carefully the side of the unilateral CB during endoscopic sinus surgery to avoid possible complications.

Ethics Committee Approval: The study was approved by University of Health Sciences, Ümraniye Training and Research Hospital Ethics Committee (B.10.1.TKH.4.34.H.GP.0.01/132, date: 21.12.2017).

Informed Consent: Since it was a retrospective study, consent was not obtained from the patients.

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Awareness of Pregnant Women About Routine Applied Screening Tests and Supportive Treatments in a University Hospital

Bir Üniversite Hastanesinde Rutin Uygulanan Tarama Testleri ve Destek Tedavileri Hakkında Gebelerin Farkındalıkları

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ABSTRACT

Introduction: In this study, we aimed to evaluate the awareness of pregnant women about routine applied screening tests and supportive treatments in a university hospital and the factors affecting this.

Methods: This observational, descriptive study was carried out between 15th April and 30th November 2018. Four hundred and ninety-three volunteer pregnant women who applied to the Necmettin Erbakan University Meram Faculty of Medicine outpatient clinic for the first time or were being followed up formed the study cohort. In order to determine socio-demographic characteristics, awareness of screening tests, and supportive therapies, and the factors affecting this, a questionnaire consisting of 36 multiple-choice, open-ended questions was applied through face-to-face interviews.

Results: The median age of the participants was 27. More than half (57.4%) were graduated from primary school or did not receive education, and 89.0% were unemployed. The majority of the participants stated that they knew screening tests (92.1%) and supportive treatments (93.9%). Forty-eight point one percent and 44.0% of screening tests and supportive treatments were learned from obstetricians, respectively. The reason stated by 57.6% of the participants who did not want to have screening tests was, "I find it unnecessary because I do not want to end my pregnancy". Participants who were 27 years old or older (p=0.021), who were at least high school graduates (p=0.016), who were employed (p=0.041), and who had given birth before (p<0.001) knew the screening tests more significantly.

Conclusion: The study results showed that the awareness of pregnant women about screening tests and supportive treatments increased with increasing maternal age, education level, employment status, and the number of births. Although the percentage of getting information from healthcare workers about screening tests and supportive treatments was higher, it was still not at the desired level. Therefore, we believe that healthcare workers should be more sensitive to informing and counseling during prenatal care, especially for young and low-educated mothers living in rural areas.

Keywords: Prenatal care, screening tests, supportive treatments, pregnancy

ÖZ

Amaç: Bu çalışmada bir üniversite hastanesinde rutin uygulanan tarama testleri ve destek tedavileri hakkında gebelerin farkındalıkları ve bunu etkileyen faktörleri değerlendirmeyi amaçladık.

Yöntemler: Bu gözlemsel, tanımlayıcı çalışma 15 Nisan-30 Kasım 2018 tarihleri arasında gerçekleştirildi. Necmettin Erbakan Üniversitesi Meram Tıp Fakültesi polikliniğine ilk kez başvuran ya da takip edilmekte olan 493 gönüllü gebe kadın çalışma grubunu oluşturdu. Sosyo-demografik özelliklerini, tarama testleri ile destek tedavileri hakkındaki farkındalıkları ve bunu etkileyen faktörleri belirlemek için çoktan seçmeli ve açık uçlu 36 sorudan oluşan anket formu yüz yüze görüşmelerle uygulandı.

Bulgular: Katılımcıların ortanca yaşı 27 yıl idi. Yarıdan fazlası (%57,4) ilkokuldan mezun olmuş veya eğitim görmemişti, %89'u çalışmıyordu. Katılımcıların çoğunluğu tarama testlerini (%92,1) ve destek tedavilerini (%93,9) bildiklerini belirtti. Sırasıyla, tarama testlerinin ve destek tedavilerinin %48,1'i ve %44'ü kadın doğum uzmanlarından öğrenilmişti. Tarama testlerini yaptırmayı istemeyen katılımcıların %57,6'sının belirttiği sebep "Gereksiz buluyorum, çünkü hamileliğimi sonlandırmak istemiyorum" idi. Yirmi yedi yaş ve üstü (p=0,021), en az liseden mezun olmuş (p=0,016), çalışan (p=0,041) ve primipar (p<0,001) olan katılımcılar tarama testlerini belirgin olarak daha fazla bildiğini ifade etmişti.

Sonuç: Çalışma sonuçları tarama testleri ve destek tedavileri hakkında gebelerin farkındalıklarının anne yaşı, eğitim düzeyi, çalışma durumu ve doğum sayısı arttıkça arttığını göstermekteydi. Sağlık çalışanlarından tarama testleri ve destekleyici tedaviler hakkında bilgi edinme yüzdesi daha yüksek olmasına rağmen, hala istenen düzeyde değildi. Bu nedenle, sağlık çalışanları doğum öncesi bakım hizmetleri sırasında, özellikle kırsal alanlarda yaşayan genç ve düşük eğitimli anneler için bilgilendirme ve danışmanlık konusunda daha duyarlı olmalılar kanaatindeyiz.

Anahtar Kelimeler: Doğum öncesi bakım, tarama testleri, destek tedavileri, gebelik

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Introduction

Complications in pregnancy, birth, and puerperium in developing countries are the leading causes of morbidity and mortality among women of reproductive age. According to the "National Maternal Mortality Study" data of the Ministry of Health in our country, 25.5% of maternal deaths during this period were due to not receiving any prenatal care (PNC), and 23.6% were due to receiving low-quality PNC (1). With the implementation of sufficient and qualified PNC services, the maternal mortality rate dropped to 14.7 in 2016 from 64 in 2002 per hundred thousand live births, and the infant mortality rate dropped to 7.3 in 2016 from 31.5 in 2002 per thousand (2).

PNC is the follow-up of the fetus and mother by trained health personnel at regular intervals during pregnancy by making necessary examinations and making recommendations in order to ensure that mothers have a healthy pregnancy and deliver their babies in a healthy way (3). In the PNC management guide created by the Ministry of Health, examinations, measurements, tests, and consultancy services that need to be performed during at least four follow-ups are defined, including one within the first 14 weeks, at 18-24th weeks, 30-32nd weeks and 36-38th weeks (2).

Periodic checks and screening tests during pregnancy allow early detection of high-risk pregnancies (4). In addition to the dual screening test on the 11th-14th week of pregnancy, ultrasonography (US) examination performed on the same day measures the nuchal thickness of the fetus and the presence of nasal bone. The triple screening tests are performed during the 16th-18th week of pregnancy (5,6). The detailed US is evaluated by experienced physicians at 18-23rd weeks (7). Gestational diabetes screening is held between 24-28th weeks. However, these tests should be performed in the first trimester in pregnant women with a history of gestational diabetes, macrosomic baby (>4.500 gr at birth), polyhydramnios, anomaly, unexplained fetal or newborn death, diabetes in close relatives, and pregnant women with a body mass index of ≥30 kg/m^2 (8,9).

Within the framework of the "neonatal tetanus elimination program", the first dose of the tetanus vaccine is applied at the 4th month of pregnancy or in the first encounter, and the second dose at least four weeks after the second dose (2,7). During pregnancy, which is an anabolic process, energy, vitamin, and mineral needs increase. For this reason, folic acid, iron, and vitamin D supplements are recommended to each pregnant woman by the Ministry of Health (10).

In our country, the level of knowledge about the pregnancies of expectant mothers and the sources from which they obtained this information are quite limited. However, it is thought that wrong or incomplete information negatively affects the view of pregnant women to screening tests and supportive treatments. For this reason, primary healthcare professionals have essential duties.

In this study, we aimed to evaluate the awareness of pregnant women about routine screening tests and supportive treatments in a university hospital and the factors affecting this.

Methods

All pregnant women who applied to Necmettin Erbakan University Meram Faculty of Medicine outpatient clinics for the first time between 15 April 2018 and 30 November 2018 or who were still in the follow-up and who agreed to participate in the study were included in this observational and descriptive study.

Since it was not known how many pregnant women would come and agree to participate in the study with the random sampling method since it was a cross-sectional study, it was assumed that the number of individuals in the universe was unknown, so it was planned to reach at least 377 pregnant women with 5% margin of error and 95% confidence interval (CI), and it was planned to include at least 414 people in the study by adding 10% to this number due to the possibility of not filling the survey questions. In keeping with the planned date range, 493 voluntary pregnant women were included. Also, the prevalence of the event was unknown since the subject being studied was not a clinical condition. According to the size of the sample determined by random sampling in this way, a pregnant applying to the outpatient clinic was a part of the universe.

The questionnaire form prepared by the researchers was applied to ten pregnant women, and the questionnaire form was finalized after the necessary corrections were made to the questions. A 36-item questionnaire was filled with face-to-face interviews, including questions aimed at obtaining socio-demographic information such as age, profession, education level, age of spouse, education status of spouse, kinship status, smoking, previous pregnancy and questions about birth process if they had given birth, knowledge about the screening tests given in options, source of this information, questions about their current pregnancy, and the recommended screening and supportive treatments (folic acid, multivitamin, vitamin D, and iron supplement) and the attitudes of pregnant women to these. One of these questions is open-ended, and it is about the reason for not wanting a screening test. Answers to this question are categorized, as "I find it unnecessary because I do not want to end my pregnancy", "I think it will harm the baby", "I do not want to have a triple test because the double test is normal", "It could not be done because the time to be done has passed" and "I do not find it reliable".

The ethical permission of the study was taken before the study, with the number of 2018/1259, dated 16.03.2018, from the Ethics Committee of Necmettin Erbakan University Meram Faculty of Medicine, Pharmaceuticals and Non-medical Researches Ethics Committee. The participants were informed about the study, and their written and verbal consents were obtained according to the Helsinki Declaration principles.

Statistical Analysis

While evaluating the findings obtained in the study, SPSS for Windows 21.0 was used for statistical analysis. Frequency, mean, standard deviation, median, minimum, and maximum values were calculated. In the comparison of categorical data, a chi-square test was used. Results were evaluated at a 95% CI and significance level at $p<0.05$.

Results

The mean age of 493 pregnant women aged 16-47 years who participated in the study was 27.6±5.8 years (median age: 27). Two hundred and

eighty-three (57.4%) had primary education or less, 89.0% (n=439) were unemployed and 11.4% (n=56) were relatives with her spouse. While 7.1% (n=35) of women stopped smoking when they learned about pregnancy, 3.3% (n=16) continued to smoke. Various socio-demographic data of the participants are shown in Table 1.

Regarding first control, 46.5% of pregnant women (n=229) applied to a state hospital, 19.7% (n=97) to a private clinic/private hospital, 17% (n=84) to a university hospital, and 14.8% (n=73) to a family health center. The mean gestational age was 22.8±10.2 weeks (median: 22 weeks). The vast majority of pregnant women (n=474, 96.1%) conceived naturally and 36.1% (n=178) were nulliparous. Four hundred and sixty-two (93.7%) women included in the study stated that they applied to the family physician they were affiliated with. When the health problems arose during pregnancy were examined, seven had hypertension, 18 had diabetes, 12 had thyroid disease, 27 had anemia, and 18 had a clotting disorder.

The majority of participants (n=454, 92.1%) stated that they knew the screening tests. When the source of information was asked to women who knew about screening tests, 52% (n=236) stated that they learned from gynecology and obstetrician, 14.5% (n=66) from a family physician, and 32.4% (n=147) from experience from a previous pregnancy. Table 2 shows the state of knowing screening tests and supportive treatments.

The most common test (n=445, 90.3%) that the pregnant women participating in the study had or asked to have was the detailed US. Table 3 shows the status of having/not having screening tests done. When women that did not want to have screening tests were asked for the reason, 57.6% (n=208) of the participants answered as "I find it unnecessary because I do not want to end my pregnancy", 19.4% (n=70) as "I think it will harm the baby", 10% (n=36) as "I do not want to have a triple test because the double test is normal", 7.8% (n=28) as "It could not be done because the time to be done has passed", and 1.9% (n=7) as "I do not find it reliable".

The relationship between the knowledge of the screening tests and educational level, age, working status, education level of the spouse, number of pregnancies, and birth status were statistically examined.

Table 1. Socio-demographic features of participants

Parameters	n	%
Age		
<27 years	260	52.7
≥27 years	233	47.3
Education status		
≤ Primary education	283	57.4
≥ High school	210	42.6
Employment status		
Unemployed	439	89.0
Employed	54	11.0
Education status of spouse		
≤ Primary education	247	50.1
≥ High school	246	49.9
Employment status of spouse		
Tradesman/self-employed	158	32.1
Officer	76	15.4
Worker	259	52.5
Kinship status with spouse		
There is kinship	56	11.4
No kinship	437	88.6
Place of residence		
Konya center	364	73.8
District and villages	129	26.2
Smoking status		
Never smoked	432	87.6
She quitted when she learned about her pregnancy	35	7.1
She quitted before pregnancy	10	2.0
Still smoking	16	3.3

Table 2. Knowledge about the screening tests and supportive treatments

Parameters	Knows		Does not know	
	n	%	n	%
Screening tests in pregnancy	**454**	**92.1**	**39**	**7.9**
Double screening test	419	85.0	74	15.0
Triple screening test	414	84.0	79	16.0
Glucose challenge test	405	82.2	88	17.8
Detailed ultrasonography	401	81.3	92	18.7
Supportive treatments during pregnancy	**463**	**93.9**	**30**	**6.1**
Folic acid supplement	393	79.7	100	20.3
Multivitamin supplement	258	52.3	235	47.7
Iron supplement	345	70.0	148	30.0
Vitamin D supplement	355	72.0	138	28.0
Vaccines during pregnancy	352	71.4	141	28.6

Table 3. Screening tests and tetanus vaccination status

Parameters	I want/I had		I want/I will have		I do not want/I did not have	
	n	%	n	%	n	%
Double screening test	206	41.8	75	15.2	212	43.0
Triple screening test	115	23.3	112	22.7	266	54.0
Glucose challenge test	112	22.7	152	30.8	229	46.5
Detailed ultrasonography	226	45.9	219	44.4	48	9.7
Tetanus vaccine	278	56.4	193	39.1	22	4.5

Awareness of screening tests was found to be statistically significantly higher in patients aged 27 years or older (p=0.021), with high school or more education (p=0.016), employed (p=0.041), and those who had given birth before (p<0.001) (Table 4).

The majority of the participants (n=463, 93.9%) stated that they knew supportive treatments. Information sources for screening tests and supportive treatments are shown in Table 5. Among the supportive treatments, the highest awareness was found in vaccines during pregnancy. Four hundred and seventy-one women (95.5%) participating in the study stated that they had the vaccines during pregnancy or wanted to have them. Table 6 shows other recommended therapies and the usage status of them. Three hundred and forty-two (69.4%) women answered the question, "Should the family physician play an effective role at the community level in promoting supportive treatment during pregnancy?" as "I totally agree".

Discussion

Mothers having a healthy pregnancy and the birth of healthy babies are the cornerstones of public health. For this reason, screening tests and supportive treatments, which are among the components of PNC, are critical. In our study, we examined the factors that affect the compliance of screening tests and supportive treatments applied to the expectant mothers who applied to the pregnancy clinic of our hospital and the factors that affect their compliance, if any.

While the highest fertility rate in our country was in the 20-24 years age group in previous studies, it was observed in the 25-29 years age group in Turkey Demographic and Health Survey (TDHS)-2013. This result indicates that pregnancies are delayed to the advanced ages in Turkey (11). Similarly, the median age of mothers was found to be 27 years in our study. According to the TDHS-2013 report, 81% of women were living in urban areas, 44% of women in the 25-29 years age group and 18% of women in the 45-49 years age group were at least high school graduates, and 31% of women were employed during the study (11). Similarly, in our study, 73.8% of the participants lived in the city center of Konya, and 49.9% of them had high school and above education. Unlike the TDHS report, in our study, 89% of pregnant women were housewives, and only 11% were employed.

According to the 2016 Turkish Statistical Institute data, rates of consanguineous marriage in our country is 23.2% (12). In our study, the rate of consanguineous marriage was found to be 11.4%. Although this result is evaluated as a positive development for our country, it is necessary to know that the study sample and the place where the study was conducted may also be effective in this difference.

Smoking during pregnancy increases risks such as miscarriage, premature birth, low birth weight, and sudden infant death, causing severe harm. In our study, 87.6% of the participants stated that they did not smoke at all, 2% stated that they quitted before pregnancy, 7.1% stated that they quitted when they learned their pregnancy, and 3.3% stated that they were still smoking. In studies conducted in England and Scotland, it was reported that one in four women smoked before or during pregnancy, and one in eight women continued smoking during their pregnancy. In a study conducted in Romania, it was reported that 30% of the interviewed mothers were smoking before pregnancy and that 43.3% of them continued smoking during pregnancy (13,14). In a study conducted with 513 pregnant women in Italy, 22.3% of the participants continued to smoke during their pregnancy and claimed that they

Table 4. Knowledge about the screening tests and related factors

	Knowledge about the screening tests in pregnancy					
	Yes		No		χ^2	p
	n	%	n	%		
Education status						
≤ Primary education	253	89.4	30	10.6	5.761	0.016
≥ High school	201	95.7	9	4.3		
Age						
<27 years	232	89.2	28	10.8	5.368	0.021
≥27 years	222	95.3	11	4.7		
Employment status						
Unemployed	401	91.3	38	8.7	4.176	0.041
Employed	53	98.1	1	1.9		
Employment status of spouse						
≤ Primary education	223	90.3	24	9.7	1.747	0.186
≥ High school	231	93.9	15	6.1		
Number of pregnancies						
≤2	247	89.9	28	10.2	3.726	0.054
>2	207	95.0	11	5.0		
Delivery status						
No	152	85.9	25	14.1	13.334	<0.001
Yes	302	95.6	14	4.4		

Table 5. Information sources for screening tests and supportive treatments in pregnancy*

Parameters	n	%
Information sources for screening tests		
Previous pregnancy		
Yes	147	32.4
No	307	67.6
Family physician		
Yes	66	14.5
No	388	85.5
Obstetrician		
Yes	236	52.0
No	218	48.0
Internet/social media		
Yes	34	7.5
No	420	92.5
Printed media/TV		
Yes	2	0.4
No	452	99.6
Other (mother, relatives, etc.)		
Yes	27	5.9
No	427	94.1
Information source for supportive treatments		
Previous pregnancy		
Yes	138	29.8
No	325	70.2
Family physician		
Yes	170	36.7
No	293	63.3
Obstetrician		
Yes	216	46.7
No	247	53.3
Internet/social media		
Yes	20	4.3
No	443	95.7
Printed media/TV		
Yes	2	0.4
No	461	99.6

*Participants gave multiple answers to these questions, TV: television

continued smoking during their pregnancy because they received very little information about this subject during their examination (15). Pirdal et al. (16) reported that 79.4% of pregnant women never smoked in their lives, 18.1% stopped smoking during pregnancy, and only 2.5% continued smoking during pregnancy. These examples in the literature highlight the importance of providing more information about the risks of smoking in pregnancy during PNC, as women planning pregnancy and currently pregnant women are a high priority target group for smoking cessation interventions.

In our study, 93.7% of the pregnant women stated that they admitted to the family physician they were affiliated with, and 46.5% stated that they admitted to the state hospital for the first examination. In the study of Kurnaz et al. (17), 94.0% of the participants stated that they had a pregnancy record in the family physician, and 43.4% stated that they went to the family health center for the first control. In the study conducted by Durusoy et al. (18) in İzmir, the rate of pregnant women who were followed up by the family physician was 85%, and the place for first control was state hospital with a rate of 33.2%. We think that this may be due to the idea that pregnant women apply to hospitals in order to learn the baby's gender or that they believe they should be examined by an obstetrician, even if the pregnancy is not risky at all.

In our study, the participants stated that they had or they wanted to have a double test (57%), triple test (46%), glucose challenge test (53.5%), and detailed US (90.3%) performed. In the study of Potur et al. (19), it was reported that 70.3% of the pregnant women had a glucose challenge test, 59.5% had a double test, and 48.6% had a triple test. In the study conducted by Desdicioğlu et al. (20), 72.25% of the pregnant women stated that they had or wanted to have all screening tests. In the study of Bilgin et al. (21), out of 300 pregnant women, 192 answered yes to only triple, 20 only to double, and 41 to both to the question whether they had any screening test during pregnancy.

In our study, the source of information was obstetricians in 52% of pregnant women, family physicians in 14.5%, internet/social media in 7.5%, and experience from previous pregnancy in 32.4%. In the study of Ruhat Karakuş, the answers given to the question of "where did you learn the double or triple screening test?" were "my physician recommended" (86.5%), "I learned from the internet" (8.1%), "my friends recommended" (2.7%), "I learned from TV or newspaper" (2.7%) (22).

In our study, the awareness of pregnant women about screening tests was found to be significantly higher in those who were 27 years of age and older, who had high school and above education, who were employed and who gave birth before. Unlike our study, the mean age of pregnant women who knew the double and triple tests was found to be

Table 6. Suggestion and usage status of supportive treatments

Parameters	Recommended I used		Recommended I am using		Recommended I will use		Recommended I do not want		Not recommended	
	n	%	n	%	n	%	n	%	n	%
Folic acid supplement	283	57.4	113	22.9	15	3.0	10	2.0	72	14.6
Multivitamin supplement	122	24.7	135	27.4	29	5.9	7	1.4	200	40.6
Iron supplement	129	26.2	159	32.3	43	8.7	5	1.0	157	31.8
Vitamin D supplement	150	30.4	179	36.3	38	7.7	6	1.2	120	24.3

lower in the study of Desdicioğlu et al. (20). In the study of Pirdal et al. (16), two independent factors affecting the knowledge level of pregnant women were found to be the education level and age. In the study of Bilgin et al. (21), the rate of obtaining information about screening tests and the level of the evaluation were increasing as the level of education increased; however, the increase in the parity seemed to decrease these. Increased awareness with age was considered to be related to the increasing number of births and information learned from previous pregnancies. Increasing awareness with education level may be the result of education facilitating learning and understanding in general.

When women that did not want to have screening tests were asked for the reason, 57.6% (n=208) of the participants answered as "I find it unnecessary because I do not want to end my pregnancy", 19.4% (n=70) as "I think it will harm the baby", 10% (n=36) as "I do not want to have a triple test because the double test is normal", 7.8% (n=28) as "It could not be done because the time to be done has passed", and 1.9% (n=7) as "I do not find it reliable". When the participants were asked about the reasons for not wanting prenatal tests in the study by Lewis et al. (23), they stated that the participants responded as "ending pregnancy is not an option" and "invasive tests increase the risk of miscarriage", which are mostly based on opinions and moral values. Desdicioğlu et al. (20) stated that 75% of the pregnant women telling that they would not have/did not have the diabetes screening test stated that they heard from the media that the test was harmful. In the same study, the most common reasons for not having double and/or triple tests were "finding the tests unnecessary" (54.7%) and "missing the time of the test" (35.7%). Although the majority of the participants stated that they knew the screening tests, the rate of pregnant women who stated that they did not have the tests due to any missing or wrong information, avoiding the risk of harm to the baby, and moral values was found to be high.

According to the Turkey Nutrition and Health Survey 2010 data, the most widely used nutritional supplement during pregnancy is iron (43.5%), followed by multivitamin/mineral (27.1%), folic acid (15.1%), and vitamin D (5.7%) (24). In our study, the participants stated using folic acid (83.4%), vitamin D (74.5%), iron (67.2%), and multivitamin (58%).

In our study, 95.5% of the pregnant women stated that the tetanus vaccine was/would be administered. In the study of Kurnaz et al. (17), 73.6% of the participants had a tetanus vaccine, and the remaining participants stated that they did not get a tetanus vaccine because they were fully vaccinated. Tetanus vaccine was administered to 71.2% of the pregnant women in the study of Çatak et al. (25) and 77.6% of the pregnant women in the study of Ergün et al. (4).

The results of the use of supportive treatments in the presented study were found to be compatible with the literature. In other studies, it was found that the pregnant women received more adequate and qualified PNC as the age of the mothers, the level of education, the number of births, and the rate of living in the urban area increased (26,27). It is known that as the awareness of healthcare professionals and information and consultancy services provided to pregnant women increase, their compliance with screening tests and supportive treatments increases (28).

Conclusion

In this study, it was found that awareness about screening tests and supportive treatments increased with increased maternal age, education level, number of births, and the employment status of the mother. Although we have found high rates of learning screening tests and supportive treatments from healthcare professionals, it is a pity that all pregnant women are not contacted, and PNC is not at the desired level. We can say that this result refers primarily to the family physicians, who are the first point of contact with pregnant women, and to the healthcare professionals in the services and information of PNC. We think that younger mothers with lower education levels in rural areas should be prioritized for information about counseling during the PNC services.

The most important limitation of the study was that the study was conducted in a tertiary healthcare institution. Therefore, there is a possibility that some of the participants were pregnant women who were referred to due to more problematic pregnancy processes. However, we can say that the absence of a referral chain in our country is a situation that eliminates this bias. Nevertheless, extensive studies in primary care are needed to cover the general population. Although the results of the study cannot reflect the general public, we believe that it is a study that can contribute to the literature and contribute to the future studies with the awareness of pregnant women about routine screening tests and supportive treatments and the possible role of family physicians in this regard.

Ethics Committee Approval: The ethical permission of the study was taken before the study, with the number of 2018/1259, dated 16.03.2018, from the Ethics Committee of Necmettin Erbakan University Meram Faculty of Medicine, Pharmaceuticals and Non-medical Researches Ethics Committee.

Informed Consent: The participants were informed about the study, and their written and verbal consents were obtained according to the Helsinki Declaration principles.

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Acute Pancreatitis Associated with Rotavirus Infection and Review of The Literature

Rotavirüs Enfeksiyonuna Bağlı Akut Pankreatit Olguları ve Literatürün Gözden Geçirilmesi

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ABSTRACT

Agents causing acute gastroenteritis are not common causes of pancreatitis etiology in children. Pancreatitis associated with rotavirus infection is very rare. Cases with acute pancreatitis during rotavirus gastroenteritis are reported due to rare associations. In this article, the causes of acute pancreatitis and cases of acute pancreatitis due to rotavirus infection were investigated. Clinical findings were mild, and complications were not observed in both of our patients, including a two-year-old female and a three-year-old male, and other cases evaluated in the literature. The patients were diagnosed with amylase-lipase elevation, imaging findings suggesting pancreatitis, and symptoms including vomiting and abdominal pain. The amylase and lipase levels of our patients decreased to normal levels in accordance with the literature, and no complication was observed in any patient. In the case of the continuation of abdominal pain in rotavirus gastroenteritis, it is essential to keep in mind the diagnosis of acute pancreatitis in terms of treatment and follow-up.

Keywords: Children, gastroenteritis, pancreatitis, rotavirus

ÖZ

Çocuklarda pankreatit etiyolojisinde akut gastroenterit etkenleri sık görülen sebeplerden değildir. Rotavirüs enfeksiyonuna bağlı görülen pankreatit ise oldukça nadirdir. Rotavirüs gastroenteriti sırasında akut pankreatit gelişen olgular, rotavirüs enfeksiyonuna bağlı akut pankreatitin nadir olması nedeniyle sunulmuştur. Bu yazıda, akut pankreatit sebepleri ve rotavirüse bağlı gelişen akut pankreatit olguları incelenmiştir. İki yaş kız ve üç yaşındaki erkek iki olgumuzda ve literatürde değerlendirilen diğer olgularda klinik bulgular hafif seyretmiş, komplikasyon görülmemiştir. Olgulara amilaz-lipaz yüksekliği, pankreatiti düşündüren görüntüleme bulguları ve kusma, karın ağrısı semptomlarıyla tanı konulmuştur. Olgularımızın amilaz ve lipaz değerleri literatür ile uyumlu günlerde normal seviyeye gerilemiş, hiçbir hastada komplikasyon gelişmemiştir. Rotavirüs gastroenteritinde karın ağrısının devamı halinde akut pankreatit tanısının akılda tutulması tedavi ve takip açısından önemlidir.

Anahtar Kelimeler: Çocuklar, gastroenterit, pankreatit, rotavirüs

Introduction

Rotavirus is the single most common and severe disease-causing cause of gastroenteritis under the age of 2 in the world. All children who have reached the age of 5 in the world have been infected with rotavirus at least once. The main symptoms of acute rotavirus gastroenteritis are fever, vomiting, watery diarrhea, abdominal cramps, and dehydration. Neither vaccine nor natural infection provides permanent immunity. The first natural infection progresses with very severe symptoms (1). Rotavirus infection is rarely seen among the causes of acute pancreatitis (2).

The annual incidence of pancreatitis in children in the United States is 13.2/100.000 (3). The annual incidence of acute pancreatitis in childhood is reported to be 3.6-13.2/100.000 in different pediatric centers from Europe (4). In our country, there is not enough data on this issue (5). The incidence of pancreatitis in children has been increasing in recent years. The reason for this is that the frequency of diagnosis is increased depending on the awareness of the disease. Causes of acute pancreatitis in children differ from adults and often include drugs, infections, trauma, and anatomical disorders (6). In some cases, a cause cannot be determined (idiopathic pancreatitis) (7). Meeting at least two of the criteria determined for the diagnosis of acute pancreatitis is sufficient for diagnosis (8). Acute pancreatitis associated with rotavirus infection is extremely rare. In this article, it was aimed to present cases with acute pancreatitis due to rotavirus infection and to review the relevant

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literature. We received written consent from the fathers of both of our cases for the case report.

Case Reports

Case 1

A two-year-old girl presented to the pediatric emergency clinic with fever for three days, watery, bloodless, mucous diarrhea, and vomiting ten times a day. There were mild fluid loss and tenderness in the epigastric region in the physical examination of the patient, who did not have any features in her medical history and family history. The patient's bowel sounds were increased, and other system examinations were normal. The patient had no trauma history or chronic disease in progress. Her laboratory results were as follows: leukocyte white blood cell (WBC): 8.230/mm^3, hemoglobin (Hb): 11.3 g/dL, platelet (PLT): 304.000/mm^3, urea: 13.3 mg/dL, creatinine: 0.17 mg/dL, sodium: 128 mmol/L, potassium: 2.8 mmol/L, alanine aminotransferase (ALT): 10 U/L, aspartate aminotransferase (AST): 44 U/L, total bilirubin: 0.2 mg/dL, direct bilirubin: 0.03 mg/dL, and C-reactive protein (CRP): 3.8 mg/L (range: 0-5). The microscopic examination of stool was normal, the rotavirus antigen was positive in stool, and there was no pathological growth in stool culture. On the third day of hospital stay, the amylase value was 122 U/L (28-100), and lipase value was 280 U/L (5-31), which were evaluated due to continuation of vomiting, abdominal pain, and tenderness in the epigastric region. The pancreas was edematous in abdominal ultrasonography (USG). Acute pancreatitis was considered in the patient, and other tests for the etiology of acute pancreatitis were negative. Oral intake was stopped. Intravenous (IV) fluid and ranitidine treatment were started. In the follow-up, the amylase and lipase values decreased to normal on the eighth day of the treatment, and the patient did not develop any complications and was discharged upon the remission of her findings.

Case 2

A three-year-old male patient presented with vomiting and watery diarrhea for five days. There was no feature in his medical history and family history. On physical examination, the tongue was dry, the turgor was impaired, and there were signs of moderate fluid loss. Intestinal sounds were increased, and there was widespread tenderness in the epigastric region and lower abdominal quadrants. There was no abdominal defense and rebound. His laboratory results were as follows: WBC: 20.720/mm^3, Hb: 12 g/dL, PLT: 502.000/mm^3, urea: 38 mg/dL, creatinine: 0.23 mg/dL, sodium: 136 mmol/L, potassium: 4.1 mmol/L, ALT: 30 U/L, AST: 50 U/L, total bilirubin: 0.22 mg/dL, direct bilirubin: 0.06 mg/dL, and CRP: 4.4 mg/L (range: 0-5). Rare leukocyte and rotavirus antigen positivity were detected in the microscopic examination of stool, and there was no pathological growth in stool culture. The pancreas was found to be heterogeneous in abdominal USG performed due to extensive tenderness in the abdomen. The amylase level was 108 U/L (range: 28-100), and lipase was 288 U/L (range: 5-31). No other factor was found in the examinations for the cause of acute pancreatitis. Acute pancreatitis due to rotavirus infection was considered in the patient. The enteral feeding of the patient was stopped, and IV fluid treatment was started. His clinical findings improved, and amylase and lipase levels decreased gradually during follow-up, and he was discharged on the tenth day of diarrhea. It was found that the amylase and lipase levels observed on the 12th day of control were normal.

Discussion

Rotavirus is a cause of diarrhea with high morbidity and mortality all over the world. While diarrhea, vomiting, and fever are common in this viral enteritis, abdominal pain is a rare finding. Acute pancreatitis has broad findings from mild abdominal pain to severe metabolic disorder and shock (9). The diagnosis of acute pancreatitis is determined by providing at least two of the three criteria including a) abdominal pain that is more prominent in the epigastric region, that can be seen in the right and left upper quadrants, and that rarely spreads to backward, unlike in the adult, b) more than three-fold increase in serum amylase and/or lipase levels, and c) radiological imaging findings of pancreatitis (Table 1) (8,10). Causes of acute pancreatitis in children differ from adults. Causes in children are generally drugs, infections, trauma, and anatomical disorders (Table 2) (4). Apart from these reasons, the cause cannot be found in 23% of the cases (7). While the sensitivity of USG, which is one of the imaging methods that support pancreatitis, is 70%, the sensitivity of computed tomography is 90% (11). Magnetic resonance cholangiopancreatography, on the other hand, is a noninvasive method that has superiority even to endoscopic retrograde cholangiopancreatography since it can easily show anatomical disorders

Table 1. Acute pancreatitis diagnostic criteria (6)

Having at least two of the three criteria below;
1. Abdominal pain compatible with acute pancreatitis,
2. Serum amylase and/or lipase levels three times higher than than the upper limit of normal,
3. Detection of findings supporting pancreatitis in radiological examinations (ultrasonography, magnetic resonance imaging, or computed tomography).

Table 2. Causes of acute pancreatitis in children (4)

1. Congenital anomalies and obstruction around the bulb: Choledochal cysts, pancreaticobiliary junction anomalies, gallstones, cholecystitis, pancreatic divisum, tumors, congestion due to Ascaris parasite,
2. Infections: Mumps, measles, Coxsackie, Echovirus, influenza, Epstein-Barr viruses, and *Mycoplasma, Salmonella, Gram-negative bacteria*,
3. Drugs: L-asparaginase, steroid, valproic acid, azathioprine, mercaptopurine, mesalazine, cytarabine, salicylic acid, indomethacin, tetracycline, chlorothiazide, isoniazid, anticoagulant drugs, alcohol,
4. Trauma: Blunt trauma, child abuse, ERCP and post-surgery,
5. Systemic diseases: Reye's syndrome, Systemic Lupus Erythematosus, Polyarteritis Nodosa, Juvenile Rheumatoid Arthritis, Sepsis, Multiple Organ Failures, Organ Transplants, Hemolytic Uremic syndrome, Henoch Schoenlein purpura, Kawasaki disease, Inflammatory Bowel disease, Chronic Intestinal Pseudo-obstruction, Gastric Ulcers, Anorexia Nervosa, Drug Allergies, Cystic Fibrosis,
6. Metabolic causes: Hyperlipoproteinemia (I, IV, V), hypercalcemia, diabetes, α1 antitrypsin deficiency,
7. Nutritional disorders: Malnutrition, high-calorie infusion, vitamin A and D deficiency,
8. Other reasons: Familial, idiopathic.

ERCP: Endoscopic retrograde cholangiopancreatography

and stone possibilities (3). Although there is not an absolute value in determining the severity of pancreatitis, a more than seven-fold increase in serum lipase is one of the crucial indicators of severity and has a sensitivity of 85-90% (12). There may be recurrences in 15-35% of acute pancreatitis cases. Acute recurrent pancreatitis is mostly due to causes such as idiopathic, genetic mutations, and biliary anomalies (13). The risk of death from this disease in children ranges from 0-11% and is lower than in adults (14).

The diagnosis of acute pancreatitis is difficult due to its rarity and heterogeneous findings. Severe pancreatitis develops less frequently in children than in adults. Although hyperamylasemia has been reported with some infectious agents after acute gastroenteritis, acute pancreatitis is rare (15). In their study, Tositti et al. (16) evaluated 507 adult gastroenteritis patients and showed that 10.2% of cases had hyperamylasemia, and only one case developed acute pancreatitis. It has been reported that hyperamylasemia is seen mostly in gastroenteritis due to *Salmonella* species, followed by rotavirus, *Clostridium difficile*, and *Campylobacter* species. However, acute pancreatitis was not detected in any adult case with rotavirus gastroenteritis (n=29) in this series (16). Retrospective studies state that adults with gastroenteritis have a temporary hyperamylasemia but that this is not pancreatitis (2).

In the article in which 87 children with acute pancreatitis were evaluated in our country, the most common complaints were sudden onset abdominal pain in 79% patients, vomiting in 12% patients, and restlessness in 6% patients. No complaints were detected in one patient, and the diagnosis was reported to be made with elevated amylase and lipase values and radiological findings. While a cause could not be determined in 25% of these patients, the most common cause was trauma. Infection was not detected among the causes. Recurrence occurred in 15% of patients, and only one patient progressed to chronic pancreatitis. Four patients died due to this disease (17).

In experimental studies in newborn mice, rotavirus has been shown to replicate in the liver, spleen, pancreas, heart, thymus, lungs, and kidneys. Histopathological changes are caused by rotavirus and include inflammation of the portal system and biliary tract (18). Nuclear Factor B (NF-B) is activated with the effect of interleukin 8 (IL-8), which is chemotactic in rotavirus infection (19). The main destructive effect in rotavirus infection is due to the inflammation caused by neutrophils, macrophages, lymphocytes, and monocytes, rather than the cytopathic virus effect, which are attracted to the environment with the effect of IL-8 (19). IL-8 is used in the clinic to distinguish rotavirus gastroenteritis from other gastroenteritis (20). IL-8 may also be the factor that initiates rotavirus pancreatitis. In the literature, it has been mentioned that measuring and monitoring serum IL-8 levels is the correct method for following prolongation and chronicization of pancreatitis (21). Recovery of rotavirus gastroenteritis is related to virus-specific interferon-gamma formation (15). Interferon-gamma plays a vital role in the control of viral infections. Interferon-gamma also protects from pancreatitis. It does this by reducing NF-B and IL-8 (22).

In order to be able to diagnose acute pancreatitis due to rotavirus infection, the causes that may have a role in etiology and which we mentioned in Table 2 should be investigated (6). In our cases, other causes of acute pancreatitis were excluded. Acute pancreatitis due to rotavirus infection has a good prognosis, and no complication develops in this pancreatitis. In cases in the literature, amylase and lipase levels returned to normal within 5-12 days (23). In accordance with the literature, our cases also had an excellent course, and the amylase and lipase levels returned to normal on the 8th and 12th days.

The first rotavirus pancreatitis was published in 2009 in India (24). Considering that rotavirus infection is widespread in the world, it can be thought that there are many cases with pancreatitis that are overlooked in young infants. In a school-age patient reported from our country, it was recently reported that rotavirus-induced pancreatitis was observed after a prolonged abdominal pain complaint (8). In the case reports of 2.5- and 3-year-old patients with diagnosed rotavirus gastroenteritis in our country, lipase levels were found twice and five times higher than the amylase levels and abdominal ultrasonographic examinations were found to be normal. On the 12th and 13th days, amylase and lipase levels returned to normal, and patients recovered without complications (25). In a publication from our country in 2018, seven cases reported in the literature were mentioned. It was observed that all of the cases had diarrhea, vomiting, abdominal pain, and the majority of the cases were between two and five years old. Our cases are also compatible with the literature regarding age groups. In half of the cases, a slightly enlarged pancreatic appearance was detected in the radiological evaluations. IV hydration or IV hydration + ranitidine treatment were given as treatment (23). We administered IV hydration plus ranitidine treatment to our case with more pronounced epigastric pain, and IV hydration treatment to our other case. After the case reports in 2018, we have not encountered any recently reported cases in the literature. The reason for not being encountered maybe since abdominal pain is considered as the prolonged symptom of gastroenteritis and that amylase and lipase levels are not examined, and mild pancreatitis improves without being diagnosed.

In conclusion, rotavirus-induced pancreatitis should be considered among the cause of abdominal pain in rotavirus gastroenteritis, which is common in children, and this should be kept in mind in terms of treatment approach and follow-up.

Informed Consent: We received written consent from the fathers of both of our cases for the case report.

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