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Importance of Biostatistics in Medical Researches

Tıbbi Araştırmalarda Biyoistatistiğin Önemi

Mehmet Ali SUNGUR

Today's research and researchers tend to reach conclusions with the help of data, ever-increasing in size. Statistical methods are essential to researchers, as they transform raw data into information and produce meaningful results. More information can be get from data if it is known how variable they are; in a way, a picture of the data can draw with the pieces of information. The most valuable information about complex causal relationships can be obtained with the help of various statistical methods. For these reasons, as in all fields of researches, physicians making researches in medical sciences need to benefit from biostatistics more and more frequently and intensively. The value of research is also related to its biostatistical value, besides its subject, being up-to-date, important, and useful results, etc. Researches should be planned and carried out following scientific principles. Compliance of a study with scientific principles is also closely related to the correct and conscious use of biostatistics methods and principles.

In every research conducted by obtaining and analyzing data, the results section is substantially based on the findings obtained with statistical methods. Therefore, the reliability and validity of the findings depend on the accuracy of the statistical methods and approaches used. The validity of the results of a research conducted without complying with the correct and appropriate biostatistical methods will be doubtful and the reliability will be questionable. In addition to data analysis, biostatistics should also be used in the main points of methodology, such as designing the research in line with the purpose, determining the sample size, and considering the measure of errors, etc. The role of biostatistics in medical research starts at the planning stage. Valid statistical inference, and therefore decision-making, depends on robust planning. Therefore, biostatistics is essential not only for determining and using the methods to analyze data but also for using and benefitting from correct and conscious statistical methods and practice for the planning stage and also methodology. If all stages in research are not well established and planned, failure may occur at any stage of the research or the whole research. In such a case, the cost, time, and effort spent can be wasted. Therefore, a large part of research time should be devoted to the planning stage. Adequate use of statistical methods and statistical analysis of data is only possible if the design of the research is statistically sound. If the planning stage is not satisfactorily resolved, inference with the data and results obtained will never be adequate. As a result, biostatistical methods should be used appropriately at every stage of research from planning to publication.

In relatively simple situations where data are analyzed at the basic level, it may be sufficient to calculate simple values such as means, standard deviations, standard errors, confidence intervals or to apply standard parametric or non-parametric tests. Despite being used widely, even these basic concepts and tests are sometimes misunderstood, even not understood, or misinterpreted by researchers with limited statistical knowledge. Also, these basic calculations and analyses are just the tip of the iceberg and are the preliminary stage of the data analysis. To extract the maximum information from the data, it is often necessary to use more complex statistical methods. More complex research models require advanced statistical methods, which require the establishment and testing of advanced statistical models to make inferences from the data obtained. Such advanced methods should be applied in such a way that it becomes clear what the result means, considering both the place and purpose of their use. One of the reasons for misuse or misunderstanding is that the no or limited knowledge about the assumptions required by statistical methods, even at a basic level. When the assumptions required by the tests are not met, the results may be partially or completely wrong, leading to inaccurate conclusions and interpretations. Biostatisticians understand these issues and are aware of how and when seriously misleading may occur when the assumptions are not fulfilled. Another reason is over-analysis. The large number of results and p-values that occur due to reasons

such as performing some analyzes even though it is not necessary or appropriate, or analyzing the data separately, can be even more confusing for researchers.

To be used and benefit from biostatistical methods and principles smoothly and effectively in the reporting and discussion phase, close cooperation with a biostatistics expert is needed, whether the researchers are professionals in the relevant field or physicians with sound statistical knowledge. Biostatisticians can give useful guidance on planning, analyzing, reporting, etc. When this cooperation is best provided, it will make a valuable contribution to the validity and reliability of the research results.

To sum up, as in all field of researches, physicians making researches in medical sciences needs to know and interpret statistical results, at least at a statistical literacy level. Knowledge at a basic level about biostatistics and statistical methods is invaluable for the assessment of findings, clinical decision-making, and critical evaluation of the implementation of findings into medical and clinical practice. Providing appropriate and adequate training in the understanding and application of statistical methods and their results in medicine is crucial.

To support this, manuscripts submitted to Duzce Medical Journal are also reviewed by a biostatistics expert during the peer-review process, and comments and recommendations are given in more thoroughness and detail to support and assist the authors in improving and revising their manuscript and ultimately lead to a publication of the highest quality as much as possible.

In addition, starting from this issue, we start to publish informative articles in the field of biostatistics. In this issue, we share with our readers an invited review titled "Development of Biostatistics: From Past to Future" by Karahan and Karaağaoğlu, experts in the field. This review only scratches the history and surface of biostatistics in medical researches. Nonetheless, hopefully, this review makes a stimulus for physicians to further develop and deepen their ability in the appropriate use, understanding, and interpreting correctly of statistical methods.

On behalf of Duzce Medical Journal, we would like to thank the authors for this review and hope it will be useful to our readers.

Mehmet Ali SUNGUR
Editor in Chief, Duzce Medical Journal
Biostatistics, Duzce University, Duzce/Turkey
orcid.org/0000-0001-5380-0819

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Development of Biostatistics: From Past to Future

Biyoistatistiğin Gelişimi: Geçmişten Geleceğe

Sevilay KARAHAN
0000-0002-8692-7266
Ahmet Ergun KARAAĞAOĞLU
0000-0002-7024-7231

Department of Biostatistics, Hacettepe University School of Medicine, Ankara, Turkey

Corresponding Author
Sorumlu Yazar
Ahmet Ergun KARAAĞAOĞLU
ekaraaga@gmail.com

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ABSTRACT

Biostatistics which is the application of statistics in the field of health and biology; provides powerful tools for creating questions, designing studies, developing measurements, and analyzing data and has an important place in determining the efficacy and safety of products such as drugs and vaccines. The impact of statistical sciences on medical and biological sciences has increased rapidly during the last few decades. Clinicians need to understand statistics well enough to follow up and evaluate empirical studies that provide an evidence base for clinical practice. Recent advances in biomedical research have created both new challenges and opportunities for statisticians and data scientists. Big data analytics, precision medicine, artificial intelligence, causal inference, and other new research resources inspire data scientists to develop modern statistical methods and innovative inference procedures. Therefore new philosophies such as causal models and prediction, new models such as graphical chain models and random effects models, faster computers and new clever algorithms for integration and maximization are needed. Without adequate investment in biostatistics, all medical research is at a significant risk of "drowning in data, but starving for knowledge".
Keywords: Biostatistics; data science; big data.

ÖZ

İstatistiğin sağlık ve biyoloji alanındaki uygulaması olan biyoistatistik; soru oluşturma, çalışma tasarlama, ölçüm geliştirme ve verilerin analizi için güçlü araçlar sağlar ve ilaç, aşı gibi ürünlerin etkinlik ve güvenliğinin belirlenmesinde önemli bir yere sahiptir. İstatistik biliminin tıp ve biyolojik bilimler üzerindeki etkisi son yıllarda hızla artmıştır. Klinisyenlerin, klinik uygulama için kanıtlar sağlayan deneysel çalışmaları izlemek ve değerlendirmek için istatistiği yeterince iyi anlamaları gerekir. Biyomedikal araştırmalardaki son gelişmeler, istatistikçiler ve veri bilimcileri için hem yeni zorluklar hem de fırsatlar yaratmıştır. Büyük veri analitiği, hassas (kişiselleştirilmiş) tıp, yapay zeka, nedensel çıkarım ve diğer yeni araştırma kaynakları; veri bilimcilerine modern istatistiksel teknikler ve yenilikçi çıkarım yöntemleri geliştirme konusunda ilham vermektedir. Bu nedenle; nedensel modeller ve tahmin gibi yeni felsefelere, grafik zincir ve rastgele etki gibi yeni modellere, daha hızlı bilgisayarlara, entegrasyon ve maksimizasyon için yeni akıllı algoritmalara ihtiyaç vardır. Biyoistatistiğe yeterli yatırım yapılmazsa, tüm sağlık araştırmaları önemli bir "veride boğulma, ancak bilgi açlığından ölme" riski altındadır.
Anahtar kelimeler: Biyoistatistik; veri bilimi; büyük veri.

INTRODUCTION

How to translate a scientific question into a statistical one, and interpreting the result of a statistical analysis, are more important than knowing how to carry out the computations involved in the analysis. Scientific theory provides a model of how nature should behave; a scientific experiment generates data that show how nature actually behaves, and statistical inference is the bridge between model and data (1).

Statistics can be defined as the science of extracting information from data in the presence of variability and uncertainty and is a key tool that relates the diversity of each patient's observations to more abstract concepts such as clinical features, natural histories, clinical response and risks (2, 3). Studies with appropriate biostatistical support, from design to analysis and reporting, are the best experimental studies, therefore it plays a critical role in health research (3). Biostatistics which is the application of statistics in the field of health and biology; provides powerful tools for creating questions, designing studies, developing measurements, and analyzing data and has an important place in determining the efficacy and safety of products such as drugs and vaccines (4). The names such as biostatistics, biometrics, biometry and even bioinformatics, direct the same general enterprise of the use and development of statistical theory and methods to address design, analysis and interpretation of information in the biological sciences. Whatever it is called, it has always been and continues to be of great importance in the conduct of scientific investigations in agriculture, life sciences in general, ecology, forestry, medicine and public health. By combining statistical reasoning with knowledge of the real scientific problems, statisticians can and have made high-profile contributions both to the science and to the policy. With the explosion of data collection in areas like genomics, medical imaging, environmental sciences, with the increasing reliance on sophisticated mathematical modeling to explain biological phenomena; and with the increasing public focus on displaying "statistics" at every turn and disseminating results of studies, gained statistical sciences increasing importance (5). A biostatistician's unique contribution to a research team is the ability to measure uncertainty and generate robust inferences from data. Due to the increasing complexity and amount of health-related data, the need for biostatistics expertise in research teams is expanding and evolving (6).

One often faces a dilemma when asked to define *biostatistics*. As indicated by Khurshid et al. (7) the problem begins with the word itself. Its roots are Greek where the component *bios* involves biology; the study of living things and the component *statistics* involves the amassing, tracking, analysis, and application of data. A large variety of terms in scientific literature are interchangeably used: biostatistics, biometry, biometrics, biological statistics, medical statistics, clinical statistics, biostatistical science, sometimes even biomedical statistics, medical biostatistics, environmental statistics, pharmaceutical statistics, biopharmaceutical statistics, and public health statistics. The terminology is inconsistent and is confusing at best. Looking at these terms without an understanding, it seems that they all deal with different topics. Despite these differences, the terms are used to mean the same thing.

Chiang (8) in his discussion on an article by M. Zelen, who indicated that it was difficult to classify biostatistics as a discipline and proposed the term *biostatistical science*, stated that he did not share his (Zelen's) difficulty in classifying biostatistics as a discipline. He defined biostatistics as a discipline that is concerned with the development and application of statistical theory and methods for the study of phenomena arising in the life sciences and whether biostatistics is or is not a discipline depends on the amount and quality of knowledge that has been developed and accumulated in the field. Although biostatistics could not be considered as a discipline till 1940's, by the drastic change experienced after then, researches shifted from descriptive statistics to the development of theoretical basis for the field. As a result, biostatistics today contains a respectable body of knowledge both in quality and quantity and is built on a solid theoretical foundation. As a conclusion Chiang (8) stated that more people in mathematical statistics will focus on biostatistical problems, and biostatistics will increase in its contribution and importance in the advancement of science (8).

Biostatistics is the application and development of statistical techniques for biological sciences and biostatistics is defined as "statistical method(s) in medicine and the health sciences" (9).

The impact of statistical sciences on medical and biological sciences has increased rapidly during the last few decades. Physicians practice on the basis of clinical knowledge, which is framed after a series of tests, treatments and statistical analyses. A physician may not have a sound knowledge of statistical principles or techniques, but the information he uses in the clinical decision-making process is undoubtedly always based on statistical evidence. However, conclusions drawn from the statistical evidence may be inaccurate or misleading and therefore, without a sound understanding of statistics, a physician may not be able to reach the most appropriate decision.

Statistics is a discipline that has changed science, medicine, and public policy. Biostatistical principles are necessary in all branches of biology and medicine, and have become a mandatory part of medical research. The Human Genome Project, for example, while relying on advanced biological techniques, also depends heavily on statistical techniques for extracting the right data out of a large pool of gene sequences. The evolution of (bio)statistics, is parallel to development in other scientific fields, particularly medicine (7).

The statistical analysis should be part of the scientific method used to test the hypothesis and should be planned before the study is started. Thus, the study of probabilities and the way they have to be interpreted in medical practice tell you something about scientific method. And scientific method is the only thing keeping you all from being quacks (10).

HISTORICAL DEVELOPMENT OF STATISTICS AND BIOSTATISTICS

In earlier times when scientists started to understand and explore the nature and their environment the very first thing they used to do is to measure and record some

important features. Therefore the history of statistics dates back to ancient times. The first appearance of statistics in the world began with the census of soldiers and keeping records of events such as marriage, birth and baptism. As it can be understood from here, the first statistical studies were mostly on descriptive statistics. The origins of probability and statistics are usually found 1650 - 1700 period by dealing with the mathematical explanation of games of chance and the study of mortality data. During the 1700's, in the Age of Enlightenment, things gradually began to change. The scientific method, based on empirical evidence about diseases and the impacts of different interventions, began to be applied. However, the study of medical treatment remained almost entirely qualitative. At the end of the eighteenth century, Gilbert Blane described the research process as hinging almost exclusively on clinical reasoning, with no hint of quantitative analysis (11). This was also the age of Scientific Revolution and many well-known scientists like Galileo and Newton, although not influencing its development, were the early users of probability and statistics (1). Not too long after, however, statistical ideas would start gaining importance. By the early nineteenth century, the concept of probability, essentially in its current form, was well known. Pierre-Simon de Laplace was the leading scientific theorist of the era, and a strong believer in the potential of statistical analysis in various fields. In medical research, he advocated comparing rates of success between alternative therapeutic interventions. With the use of quantitative data, scientists such as John Graunt, William Patty, Edmund Halley, Jacob Bernoulli started to work in the field of mathematical statistics. Graunt modeled population growth, and Halley applied statistical models to the insurance industry. Deparsier calculated the average life expectancy by creating life tables. Bernoulli's law of large numbers is an important milestone. Theorems developed by important scientists such as Laplace, Legendre, Gauset and Poisson are still used today (12).

The person who laid the foundations of biometrics is Quetelet, who is considered the father of modern statistics. Quetelet was the first to combine the methods of anthropology and social statistics with results from probability theory and mathematical statistics. He produced works such as "On the development of man and his abilities or the experience of social physics", "On the social system and the laws that govern it" and "Anthropology". Russian statisticians such as Chebyshev, Markov and Kolmogorov also produced very important works on statistics (12).

Two of those who played an important role in the application of statistics to biology are the British statisticians Francis Galton and Karl Pearson. Darwin's cousin, Galton, published work on anthropology and genetics. Similarly, Pearson successfully studied the heredity and variability problems of organisms. He is the developer of methods such as correlation coefficient and chi-square statistics, which are still widely used today (13). He is also the founder of Biometrika, one of the most important journals in the field, which has been published since 1901. Gosset, a student of Pearson and publishing his work under the pseudonym "student"; worked on small sample theory (12).

Another person who made great contributions to biometrics is Ronald Yelmer Fisher. As both a biologist-experimenter and mathematician-statistician, Fisher brought not only new methods but also new ideas to biometrics. He revolutionized the theory and practice of statistics, especially as it applies to agricultural experimentation. Fisher is revered not only as a great statistician but also as one of the great geneticists of the twentieth century. Two of Fisher's fundamental contributions to experimental design were the ideas of randomization and blocking (1).

Fisher's revolutionary innovations in experimental design and analysis proved enormously successful in several fields, especially in agricultural research and industrial engineering. Fisher's methods of significance testing, building upon W.S. Gossett's initial breakthrough of the t-test in 1908, finally solved the problem of how to analyze experiments with modest sample sizes. His designs based on random assignment of treatments to experimental units provided not only a firm basis for calculating p-values for significance tests, but also a means of eliminating bias resulting from uncontrolled "causes" influencing the outcome. One very important but controversial direction of this development was initiated by Jerzy Neyman and E.S. Pearson. These pioneers regarded statistics primarily as a way to guide decisions, an approach that Fisher found inappropriate for scientific research. However, the Neyman-Pearson decision-theoretic methods, including such concepts as confidence intervals, Type I and Type II error rates and statistical power, became widely accepted. These tools proved particularly useful for research related to industrial product development, production and testing. The first randomized clinical trial, directed by Hill in 1946 under the auspices of the British Medical Council, demonstrated clearly the benefits conferred by streptomycin for the treatment of tuberculosis. Hill's pioneering efforts began shifting medical opinion toward a greater appreciation of the value to society of definitive findings that could be obtained via randomized clinical trials (11).

When it comes to today, Marvin Zelen noted the emergence of a field called the "science of biostatistics", referring to the application of statistics, probability, computation, and mathematics to a subject area. Also, Zelen said, "The future of Biostatistics Science will be closely related to computation" (13).

Till 1980's, "pure science" was considered "better science." Important break-throughs in the beginning of 1990's, primarily in the field of molecular biology, have made it increasingly clear that the future is bright for interdisciplinary and multidisciplinary efforts and that academic institutions should prepare themselves for this trend (5).

FUTURE OF BIOSTATISTICS

Recent advances in biomedical research have created both new challenges and opportunities for statisticians and data scientists. Big data analytics, precision medicine, artificial intelligence, causal inference, and other new research resources inspire data scientists to develop modern statistical methods and innovative inference procedures (14). Nowadays scientists are confronted with larger data sets and more complex data. Therefore new philosophies

such as causal models and prediction, new models such as graphical chain models and random effects models, faster computers and new clever algorithms for integration and maximization are needed (15).

The recently popularized concept of data science is a combination of mathematics-statistics, field knowledge and computer knowledge (Figure 1). Data is the most precious mine of the information age. Data scientists are needed to process this mine and turn data into information. Data science requires fusion of statistical thinking and information technology (16).

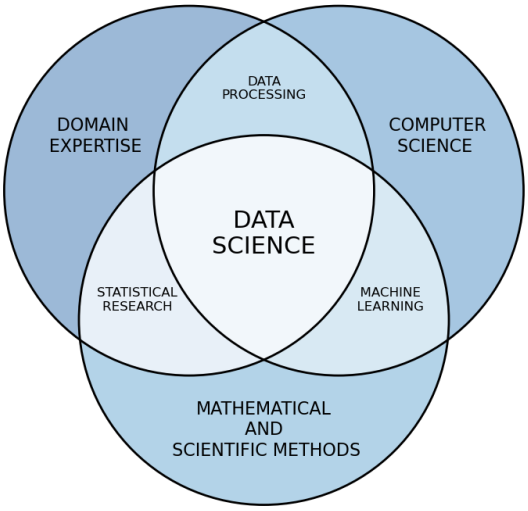

Figure 1. Components of data science (16)

Millions of dollars are spent on medical and public health research around the world each year. The correct use of this expenditure depends on the correct analysis and interpretation of the data. Investments in this research are at risk if insufficient attention is paid to biostatistics. As the data collected grows, the risk of not understanding the structure of this data and not using the right statistical approaches in the analysis will also increase (3).

Clinicians need to understand statistics well enough to follow up and evaluate empirical studies that provide an evidence base for clinical practice. They should realize the statistical aspects of the clinical literature, evaluate the strengths and weaknesses of the presented analyzes, and strengthen their active participation in research. A perfectly chosen and applied analysis would be misleading best if it is done from data collected using incorrect data or data collected using an incorrect measurement technique, or at the wrong time. To quote the aphorism often uttered in introductory statistics classes, "garbage in, garbage out" (2). An in-depth education is needed to establish the connection between health problems and statistical methods. Biostatistics is a basic discipline required by the clinician, master's or doctoral student / graduate who conducts research in a very wide area. This situation sometimes causes biostatistics to be seen as a simple technical tool rather than a basic discipline (3). Although many experienced researchers value collaboration with biostatisticians, biostatistics is sometimes considered an ancillary service rather than an academic discipline. To maximize the contribution of biostatisticians to research, biostatistics must be integrated with the purpose of research (6). Shallow and misuse of statistics can easily lead to unscientific applications. Biostatistics requires not only knowledge of statistical methods but also the use of technical skills, including computation (3). On the other hand, sometimes a large gap occurs between biostatistician's advanced statistical model and clinicians' perspective. Both sides should strive to build a bridge that will bridge this gap (15).

In the twenty-first century, there has been a significant increase in data collection at low costs. Large and frequent data are collected in the fields of molecular biology, health science, engineering, geology, climatology, economics, finance and humanities. For example, in biomedical research, MRI, fMRI, microarray, and proteomics data are often collected for each subject, involving hundreds of subjects; in molecular biology, large sequencing data is rapidly becoming available; thousands of high-resolution images are collected in natural resource exploration and agriculture; millions of transactions are recorded every day in business and finance. Huge volumes of data are recorded on social media platforms. The frontiers of science, engineering, and the humanities differ in problems in studies, but still share a common theme: big or complex data is being collected and new information needs to be discovered. Big data and new scientific research have a strong influence on statistical thinking, methodological development and theoretical work. It also challenges traditional statistical theory, methods, and computation. Many new insights and phenomena need to be discovered and new statistical tools need to be developed (17, 18).

The application of data-intensive biomedical assays and technologies, such as DNA sequencing, proteomics, imaging protocols, and wireless health monitoring devices have revealed a great deal of inter-individual variation with respect to mechanisms and factors that influence disease. This has led to the belief that interventions must be tailored (i.e., 'personalized') to the features each patient possesses. In the context of personalized medicine (precision medicine), if is not known a priori what intervention might 'match' a patient's profile, then it becomes an empirical question as to which intervention might be most appropriate for that patient (19).

CONCLUSION

With the onset of the big data era, more and more people are trying to draw conclusions from this data. However, the spread of software developed by those who do not have sufficient knowledge about statistical methods poses an increasing risk. Big data requires both an advanced understanding of basic statistical concepts and methods and a mastery of computational tools such as dimension reduction and machine learning. More data does not always mean better data, and more analysis does not necessarily mean better science. The quality and reproducibility of research findings depend on the design of the data collection process and the sources of limitations and biases of the research. Without adequate investment in biostatistics, all medical research is at a significant risk of "drowning in data, but starving for knowledge" (3).

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How Important Is Liver Damage During COVID-19?

COVID-19 Sırasında Gelişen Karaciğer Hasarı Ne Kadar Önemli?

Berat EBİK[1]
0000-0002-0012-2505
Nazım EKİN[1]
0000-0001-5302-8953
Ferhat BACAKSIZ[1]
0000-0002-9670-3290
Jihat KILIÇ[2]
0000-0003-3722-350X

[1]Department of Gastroenterology, Gazi Yaşargil Education and Research Hospital, Diyarbakır, Turkey

[2]Department of Internal Medicine, Gazi Yaşargil Education and Research Hospital, Diyarbakır, Turkey

Corresponding Author
Sorumlu Yazar
Berat EBİK
beratebik@gmail.com

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ABSTRACT

Aim: The present study aimed to examine the prevalence of liver dysfunction in patients hospitalized due to coronavirus disease 2019 (COVID-19) and to investigate the role of liver dysfunction in predicting intensive care unit admission and mortality.
Material and Methods: A total of 2168 patients who had no previously known chronic liver disease and were found to be COVID-19 positive on polymerase chain reaction test were divided into mild and severe COVID-19 groups. The effect of the development of liver damage on the course and prognosis of COVID-19 was investigated.
Results: Elevated liver enzymes developed in 26.3% (n=461) of the patients with mild COVID-19 and in 45.3% (n=189) of the patients with severe COVID-19. The highest ALT and AST elevation was detected in patients using more than one drug with antibiotics ($p<0.001$). Severe hepatitis developed in 1.4% (n=25) of the patients with mild COVID-19 and 6.5% (n=27) of the patients with severe COVID-19 ($p<0.001$). There was a weak negative correlation between ALT and albumin ($p=0.017$, $r=-0.497$), while a weak positive correlation with bilirubin ($p=0.024$, $r=0.352$), a moderate positive correlation with ferritin ($p=0.016$, $r=0.504$), and a weak positive correlation with INR ($p=0.022$, $r=0.383$) were found in patients with severe COVID-19.
Conclusion: The results showed that 30% of COVID-19 patients had impaired liver function of varying severity and that liver damage was more common in patients with severe COVID-19. It was also determined that liver damage occurring during COVID-19 was an indicator of intensive care requirement and the mortality risk.
Keywords: COVID-19; liver; prognosis.

ÖZ

Amaç: Bu çalışma, koronavirüs hastalığı 2019 (coronavirus disease 2019, COVID-19) nedeniyle hastaneye yatırılan hastalarda karaciğer fonksiyon bozukluğu prevalansını incelemeyi ve yoğun bakım ünitesine yatış ve mortaliteyi öngörmede karaciğer fonksiyon bozukluğunun rolünü araştırmayı amaçlamaktadır.
Gereç ve Yöntemler: Önceden bilinen bir kronik karaciğer hastalığı olmayan ve polimeraz zincir reaksiyonu testi ile doğrulanmış COVID-19 pozitif olan toplam 2168 hasta, hafif ve şiddetli COVID-19 olarak gruplara ayrıldı. Karaciğer hasarı gelişiminin COVID-19'un seyrine ve prognozuna etkisi araştırıldı.
Bulgular: Hafif COVID-19'lu hastaların %26,3'ünde (n=461) ve şiddetli COVID-19'lu hastaların %45,3'ünde (n=189) karaciğer enzimlerinde yükselme meydana geldi. En yüksek ALT ve AST artışları, antibiyotikler ile birlikte birden fazla ilaç kullanan hastalarda tespit edildi ($p<0,001$). Hafif COVID-19'lu hastaların %1,4'ünde (n=25) ve şiddetli COVID-19'lu hastaların %6,5'inde (n=27) şiddetli hepatit gelişti ($p<0,001$). Şiddetli COVID-19'lu hastalarda, ALT ile albumin arasında zayıf düzeyde negatif bir korelasyon ($p=0,017$; $r=-0,497$) saptanırken, bilirubin ile zayıf düzeyde pozitif bir korelasyon ($p=0,024$; $r=0,352$), ferritin ile orta düzeyde pozitif bir korelasyon ($p=0,016$; $r=0,504$) ve INR ile zayıf düzeyde pozitif bir korelasyon ($p=0,022$; $r=0,383$) olduğu saptandı.
Sonuç: Sonuçlar, COVID-19 hastalarının %30'unda farklı şiddette karaciğer fonksiyon testlerinde bozulma meydana geldiğini ve şiddetli COVID-19 hastalarında karaciğer hasarının daha yaygın olduğunu göstermektedir. Ayrıca COVID-19 sırasında meydana gelen karaciğer hasarının yoğun bakım gereksinimi ve ölüm riskini artıran bir etken olduğu da belirlenmiştir.
Anahtar kelimeler: COVID-19; karaciğer; prognoz.

INTRODUCTION

Coronaviruses are a virus family that primarily affect the human respiratory system, causing upper respiratory tract disease and pneumonia (1). To date, 7 types of coronaviruses that infect humans have been identified, including severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), which is known to cause coronavirus disease 2019 (COVID-19) (2). COVID-19 was first detected in 2019 in China and rapidly spread to the entire world and then was declared a global pandemic. At the time of writing, approximately 160 million COVID-19 cases had been diagnosed and more than 3.2 million deaths had been recorded (3). COVID-19 usually leads to death through respiratory failure, septic shock, and multi-organ failure, and over time more information and experience has been gained regarding the effect of COVID-19 on other organs. Liver damage in COVID-19 does not occur through a single mechanism. A leading cause of this damage is immunity-mediated damage which is formed as a result of a severe inflammatory response associated with COVID-19. All inflammation markers primarily including C-reactive protein (CRP), ferritin, lactate dehydrogenase (LDH), D-dimer, and interleukin (IL)-6 have been shown to be elevated in such patients (4). Another mechanism of damage is the replication of the virus within hepatocytes and cholangiocytes on angiotensin-converting enzyme 2 (ACE-2) receptors found in the liver (5). Additionally, a frequently seen cause is drug-related liver disease and the reactivation of existing liver disease associated with the drugs used (6). In the liver biopsies of patients who died due to COVID-19, drug-related liver damage and abnormalities associated with shock and sepsis have been observed more often compared to other patients (7). In a previous study, presence of cellular apoptosis in different organs (lungs, liver, kidneys) was shown to be stimulated through pathways dependent on the caspase of protein 7a, which is specific to the coronavirus family. The study also confirmed that the hepatocytes that were directly attacked by the SARS-CoV viruses caused liver damage (8).

The present study aimed to examine the prevalence of liver dysfunction in patients hospitalized due to COVID-19 and to investigate the role of liver dysfunction in predicting intensive care unit (ICU) admission and mortality.

MATERIAL AND METHODS

The study included 2168 patients that were hospitalized with a diagnosis of COVID-19 between April and December 2020. All the patients were found to be positive for COVID-19 on polymerase chain reaction (PCR) assay. An approval was obtained from the local ethics committee (16.10.2020, 611) and an informed consent was obtained from each patient.

Patients that were previously diagnosed with chronic liver disease, patients that developed liver enzyme elevation and liver dysfunction during hospitalization, liver transplantation patients, patients with immunosuppressive therapy, patients with malignancy, and patients that had COVID-19 symptoms but had a negative PCR test were excluded from the study.

Patients that only had symptoms of fever, cough, headache, widespread joint pain, muscle pain, loss of smell and taste, and mild shortness of breath (respiratory count <20/min and oxygen saturation >92%) were accepted as having mild COVID-19. As recommended in the guidelines, patients with severe tachypnea, oxygen saturation <90%, and low PaO_2 according to blood gas analysis and severe respiratory and circulation problems requiring intensive care treatment were accepted as having severe COVID-19 (9). Based on this distinction, we examined the difference between patients with mild and severe COVID-19 in terms of liver dysfunction and the frequency of liver damage. In addition, we compared the liver functions of surviving and non-surviving patients as well.

Alanine aminotransferase (ALT) >35 U/L, aspartate aminotransferase (AST) >40 U/L, gamma-glutamyltransferase (GGT) >45 U/L, alkaline phosphatase (ALP) >125 U/L, total bilirubin (TBIL) >1.2 mg/dL, direct bilirubin (DBIL) >1 mg/dL, lactate dehydrogenase >225 U/L, Albumin <4 mg/dL, international normalized ratio (INR) >1.2, ferritin >150 ng/ml, and D-dimer >243 ng/ml were accepted as abnormal values (10).

Statistical Analysis

Data were analyzed using SPSS v.26.0 for Windows (Armonk, NY: IBM Corp.). Normal distribution of data was assessed using Kolmogorov Smirnov test, skewness, and kurtosis. Categorical variables were expressed as frequencies (n) and percentages (%). Continuous variables with normal distribution were expressed as mean±standard deviation (SD), and median (minimum-maximum) used for non-normal distribution. Normally distributed continuous variables were compared using Student's t test and parameters with non-normal distribution were compared using Mann-Whitney U test dichotomously. The difference in the distribution of categorical data between the groups was evaluated with the Pearson chi-square or Fisher's exact test, considering the number of patients in the categories. Post hoc Bonferroni correction was made in case of significance. Pearson correlation analysis was used according to their distribution to determine the correlation between continuous parameters. A two-tailed p value of <0.05 was considered significant in all tests.

RESULTS

The 2168 patients comprised of 1125 (51.9%) male and 1043 (48.1%) female. Mild COVID-19 was diagnosed in 1751 (80.8%) patients and severe COVID-19 was diagnosed in 417 (19.2%) patients. Mortality developed in 252 (11.6%) patients. Patients with severe COVID-19 had a significantly higher mean age (67.6 vs 43.6 years) and a significantly longer hospitalization duration (14.3 vs 7.1 days) compared to the patients with mild COVID-19 ($p<0.001$). No comorbidity was determined in 70.3% (n=1525) of the patients and at least one comorbidity was present in the remaining 29.7% (n=643) of the patients. Most commonly seen two comorbidities were essential hypertension and diabetes mellitus (Table 1).

Elevated liver enzymes developed in 15.6% (n=32) of those using only hydroxychloroquine (HCQ); this rate was 13.8% (n=53) in patients who received favipiravir only. While ALT and AST elevation occurred in 28% (n=254) of the patients taking antibiotics; the highest ALT and AST elevation was detected in patients using more than one drug with antibiotics ($p<0.001$). Severe hepatitis developed

in 1% of mild COVID-19 patients that used HCQ only and in 0.8% of patients that used favipiravir only. However, severe hepatitis developed in 1.8% of patients in whom antibiotics were added to the treatment (Table 2).

Elevated liver enzymes occurred in 26.3% (n=461) of patients with mild COVID-19, while in 45.3% (n=189) of severe COVID-19 patients. Severe hepatitis developed in 1.4% (n=25) of the patients with mild COVID-19 and 6.5% (n=27) with severe COVID-19 (p<0.001, Table 3).

On admission, the ALT, AST, ALP, and GGT levels were similar in surviving and non-surviving patients. At the end of treatment these levels were found to be significantly higher in non-surviving patients compared to surviving patients (Table 4).

There was a weak negative correlation between ALT and albumin (p=0.017, r=-0.497), while a weak positive correlation with bilirubin (p=0.024, r=0.352), a moderate positive correlation with ferritin (p=0.016, r=0.504), and a weak positive correlation with INR (p=0.022, r=0.383) were found in patients with severe COVID-19. However no such correlation was found in patients with mild COVID-19 (Table 5).

Table 1. Demographic and clinical characteristics

	Mild COVID-19 (n=1751)	Severe COVID-19 (n=417)	p
Age (years), mean±SD (min-max)	43.6±17.8 (9-84)	67.6±16.7 (18-100)	**<0.001**
Gender, n (%)			
Male	885 (50.5%)	240 (57.6%)	**0.010**
Female	866 (49.5%)	177 (42.4%)	
Days of hospitalization (days), median (min-max)	7.1 (1-30)	14.3 (3-79)	**<0.001**
Comorbid diseases, n (%)			
None	1376 (78.6%)	149 (35.7%)	**<0.001**
DM	162 (9.2%)	71 (17.0%)	**<0.001**
HT	187 (10.7%)	80 (19.2%)	**<0.001**
More than one	26 (1.5%)	117 (28.1%)	**<0.001**
Used Drugs, n (%)			
HCQ	398 (22.7%)	45 (10.8%)	**<0.001**
Favipiravir	1337 (76.3%)	401 (96.1%)	**<0.001**
Antibiotics	1274 (72.7%)	398 (95.4%)	**<0.001**
Tocilizumab	6 (3.4%)	154 (36.9%)	**<0.001**
High dose steroid	165 (9.4%)	316 (75.7%)	**<0.001**
Oseltamivir	78 (4.4%)	46 (11.0%)	**<0.001**
Remdesivir	1 (0.05%)	17 (4.0%)	**<0.001**
İmmunoglobulin	2 (0.1%)	13 (3.1%)	**<0.001**

COVID-19: coronavirus disease 2019, SD: standard deviation, DM: diabetes mellitus, HT: hypertension, CAD: coronary artery disease, CVD: cerebrovascular disease, HCQ: hydroxychloroquine

Table 2. Relationship between the drugs used and the ALT levels in patients with mild COVID-19 (n=1751)

	HCQ (n=205)	Favipiravir (n=386)	Antibiotics* (n=908)	Others** (n=252)	p
ALT					
Normal	173 (84.4%)	333 (86.2%)	654 (72.0%)#	130 (51.6%)#	**<0.001**
1-3 times higher (mild)	28 (13.6%)	45 (11.7%)	223 (24.6%)#	107 (42.4%)#	
3-5 times higher (moderate)	2 (1.0%)	5 (1.3%)	15 (1.6%)	11 (4.4%)	
>5 times higher (severe)	2 (1.0%)	3 (0.8%)	16 (1.8%)	4 (1.6%)	

ALT: alanine aminotransferase, HCQ: hydroxychloroquine, *: with or without favipiravir and HCQ, **: tocilizumab, remdesivir, immunoglobulin, high-dose steroid, #: significantly different

Table 3. Increase rates in ALT levels in patients with mild and severe COVID-19

	Mild COVID-19 (n=1751)	Severe COVID-19 (n=417)	p
ALT			
Normal	1290 (73.7%)	228 (54.7%)#	**<0.001**
1-3 times higher (mild)	403 (23.0%)	130 (31.1%)#	
3-5 times higher (moderate)	33 (1.9%)	32 (7.7%)#	
>5 times higher (severe)	25 (1.4%)	27 (6.5%)#	

ALT: alanine aminotransferase, COVID-19: coronavirus disease 2019, #: significantly different

Table 4. Liver functions at the beginning and end of the treatment*

	Surviving (n=1916)		Non-surviving (n=252)		p_B	p_A
	Before	After	Before	After		
ALT	25.9±25.6	35.2±34.1	25.5±16.8	125.0±109.5	0.309	**<0.001**
AST	24.1±20.6	38.8±16.2	28.6±24.1	181.3±171.2	0.125	**<0.001**
GGT	30.6±28.8	36.9±42.8	32.9±32.8	58.1±48.7	0.767	**0.003**
ALP	87.5±30.3	90.9±43.4	91.2±38.7	103.7±47.2	0.098	**0.028**

*: patients who recently developed ischemic hepatitis were not included in the table, ALT: alanine aminotransferase, AST: aspartate aminotransferase, GGT: gamma-glutamyltransferase, ALP: alkaline phosphatase, p_b: before treatment, p_a: after treatment between group p value

Table 5. Correlation among liver functions

		Mild COVID-19 (n=1751)				Severe COVID-19 (n=417)			
		Albumin	**TBIL**	**Ferritin**	**INR**	**Albumin**	**TBIL**	**Ferritin**	**INR**
ALT	r	-0.041	0.106	0.015	0.014	**-0.497**	**0.352**	**0.504**	**0.383**
	p	0.311	0.120	0.850	0.910	**0.017**	**0.024**	**0.016**	**0.022**
GGT	r	-0.104	0.239	0.067	**0.337**	**-0.308**	**0.706**	**0.394**	**0.463**
	p	0.122	0.053	0.190	**0.037**	**0.027**	**0.011**	**0.021**	**0.018**

COVID-19: coronavirus disease 2019, ALT: alanine aminotransferase, GGT: gamma-glutamyltransferase, TBIL: total bilirubin, INR: international normalized ratio

DISCUSSION

In this study, we found that 30% of patients who received COVID-19 treatment had elevated liver enzymes of varying degrees and we also noted that liver damage was more common in patients with severe COVID-19. Of note, the most common cause of liver enzyme elevation in patients with mild COVID-19 was the drugs used for the treatment. Isolated ALT and AST elevations were highly common in patients with mild COVID-19 and there was no correlation between these enzyme elevations and inflammation parameters. However, liver enzyme elevation was generally mild and transient in all patients. We also noted that patients with severe COVID-19 developed more severe liver dysfunction compared to patients with mild COVID-19. The most common cause of liver damage in these patients was systemic inflammation associated with cytokine storm and liver damage due to sepsis. We also found a correlation between inflammation markers and the severity of liver dysfunction in these patients, which implicates that the negative effect of increased inflammation on the liver is reflected in mortality.

A previous study investigated the relationship between COVID-19 and the liver and concluded that the liver dysfunction and liver damage in COVID-19 patients have a significant role in the prognosis of the disease (11). Another study reported that liver enzyme elevation that occurred during the disease indicated serious disease and the study also noted that albumin, which is a negative acute phase reactant, has a prognostic significance. In the present study, we found that the liver damage in COVID-19 pneumonia is more severe when compared to that of other viral pneumonias (12). In a meta-analysis with a large number of patients, liver function changes were reported in 47% of patients with COVID-19 and this situation was associated with in-hospital mortality (13).

Another meta-analysis reviewed 12,882 cases published in 24 studies that examined the relationship between COVID-19 and the liver, and found that 26.5% of the patients had liver damage. In addition, the study also showed that acute liver injury and elevated liver enzymes are significantly associated with the severity of COVID-19 (14).

It has been shown that liver damage due to COVID-19 is due to the direct cytopathic effect of the virus that is induced by the binding of virus to hepatocytes and cholangiocytes through ACE2 receptors. This damage predominantly occurs in cholangiocytes, which have an important role in both cell renewal and immune response (15). Meaningfully, this shows that GGT may elevate in severe COVID-19 patients (16). Similarly, in our study, a significant increase in GGT was noted, particularly in non-surviving patients.

CONCLUSION

In conclusion, the results indicated that liver functions should be closely monitored in patients hospitalized due to COVID-19. Liver is known to have an extremely high functional reserve and it is also known that liver damage in COVID-19 can be in many different forms and can have a broad spectrum of manifestations. Abnormal liver functions that develop in patients with mild COVID-19 are typically mild and temporary and often do not pose a clinical problem. However, liver function abnormalities that develop in severe COVID-19 patients affect the prognosis of the disease. Therefore, attention should be paid to liver function disorders that develop in patients with severe COVID-19. It should also be noted that the drugs used in the treatment of patients with liver dysfunction have the potential of hepatotoxicity and widespread systemic inflammation, and thus alternatives for the treatment should always be readily available.

Ethics Committee Approval: The study was approved by the Ethics Committee of Gazi Yaşargil Education and Research Hospital (16.10.2020, 611).

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Investigation of Toxin Profiles of Methicillin Resistant and Sensitive *Staphylococcus aureus* Strains Isolated from Various Clinical Specimens

Çeşitli Klinik Örneklerden İzole Edilen Metisiline Dirençli ve Duyarlı *Staphylococcus aureus* Suşlarının Toksin Profillerinin Araştırılması

Mücella BAYIRLI[1]
0000-0001-6120-0071
Özkan ASLANTAŞ[2]
0000-0003-0407-8633
Burçin ÖZER[3]
0000-0001-8965-9335

[1]Hatay Mustafa Kemal University, Graduate School of Health Sciences, Hatay, Turkey

[2]Department of Microbiology, Hatay Mustafa Kemal University Faculty of Veterinary Medicine, Hatay, Turkey

[3]Department of Medical Microbiology, Hatay Mustafa Kemal University Faculty of Medicine, Hatay, Turkey

Corresponding Author
Sorumlu Yazar
Özkan ASLANTAŞ
ozkanaslantas@yahoo.com

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ABSTRACT

Aim: This study aimed to investigate the superantigenic (SAg) toxin, exfoliative toxin (ET), hemolysin (HLY), leukotoxin (LUK) genes and accessory gene regulator (*agr*) types in *Staphylococcus aureus* isolates from various clinical materials.
Material and Methods: A total of 190 *S. aureus* isolates were investigated for the presence of toxin genes, *mec*A gene and *agr* types using by polymerase chain reaction (PCR).
Results: *mec*A gene was detected in 87 (45.8%) isolates. Of the 190 *S. aureus* isolates examined, 83.7% (n=159) were found to be positive for SAg genes. The *seg* (41.1%) was determined to be the most common toxin gene, followed by *sei* (38.9%), *selo* (38.9%), *selm* (28.4%), *sea* (%25.8), and *tst* (18.4%) genes, respectively. Seventy one different SAg toxin profiles were identified. Type I νSaβ encoding *seg*, *sei*, *selm*, *seln* and *selo* was the most common mobile genetic element (MGE), which was detected in 37 isolates (19.5%). The *hla*, *hlb*, *hld*, *hlg* and *hlg2* genes were detected in 92.6% (n=176), 1.6% (n=3), 98.9% (n=188), 1.1% (n=2) and 31.6% (n=60) of the isolates, respectively. The *pvl* gene was detected in 12.6% (n=11) of methicillin resistant *S. aureus* (MRSA) and 14.6% (n=15) of methicillin sensitive *S. aureus* (MSSA), respectively (p=0.701). While none of the isolates carried *luk*M gene, 67% (n=69) of MSSA and 69% (n=60) of MRSA isolates were found to be positive for *luk*ED gene (p=0.519).
Conclusion: High occurrence and diversity of toxin genes among *S. aureus* isolates could be explained by horizontal transmission of toxin genes through MGEs.
Keywords: *Staphylococcus aureus*; methicillin resistance; toxin; gene.

ÖZ

Amaç: Bu çalışmada çeşitli klinik materyallerden elde edilen *Staphylococcus aureus* izolatlarında süperantijenik (SAg) toksin, eksfoliatif toksin (ET), hemolizin (HLY) ve lökotoksin (LUK) genleri ve aksesuar gen regülatör (*agr*) tiplerinin araştırılması amaçlandı.
Gereç ve Yöntemler: Toplam 190 *S. aureus* izolatında toksin genleri, *mec*A geni ve *agr* tipleri polimeraz zincir reaksiyonu (PZR) kullanılarak incelendi.
Bulgular: *mec*A geni 87 (%45,8) izolatta tespit edildi. İncelenen 190 *S. aureus* izolatının %83,7'si (n=159) SAg genleri yönünden pozitif bulundu. En yaygın toksin geni *seg* (%41,1) olarak belirlenirken, bunu sırasıyla *sei* (%38,9), *selo* (%38,9), *selm* (%28,4), *sea* (%25,8) ve *tst* (%18,4) genleri izledi. Yetmiş bir farklı SAg toksin profili belirlendi. *sei*, *seg*, *selm*, *seln* ve *selo* genlerini taşıyan tip I νSaβ 37 (%19,5) izolatta tespit edilerek en yaygın mobil genetik element (MGE) olarak belirlendi. *hla*, *hlb*, *hld*, *hlg* ve *hlg2* genleri izolatların sırasıyla %92,6 (n=176), %1,6 (n=3), %98,9 (n=188), %1,1 (n=2) ve %31,6'sında (n=60) tespit edildi. *pvl* geni sırasıyla, metisilin dirençli *S. aureus* (methicillin resistant *S. aureus*, MRSA) izolatlarının %12,6'sında (n=11) ve metisilin duyarlı *S. aureus* (methicillin sensitive *S. aureus*, MSSA) izolatlarının ise %14,6'sında (n=15) saptandı (p=0,701). İzolatların hiçbiri *luk*M geni taşımazken, MSSA izolatlarının %67'si (n=69) ve MRSA izolatlarının %69'u (n=60) *luk*ED geni için pozitif bulundu (p=0,519).
Sonuç: *S. aureus* izolatları arasında toksin genlerinin yüksek oranda bulunması ve çeşitliliği, toksin genlerinin MGE'ler aracılığıyla horizontal transferi ile açıklanabilir.
Anahtar kelimeler: *Staphylococcus aureus*; metisilin direnci; toksin; gen.

INTRODUCTION

Staphylococcus aureus is a versatile pathogen capable of causing a wide range of infections (1). This feature of the agent is due to its genome plasticity of acquiring and incorporating genetic materials from other bacterial species that may have antimicrobial resistance and virulence. Thus, outcome of the infections caused by *S. aureus* strains are closely related with their resistance and virulence properties (2). *S. aureus* has the ability to produce several virulence factors such as superantigens (SAgs), hemolysins (HLYs), leukotoxins (LUKs), and exfoliative toxins (ETs). Among the exotoxins secreted by *S. aureus*, staphylococcal enterotoxins (SEs) and toxic shock syndrome toxin-1 (TSST-1) have SAg activity. These toxins are important virulence factors that contribute a variety of pathological conditions, including pneumonia, soft tissue infections, toxic shock syndrome, and infective endocarditis (2,3). Moreover, it has been recently reported that SAgs particularly SEs play a prominent role in the development of asthma of hospitalized patients via induction of IgE (4). So far, *S. aureus* strains are reported to secrete at least 26 or more types of SEs and staphylococcal enterotoxin-like toxins (SEls). SAgs have the ability to stimulate synthesis of cytokines by binding concurrently to MHC-II complex and Vβ element of TCR (5).

S. aureus is also capable of producing several pore forming toxins (PFTs) that target leukocytes. The *S. aureus* isolates associated with human infections can produce up to five bi-component leukocidins, also known PFTs: Panton-Valentine Leukocidin (PVL), gamma-hemolysin AB and CB (HlgAB and HlgCB), Leukocidin ED (LukED), and Leukocidin AB (LukAB). These toxins protect *S. aureus* from being killed by phagocytes of the host (6).

Exfoliative toxins (ETs), also known as epidermolytic toxins, are highly specific serine proteases secreted by *S. aureus* that are responsible for the clinical manifestation of staphylococcal scalded skin syndrome (SSSS) (7). So far, four types of ET have been reported as exfoliative toxin A/B/C/D (ETA, ETB, ETC, and ETD). Of these, while ETA and ETB were the most common types in humans, ETC was only detected in isolates from horse infections. In 2002, ETD was only defined in clinical *S. aureus* isolates in humans (8).

Among the wide variety of Staphylococcal cytolytic exotoxins produced, HLYs are the most prominent and well-characterized ones which play an important role in the pathogenesis of staphylococcal infections. HLYs α, β, γ, and δ cause pore formation on various cell membranes including immune cells that lead leakage of cellular molecules and metabolites outside of the cell in favor of the survival of the pathogen and progression of the disease (9).

The accessory gene regulator (*agr*) system is one of the main regulatory and control factors involved in the control of pathogenesis of *S. aureus* by regulating virulence factors, biofilm formation and resistance. *S. aureus* is divided into 4 different *agr* groups (*agr* I, *agr* II, *agr* III, and *agr* IV) based on mutations and polymorphisms of *agr*C and *agr*D genes (10). It was stated that prevalence of *agr* types vary according to geographical areas (11). Moreover, a link between *agr* types and certain diseases manifestations and clinical outcome has been reported (12). Jarraud et al. (13) suggested that *agr* type III is more prevalent in TSST-1 producing isolates and *agr* type IV is more prevalent in ET producing isolates.

The main aim of the current study was to investigate the prevalence of SAg (SE, TSST-1), ET, HLY, and LUK genes in methicillin resistant *S. aureus* (MRSA) and methicillin sensitive *S. aureus* (MSSA) isolates.

MATERIAL AND METHODS

Ethical Considerations

This study was approved by the non-interventional clinical researches ethics committee of Hatay Mustafa Kemal University (05.09.2019, 14/2019).

S. aureus Isolates

All *S. aureus* strains isolated from different clinical materials submitted to the Microbiology Laboratory of the Hatay Mustafa Kemal University Hospital from January to September 2020 were included in the study. Suspected colonies were identified by hemolysis properties, Gram staining, catalase, coagulase and other biochemical tests (14). Identification of the strains and antibiotic susceptibilities of these isolates were determined with the Vitek 2 compact system (bioMe´rieux, France) and evaluated according to the breakpoints of the European Committee for Antimicrobial Susceptibility Tests (EUCAST) (15). They were confirmed by polymerase chain reaction (PCR) using primers specific to the *nuc* gene (16).

DNA Isolation

DNA isolation from the isolates was performed using a commercial extraction kit (InstaGeneTM Matrix, Bio-Rad, France). The resulting template DNAs were stored at -20 °C until use.

Detection of *mec*A Gene

The *mec*A gene in phenotypically methicillin resistant isolates were investigated as previously described by Choi et al. (17).

Detection of Toxin Genes by PCR

The genes encoding SEs (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *selj*, *selk*, *sell*, *selm*, *seln*, *selo*, *selp*, *selq*, and *selr*), TSST-1 (*tst*), ET (*eta*, *etb*), leukocidins (*pvl*, *luk*ED, and *luk*M) and HLYs (*hla*, *hlb*, *hld*, *hlg*) were examined as previously described by Omoe et al. (18), Jarraud et al. (13), Mehrotra et al. (19) and Lina et al. (20), respectively.

agr Typing

Determination of the *agr* types of *S. aureus* isolates was performed using multiplex PCR method as previously described by Gilot et al. (21).

Statistical Analysis

The statistical analyses was carried out using SPSS v.16 (SPSS Inc., Chicago, IL, USA). The frequencies of the variables was presented as numbers and percentages. The Pearson chi-square test, Fisher's exact test, and Fisher-Freeman-Halton test were used to compare categorical variables where appropriate. $p<0.05$ was considered as statistically significant.

RESULTS

PCR results for *mec*A

All *S. aureus* isolates were positive for *nuc* gene. Based on PCR amplification of *mec*A gene, 87 isolates were identified as MRSA and 103 as MSSA. The distribution of

MRSA isolates according to the clinical materials is given in Table 1. No statistically significant difference was found in terms of the type of clinical materials from which the MRSA strains were isolated (p=0.577).

Distribution of SAg and ET Genes in MSSA and MRSA Isolates

Distribution of SAg toxins and ET genes detected in MSSA and MRSA isolates is given in Table 2. Thirty-one (16.3%) isolates did not have any SAg's toxin genes examined. The most common SE genes were *seg* (41.1%), *sei* (38.9%), *selo* (38.9%), *selm* (28.4%), and *sea* (25.8%). While *eta* was observed in 9 (4.7%) isolates (6 MSSA and 3 MRSA isolates), *etb* was carried by seven MSSA and one MRSA isolates. SAg toxin genotypes and their relationship with MGEs are shown in Table 3.

Distribution of Hemolysin Genes

Nearly all isolates at least carried one of the HLY genes examined, except one MSSA isolate. There was no statistically significant difference between MSSA and MRSA isolates in terms of distribution of HLY genes (p=0.309). The distribution of HLY genes is given in Table 4.

Distribution of Leukocidin Genes

While all isolates were negative for *luk*M gene, the *luk*ED gene was detected in 67% (n=69) of the MSSA isolates and in 69% (n=60) of the MRSA isolates (p=0.519). As seen in Table 5, the *pvl* gene was detected in 14.6% (n=15) of the MSSA isolates and in 12.6% (n=11) of the MRSA isolates (p=0.701).

Distribution of *agr* Types

As seen in Table 6, *agr* type I (56.8%) was the most common type among the isolates, followed by *agr* type III (22.1%) and *agr* type II (16.8%). Distribution of *agr* types between MSSA and MRSA isolates was statistically significant (p=0.001).

Table 1. The distribution of *S. aureus* isolates according to the clinical materials

Clinical Material	MSSA	MRSA	p	Total
Throat swab	2 (1.9)	2 (2.3)		4 (2.1)
Nasal Swab	4 (3.9)	8 (9.2)		12 (6.3)
Wound	27 (26.2)	19 (21.8)		46 (24.2)
Blood	30 (29.1)	27 (31.0)		57 (30.0)
Urine	32 (31.1)	21 (24.1)	0.609	53 (27.9)
Sputum	3 (2.9)	4 (4.6)		7 (3.7)
Cerebrospinal fluid	2 (1.9)	5 (5.7)		7 (3.7)
Vaginal Discharge	2 (1.9)	1 (1.1)		3 (1.6)
Tracheal Aspirate	1 (1.0)	0 (0.0)		1 (0.5)
Total	**103**	**87**		**190**

MSSA: methicillin sensitive *S. aureus*, MRSA: methicillin resistant *S. aureus*

Table 2. Distribution of SAg toxin and ET genes detected in MSSA and MRSA isolates

SAg	MSSA (n=103)	MRSA (n=87)	p	Total
sea	25 (24.3)	24 (27.6)	0.603	49 (25.8)
seb	5 (4.9)	2 (2.3)	0.456	7 (3.7)
sec	3 (2.9)	1 (1.1)	0.626	4 (2.1)
sed	9 (8.7)	6 (6.9)	0.639	15 (7.9)
selq	13 (12.6)	13 (14.9)	0.643	26 (13.7)
selo	40 (38.8)	34 (39.1)	0,972	74 (38.9)
selm	32 (31.1)	22 (25.3)	0,378	54 (28.4)
selr	10 (9.7)	6 (6.9)	0.487	16 (8.4)
selk	16 (15.5)	10 (11.5)	0.420	26 (13.7)
seln	17 (16.5)	10 (11.5)	0.324	27 (14.2)
sell	6 (5.8)	6 (6.9)	0.762	12 (6.3)
seg	48 (46.6)	30 (34.5)	0.091	78 (41.1)
sei	44 (42.7)	30 (34.5)	0.246	74 (38.9)
seh	6 (5.8)	20 (23)	**0.001**	26 (13.7)
selj	8 (7.8)	6 (6.9)	0.819	14 (7.4)
selp	5 (4.9)	4 (4.6)	0.999	9 (4.7)
tst	14 (13.6)	21 (24.1)	0.062	35 (18.4)
eta	6 (5.8)	3 (3.4)	0.512	9 (4.7)
etb	7 (6.8)	1 (1.1)	0.072	8 (4.2)

SAg: superantigen, ET: exfoliative toxin, MSSA: methicillin sensitive *S. aureus*, MRSA: methicillin resistant *S. aureus*

DISCUSSION

The pathogenicity of *S. aureus* has been attributed to their ability to evade both innate and acquired immune mechanisms of the host (22). Apart from virulence repertoire of *S. aureus*, which plays an important role in the pathogenesis, the emergence and increasing prevalence of MRSA in healthcare institutions is one of the most important challenges encountered in the treatment of *S. aureus* infections. Methicillin resistance is one of important resistance mechanisms observed in *S. aureus* isolates. Based on *mec*A PCR results, a higher prevalence (55.8%) of MRSA was detected. In Iran, Motamedifar et al. (23) reported a similar prevalence rate (57.7%). In previous studies carried out in Turkey, prevalence of MRSA was reported as 17.9% by Özel et al. (24), 22.9% by Tanrıverdi Çaycı et al. (25), 24% by Arıcı and Aksaray (26), 12.5% in hospital acquired (HA)- *S. aureus* isolates and 43% in community acquired (CA)- *S. aureus* isolates by Duman et al. (27), 30.8% by Kılıç et al. (28), and 44% by Şahin et al. (29). In contrast, Karahan et al. (30) reported higher a prevalence rate in HA- *S. aureus* isolates (83.9%) and CA- *S. aureus* isolates (91.9%). Compared to most of the MRSA rates mentioned above, the high rate of MRSA detected in this study can be explained by the nosocomial infections or widespread use of beta-lactams in the region. It has been suggested that *S. aureus* isolates expressing PVL cause severe skin and soft tissue infections, necrotizing fasciitis and life-threatening infections such as hemorrhagic pneumonia (31). In previous studies conducted in Turkey, it was reported that the prevalence rates of *pvl* gene varied between 1.9-5.4% in MSSA isolates and 1.5-9.5% in MRSA isolates (28,30,32). Another study conducted by Duman et al. (27), showed that *pvl* gene was detected in 15% (6/88) of the CA- *S. aureus* isolates and in 3% (6/177) of the HA- *S. aureus* isolates.

In this study, *eta* and *etb* genes were detected in 4.7% and 4.2% of the isolates, respectively. Although isolation rates were higher in MSSA isolates (1.7% for *eta* and 6.7% for *etb*) compared to MRSA isolates (2.9% for *eta* and 1.1% for *etb*), this was not statistically significant. In previous studies, absence or low prevalence rates of ET genes has been reported. Xie et al. (33) reported the prevalence of *eta*, *etb* and *etd* genes in 108 clinical *S. aureus* isolates as 1.9%, 0% and 8.3%, respectively. Jiménez et al. (34) could

Table 3. SAg toxin genotypes identified in MSSA and MRSA isolates and their relationship with MGE

SAg Toxin	MSSA	MRSA	Total	Possible MGE
sea	5	4	9	φSa3mu
sea, tst	-	1	1	φSa3mu+*tst*
sea, selq	-	1	1	φSa3mu+*selq*
sea, sed	1	1	2	φSa3mu+*sed*
sea, selo	-	1	1	φSa3mu+*selo*
sea, seg, sei	2	1	3	φSa3mu+*seg+sei*
sea, selk, selq	2	-	2	φSa3mw+*selk+selq*
sea, selm, selo	1	1	2	φSa3mu+*selm+selo*
sea, seg, selm, selo	2	-	2	φSa3mu+*seg+selm+selo*
sea, seg, sei, tst	1	-	1	φ**S**a3mu+*seg+sei+tst*
sea, selm, selo, selr	-	1	1	φSa3mu+*selm+selo+selr*
sea, selo, tst	-	1	1	φSa3mu+*selo+tst*
sea, seb, seg, sei, seh, tst	1	-	1	φSa3mu+*seb+seg+sei+seh+tst*
sea, seg, sei, selm, seln, selo	1	1	2	φSa3mu, Tip I νSaβ
sea, seg, sei, seh, tst	2	-	2	φSa3mu+*seg+sei+seh+tst*
sea, seg, sei, selm, seln, selo	1	-	1	φSa3mu, Tip I νSaβ
sea, sed, selj, seln, selk, selr	-	1	1	φSa3mu, pIB485
sea, sec, seg, sei, sell, selm, selo	-	1	1	φSa3mu, Tip II νSa3, Tip I νSaβ
sea, seg, sei, sell, selm, selo, selr, tst	-	1	1	φSa3mu, Tip I νSaβ
sea, seg, sei, selm, seln, selo, tst	1	-	1	φSa3mu, Tip I νSaβ+*tst*
sea, sed, selj, selk, selq, selr	4	-	4	φSa3mw, pIB485
sea, seh, selk, selq	-	2	2	φSa3mw+*seh*
sea, seg, sei, selk, selq, tst	-	2	2	φSa3mw+*seg+sei+tst*
sea, seg, sei, selk, selq	-	2	2	φSa3mw+*seg+sei*
seb	3	-	3	ND
seb, seg, sei, selk, selm, selo, selq, tst	1	-	1	Tip I νSaβ, νSa1 (SaPI3)+*tst*
seb, seg, sei, selk, selq	-	1	1	νSa1 (SaPI3)+*seg+sei*
sec, seg, sei, selm, seln, selo, selr	2	-	2	Tip I νSaβ+*sec+selr*
sec, seg, sei, sell, selm, selo, selp	1	-	1	Tip II νSa3, Tip I νSaβ, φSa3n
sed, selj, selr	1	-	1	pIB485
sed, seg, sei, seln, tst	-	2	2	ND
sed, seg, sei, selm, seln, selo	2	-	2	Tip I νSaβ+*sed*
sed, seg, sei, selm, seln, selo, tst	-	1	1	Tip I νSaβ+*sed+tst*
sed, seg, sei, selj, selm, selo, selr	-	1	1	Tip I νSaβ, pIB485
sed, seg, sei, selk, seln	1	-	1	ND
sed, selm, seln, selo, tst	-	1	1	ND
seg, sei	4	3	7	ND
seg, sei, tst	4	-	4	ND
seg, sei, selk, selr	2	-	2	ND
seg, sei, selm, seln, selo	3	1	4	Tip I νSaβ
seg, sei, selj, selm, seln, selo, tst	2	1	3	Tip I νSaβ+*selj+tst*
seg, sei, selj, selm, selq, selo, tst	-	2	2	Tip I νSaβ+*selj+tst*
seg, sei, selm, selo, selq	-	1	1	Tip I νSaβ
seg, sei, selo, selm	9	3	12	Tip I νSaβ
seg, sei, selp, seln, selk	1	-	1	φSa3n
seg, sei, seh, selm, selo	1	-	1	Tip I νSaβ
seg, sei, selm, seln, selo, tst	1	-	1	Tip I νSaβ
seg, sei, sell, selm, selo, selr, tst	-	1	1	Tip I νSaβ+*sell+selr+tst*
seg, sei, selp, sell, selm, selr, selo	-	1	1	Tip I νSaβ, φSa3n
seg, sei, selk, seln, tst	-	2	2	ND
seg, sei, sell, selm, selo, selr	1	1	2	Tip I νSaβ
seg, sei, selj, selm, selo, tst	1	2	3	Tip I νSaβ+*selj+tst*
seg, sell, selm, selo, selr	1	-	1	ND
seh	1	10	11	ND
seh, sell	-	2	2	ND
seh, selm, selo	-	2	2	ND
seh, selq, tst	-	1	1	ND
seh, selo, selk, tst	-	1	1	ND
seh, selm, selo, tst	-	1	1	ND
selo	2	2	4	ND
selm, selo	1	1	2	ND
selm, selo, tst	2	1	3	ND
selp	1	1	2	φSa3n
selp, selq	-	1	1	φSa3n, *selq*
selq	1	-	1	ND
selq, selk	3	-	3	ND
selm, selo, selq	2	0	2	ND
selk, selm, selo, selq, tst	-	1	1	ND
selk, selq	3	-	3	ND
tst	1	-	1	ND
sell	1	-	1	ND
Negative	17	14	31	

SAg: superantigen, MSSA: methicillin sensitive *S. aureus*, MRSA: methicillin resistant *S. aureus*, MGE: mobile genetic element, ND: Since combination of SAg genes was incomplete, any association was not established between suspected MGE and toxin profile

Table 4. Hemolysin gene combinations determined in MSSA and MRSA isolates

Hemolysin	MSSA (n=103)	MRSA (n=87)	p	Total (n=190)
*hly*A	1 (1.0)	0 (0.0)		1 (0.5)
*hly*D	8 (7.8)	4 (4.6)		12 (6.3)
*hly*A-*hly*D	59 (57.3)	52 (59.8)		111 (58.4)
*hly*A-*hly*D-*hly*G2	32 (31.1)	27 (31.0)		59 (31.1)
*hly*A-*hly*D-*hly*B	0 (0.0)	2 (2.3)	0.309	2 (1.1)
*hly*A-*hly*D-*hly*G	2 (1.9)	0 (0.0)		2 (1.1)
*hly*D-*hly*G2	0 (0.0)	1 (1.1)		1 (0.5)
*hly*A-*hly*D-*hly*B-*hly*G2	1 (1.0)	0 (0.0)		1 (0.5)
Negative	0 (0.0)	1 (1.1)		1 (0.5)

hly: Hemolysin, MSSA: methicillin sensitive *S. aureus*, MRSA: methicillin resistant *S. aureus*

Table 5. Distribution of *pvl* gene among the MSSA and MRSA isolates

pvl	MSSA (n=103)	MRSA (n=87)	p	Total (n=190)
Negative	88 (85.4)	76 (87.4)	0.701	164 (86.3)
Positive	15 (14.6)	11 (12.6)		26 (13.7)

MSSA: methicillin sensitive *S. aureus*, MRSA: methicillin resistant *S. aureus*

Table 6. Distribution of *agr* types according to MSSA and MRSA isolates

agr Type	MSSA (n=103)	MRSA (n=87)	p	Total (n=190)
agr type I	53 (51.5)	55 (63.2)		108 (56.8)
agr type II*	27 (26.2)	5 (5.7)		32 (16.8)
agr type III*	17 (16.5)	25 (28.7)	**0.001**	42 (22.1)
agr type I-II	4 (3.9)	1 (1.1)		5 (2.6)
agr type I-III	1 (1.0)	1 (1.1)		2 (1.1)
agr type II-III	1 (1.0)	0 (0.0)		1 (0.5)

agr: accessory gene regulator, MSSA: methicillin sensitive *S. aureus*, MRSA: methicillin resistant *S. aureus*, *: statistically significant differences for these two *agr* types

not detect the *eta* and *etb* genes in MSSA (n=30) isolates, but detected the *eta* gene in only one the MRSA (n=30) isolate. de Souza et al. (35) did not find the *eta* and *etb* genes in any of MRSA isolates, but detected the *eta* gene in only 2.3% (3/130) of the MSSA isolates. Similarly, Nhan et al. (36) reported a low prevalence rate for *eta* (1/1186) among clinical *S. aureus* isolates. In contrast, high prevalence rates of ET genes among *S. aureus* isolates was reported by Demir et al. (37), who found that 20% (n=24) of the isolates were positive for the *eta* and *etb* genes.

It has been noted that the prevalence of *tst* gene among MRSA isolates has increased in recent years. Similarly, in this study, the frequency of *tst* gene was found to be higher in MRSA isolates in comparison to MSSA isolates (24.1% vs 13.6%, p=0.062). Hu et al. (38) reported higher prevalence rate and found the *tst* gene in 85.6% (101/118) of MRSA isolates and 10% (14/140) of MSSA isolates. However, a contradictory result was reported by Motamedifar et al. (23), who detected the *tst* gene in 18.1% (36/199) of MSSA isolates and 11.6% (17/146) of MRSA isolates.

The presence of SE genes among MRSA and MSSA isolates was found to be abundant and diverse as 83.7% (n=159) of the isolates (Table 2, Table 3). Xie et al. (33) found that 85.2% (92/108) of the *S. aureus* isolates were positive for SE genes and *sea* (44.4%), *selk* (42.6%) and *selq* (40.7%) as the most common SE genes. The researchers also reported *sea-selk-selq* (41.3%, 38/92) as the most common SE combination. In another study, Demir et al. (37) detected that 69 of 120 (57.5%) *S. aureus* isolates had one or more SE genes, with a dominance of *seg* and *sei* (49/120, 40.8%) genes. Hu et al. (38) investigated MRSA and MSSA isolates for classical and newly described SE genes, and found that 78% (92/118) of the MRSA isolates and 75.7% (89/140) of MSSA isolates were positive for SE genes. The authors also noted that *sea* (45%) and *sec* (39%) genes were as the most common SE genes and *sec-seg-sei-sell-selm-seln-selo-tst* as the dominant genotype (52/118, 44.1%) among MRSA isolates; on the other hand, *sea* (42%), *selk* (38%) and *sec* (35%) genes were as the most common SE genes and *seg-sei-sell-selm-seln-selo* as dominant genotype (18/140, 12.9%) among MSSA isolates. Tekeli et al. (39) investigated 100 MRSA isolates from the blood cultures of hospitalized patients for the SE genes, and detected the SE genes in 86% of the isolates and distribution of SE as *sea* (77%), *sea-seg* (4%), *sea-seg-sec* (1%) and *seg-sei* (2%).

Of the exotoxins synthesized by *S. aureus* isolates, HLYs are among the toxins that play important roles in the pathogenesis of staphylococcal infections. These toxins exert a lethal effect on different host cell populations, including immune cells, and have the ability to help the spread of bacteria within the host body (40). Previous studies have revealed that HLY genes are widely distributed among MSSA and MRSA isolates. In this study, nearly all isolates were found to be positive for one or more investigated HLY genes. Similar observations have also been reported in previous studies (41-44).

S. aureus is one of notorious bacteria that has the ability to produce PFTs targeting leukocytes (6). The *luk*ED gene, located in mobile staphylococcal pathogenicity island called vSAβ (45), was detected in 67.9% (129/190) of the isolates, of which 69 (67%) were MSSA and 60 (69%) were MRSA. However, none of the isolates was positive for *luk*M gene. Comparable prevalence rate was also reported by Havaei et al. (46), who detected *luk*ED in 73.8% (110/149) of the isolates. On the other hand, He et al. (47) detected *luk*ED in 81.4% (144/177) of the isolates, but *luk*M was not detectable in any isolates.

In this study, most of the isolates (56.8%) belonged to *agr* type I, followed by *agr* type III (22.1%) and *agr* type II (16.8%), respectively. The *agr* type IV was not detected in any of the isolates. Similarly, Peerayeh et al. (48) reported that most of the *S. aureus* isolates belonged to *agr* type I (55.1%), followed by *agr* type II (16.9%), *agr* type III (16.5%) and *agr* type IV (9.4%), respectively. Similar observations were also reported by Shopsin et al. (49) and van Leeuwen et al. (50), who reported that 42.1% and 71% of the isolates were belonged to *agr* type I, respectively. In contrast, Tekeli et al. (39) reported a higher rate (91%) of *agr* type I in 100 MRSA strains isolated from blood cultures. In this study, the authors did not detect *agr* type IV among the isolates. The absence of *agr* type IV has also been reported in previous studies (49-52). The differences observed in the distribution of *agr* types among *S. aureus* isolates could be explained by ecological and geographical

differences. Interestingly, some isolates carried more than one *agr* type in this study. A similar observation was also reported by Yoon et al. (53), who detected co-presence of *agr* type I-II and *agr* type I-III in 4.4% and 0.6% of the isolates, respectively. Ji et al. (54) explained this phenomenon with significant sequence changes in the domain encoding the *agr*D signal peptide.

Jarraud et al. (13) reported that there is a relationship between *agr* groups and infection types, and suggested that the strains belong to *agr* type IV are mostly associated with generalized exfoliative and suppurative infections, whereas the strains belonged to *agr* type I and II are associated with endocarditis cases. Moreover, Ji et al. (54) reported that TSST-1 producing isolates were associated with *agr* type III. In this study, ET and TSST-1 producing isolates were mainly found to belong to *agr* types I and II. The SAg toxin genes have been reported to be largely associated with MGE, such as pathogenicity islands, prophages, SCC*mec* element and plasmids (55). Seventy one SAg toxin gene profile determined in the study suggested possible presence of MGEs encoding SAg toxins. Type I νSaβ (in 24 MSSA and 13 MRSA) and φSa3mu (in 18 MSSA and 16 MRSA) were identified as the most common MGEs, followed by φSa3mw (in 4 MSSA and 7 MRSA), φSa3n (in 3 MSSA and 3 MRSA), pIB485 (in 5 MSSA and 1 MRSA) and Type II νSa3 (in 1 MSSA and 1 MRSA). Of the determined MGEs, φSa3mu encodes *sea*; type I νSaβ *seg*, *sei*, *selm*, *seln* and *selo*; type II νSa3 *sec*, *sell*; νSa1 (SaPI3) *seb*, *selk*, *selq*; type I νSa4 *sec*, *sell*, *tst*; φSa3mw *sea*, *selk*, *selq*; φSa3n *selp*; pIB485 *sed*, *selj*, *selr* and SaPI1 *seb*, *selk*, *selq* genes. In this study, MGEs were detected at a higher frequency in MRSA isolates. Similarly, while Hu et al. (37) found type I νSaβ and type I νSa4 in 83.1% and 77.1% of the MRSA isolates, and also these MGEs were found in 25% and 2.1% of MSSA isolates.

The clinical outcome of *S. aureus* infections is influenced not only by the presence of antimicrobial resistance, but also virulence factors. The acquisition of antibiotic resistance genes in *S. aureus* isolates has been reported to cause changes in the expression of virulence genes depending on the fitness cost associated with the expression of resistance genes leading to a decrease in toxin expression (34). Therefore, it is not surprising in this respect that virulence genes were detected at a higher rate in MSSA isolates than MRSA isolates.

In this study, we aimed to search only the *mec*A gene, not the *mec*C gene among the fenotipitic resistant isolates. Therefore, this situation can be considered as a limitation of the study.

CONCLUSION

The results of this study showed that there is a high genetic diversity in terms of toxin genes among the clinical *S. aureus* isolates, and that the presence and combination of toxin genes are not limited to MSSA or MRSA, indicating frequent transfer of toxin gene-containing MGEs among *S. aureus* populations. In addition, the novel SE gene combinations observed herein suggest the existence of variants or novel types of MGEs. Comparative molecular studies involving large MSSA and MRSA populations are needed to understand how native MSSA and MRSA populations arise and interact.

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Overexpression of PD-L1 in Papillary Carcinoma and Its Association with Clinicopathological Variables

Papiller Karsinomda PD-L1'in Aşırı Ekspresyonu ve Klinikopatolojik Değişkenlerle İlişkisi

Servet KOCAÖZ[1]
0000-0002-0085-2380
Gülay TURAN[2]
0000-0002-3702-8811

[1]Department of General Surgery, Ankara City Hospital, Ankara, Turkey
[2]Department of Medical Pathology, Balıkesir University Faculty of Medicine, Balıkesir, Turkey

Corresponding Author
Sorumlu Yazar
Servet KOCAÖZ
servet.kocaoz@gmail.com

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ABSTRACT

Aim: Standard treatment may not be sufficient in patients with metastatic papillary thyroid cancer (PTC), and it may be beneficial to add PD-1 agents to the treatment. Therefore, this study was conducted to compare and evaluate the expression of PD-L1 in patients with nodular goiter (NG) and Hashimoto's thyroiditis (HT) within the scope of PTC carcinoma.
Material and Methods: Thirty-five patients from each group who underwent thyroidectomy for NG, HT, and PTC, between January 2011 and December 2017 were identified. Immunohistochemically, an anti-PD-L1 stain was applied by taking new sections from the tissue samples of 105 patients. The histological type, tumour diameter, capsule invasion (CI), and lymphovascular invasion (LVI) were evaluated.
Results: Nine (25.7%) of the patients with PTC were defined as classic, 10 (28.6%) as follicular, 6 (17.1%) as oncocytic, 5 (14.3%) as encapsulated, 2 (5.7%) as solid, 1 (2.9%) tall cell, and 2 (5.7%) as Warthin-like. The expression of PD-L1 in the patients with PTC was significantly higher than in the patients with NG and HT ($p<0.001$). There was a significant relationship between the increased PD-L1 expression and staining intensity and presence of thyroid LVI in the patients with PTC ($p=0.047$). In addition, the PD-L1 expression and LVI were observed to be more severe in patients with follicular, tall cell, and oncocytic subtype PTC ($p=0.011$, $p=0.026$, respectively).
Conclusion: It was observed that the PD-L1 expression increased in patients with PTC. PD-L1 expression and LVI were more severe in patients with follicular, tall cell, and oncocytic subtype of PTC.
Keywords: Papillary thyroid carcinoma; Hashimoto thyroiditis; nodular goiter; PD-L1.

ÖZ

Amaç: Metastatik papiller tiroid kanserli (papillary thyroid cancer, PTC) hastalarda standart tedavi yeterli olmayabilir ve tedaviye anti-PD-1 ajanların da eklenmesi faydalı olabilir. Bu nedenle bu çalışma, PTC karsinomu kapsamında, nodüler guatr (NG) ve Hashimoto tiroiditi (HT) hastalarında PD-L1 ekspresyonunun karşılaştırılması ve değerlendirilmesi amacıyla yapılmıştır.
Gereç ve Yöntemler: Ocak 2011 ile Aralık 2017 tarihleri arasında, NG, HT ve PTC nedeniyle tiroidektomi yapılmış olan, her hasta grubundan otuz beş hasta belirlendi. 105 hastanın doku örneklerinden yeni kesitler alınarak immünohistokimyasal olarak anti-PD-L1 boyası uygulandı. Histolojik tip, tümör çapı, kapsül invazyon (capsule invasion, CI) ve lenfovasküler invazyon (lymphovascular invasion, LVI) değerlendirildi.
Bulgular: PTC tanısı alan hastaların 9'u (%25,7) klasik, 10'u (%28,6) foliküler, 6'sı (%17,1) onkositik, 5'i (%14,3) enkapsüle, 2'si (%5,7) solid, 1'i (%2,9) uzun hücreli ve 2'si (%5,7) Warthin benzeri tip olarak tanımlandı. PTC'li hastalarda PD-L1 ekspresyonu, NG ve HT olan hastalardan anlamlı olarak daha yüksekti ($p<0,001$). PTC'li hastalarda artmış PD-L1 ekspresyonu ve boyama yoğunluğu ile tiroid LVI varlığı arasında anlamlı bir ilişki vardı ($p=0.047$). Ayrıca foliküler, uzun hücreli ve onkositik alt tip PTC'li hastalarda PD-L1 ekspresyonu ve LVI'nın daha şiddetli olduğu gözlendi (sırasıyla $p=0,011$, $p=0,026$).
Sonuç: PTC'li hastalarda PD-L1 ekspresyonunun arttığı gözlendi. Foliküler, uzun hücreli ve onkositik alt tip PTC'li hastalarda PD-L1 ekspresyonu ve LVI'nın daha şiddetli olduğu gözlendi.
Anahtar kelimeler: Papiller tiroid karsinomu; Hashimoto tiroiditi; nodüler guatr; PD-L1.

INTRODUCTION

According to 2013 data, the annual thyroid cancer (TC) incidence in the United States (USA) is 14.42 per 100,000, and an increase in the incidence of TC and mortality related to TC rate can be seen over the years (1). Among types of cancer, TC ranks as the 4th most commonly seen type among female in the USA (2). Thyroid follicular epithelial-derived cancers are: papillary, follicular and anaplastic. Other thyroid cancers are those that spread from the medullary thyroid cancer, primary lymphoma, sarcomas, and other organs to the metastases to the thyroid gland (3). The most common of these is papillary TC (PTC), accounting for approximately 80% of TCs (4). Patients with PTC or follicular TC (FTC), among the types of TC, can often be treated without problem. In patients with medullary TC or anaplastic TC (ATC), the tumour is more aggressive and tends to metastasize earlier (5).

The role of immune control point regulators is to prevent auto-immunity, minimize collateral tissue damage, and modulate the immune system's response in chronic infection. Since most of the immune control points are initiated through the interactions of ligand-receptors, they can easily be blocked by antibodies. In addition, the recombinant form of ligands and/or receptors can be obtained (6). Among the immune checkpoint regulators, the CTLA-4 co-inhibitory receptor was first identified (7,8). However, the most studied was the interaction that occurred between PD-1, which is a T-cell inhibitor receptor, and the PD-L1 (8,9). PD-L1, which is also well-known as CD274, is a glycoprotein that occurs on the cell surface, expressed in various tissues, including tumours. Tumours that express PD-L1 may render the cytotoxic T lymphocytes (CTL) inactive through the merging of inhibitor receptor and PD-1 (10). The expression of PD-L1 increased due to cancer cells, which is a primary host immune escape system. The discovery of the interaction of binding the PD-1 and PD-L1 resulted in the development of immunotherapy as a novel, inspirational treatment for resistance against cancer. It is promising for aggressive differentiated TC patients and ATC patients to utilize immunotherapy (11).

In the recent literature, immune checkpoint inhibitors have been investigated in aggressive forms of TC (10,12,13). The expression of programmed death-ligand 1 (PD-L1) has been determined to be at a lower rate in PTC patients than in FTC and ATC patients (13). It has been reported that the expression of PD-L1 has been higher in patients who had lymph node metastases (10). It has been reported that there is no difference in the expression of PD-L1 in PTC patients who have and do not have lymphocytic thyroiditis (LT) (10,13). In another study, it was reported that the expression of PD-L1 was higher in PTC patients with LT, but there was no difference between the stage of the disease and the PD-L1 expression (14). This study was conducted to analyze the expression of PD-L1 in patients with nodular goiter (NG), Hashimoto's thyroiditis (HT), and PTC and the relation with clinicopathological characteristics.

MATERIAL AND METHODS

In this study, 35 patients from the NG, HT, and PTC groups were identified by analyzing the histopathological reports of patients who underwent thyroidectomy between January 2011 and December 2017 at Balıkesir Atatürk State Hospital. Necessary permission for the study was obtained from Balıkesir University Medical Faculty Clinical Research Ethics Committee (114, decision dated 28 August 2019). Patients with micro-carcinoma were not included in the study while identifying the patients in the PTC group. The thyroidectomy samples of 105 patients in total were obtained from the pathology archive and re-examining the new sections. These pathology samples were sealed in paraffin after fixation in 10% neutral buffered formaldehyde. Sections of a thickness of 4 microns were sliced from the samples; they were stained with hematoxylin and eosin. To confirm the previous diagnosis, they were re-examined under a routine light microscope. The prognostic parameters were re-evaluated and recorded concerning the PTC status of the patients. Afterwards, immunohistochemical PDL-1 stain was applied to the new sections taken from tissue samples of the patients in the NG, HT, and PTC groups (Figure 1a, 1b, and 1c). The recovery of antigen was performed through using CC1, which is an antigen intake solution (as default; pH 8.0) (ref. 950 CC124, Ventana Medical Systems, Inc. Tucson, AZ, ABD). The samples were incubated using the PD-L1 (E1J2J, Cell Signaling Technology, Inc., Danvers MA, 1:100 dilution) monoclonal antibodies at 37 °C for 32 min. The OptiView DAB Immunohistochemical Retaining Kit (Ventana) and OptiView Amplification Kit (Ventana) were used for visualization for 12 min. The staining process was performed for 120 min in a VENTANA BenchMark ultra-auto slide staining device (Roche Group, Basel, Switzerland) at 90 °C according to the manufacturer protocols within the user manual for antibodies.

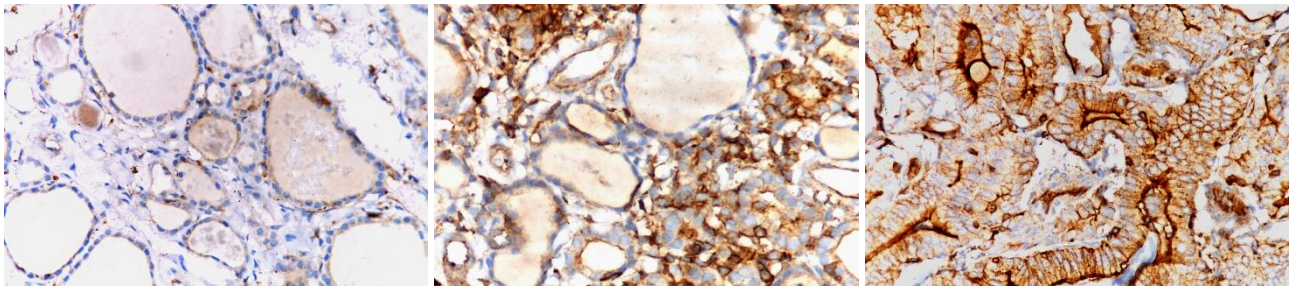

Figure 1. a) Poor expression of PDL-1 in the thyroid follicular epithelial cells in the nodular colloidal goiter, x200. **b)** Moderate expression of PDL-1 in the thyroid follicular epithelial cells, despite strong expression in the lymphoid cells in HT, x200. **c)** Increased strong PDL-1 expression in the thyroid follicular epithelial cells in a PTC, x400.

An experienced pathologist interpreted the staining results of the immunohistochemical PD-L1. Evaluation of the PD-L1 expression was semi quantitatively scored according to the staining density that could be separated from the background density (complete staining, and/or partial staining, circumferential staining and/or linear staining) of the membrane of the plasma and cytoplasmic staining. The scoring system was designed based on a scale that ranged from 0 to 3+, in which 0 indicated negative (meaning that there were 0 positive cells), 1+ indicated low (meaning that there was less than 25% positive cells), 2+ indicated medium (meaning that there were 25% to 50% positive cells), and 3+ indicated high (meaning that there was more than 50% positive cells). The patient's clinical information, such as age, sex, serum thyroid auto-antibody levels, radiological findings, and treatment method data, were obtained from their medical records.

Statistical Analysis

The data analyses in this study were performed using the SPSS software program (25.0, IBM Corp., Armonk, NY). Shapiro-Wilk test was used to analyze normality assumption for numerical variables. Numbers, percentage, mean, and standard deviation or median, interquartile range (IQR), and minimum-maximum values were used to present descriptive statistics. The Mann-Whitney U test was used in comparison of tumour diameter in terms of lymphovascular invasion (LVI) and capsule invasion (CI). The Kruskal-Wallis test was used to compare tumour diameter between PD-L1 levels. The Pearson chi-square and Fisher-Freeman-Halton test was used to analyze the difference between the groups in PD-L1 staining scores.

RESULTS

The mean age of the patients was 53.6±9.01, 47.82±13.2, and 50.26±15.51 years in the NG, HT, and PTC groups, respectively. Moreover, 21 (60%) of the participants in the NG group and 25 (71.4%) of the HT and PTC groups were female. Anti-TPO (thyroid peroxidase) and anti-TG (thyroglobulin) levels of serum thyroid autoantibodies were higher than laboratory reference values in patients in the HT group. In the radiological findings of the patients who were operated in our hospital, multinodular goiter or the presence of a giant nodule in the thyroid were detected. In the neck ultrasonography examination performed on patients with malignant fine-needle aspiration biopsy (FNAB), the presence of cervical lymph nodes with pathological appearance was determined in 7 patients. It was observed that a patient who had a lateral neck dissection and a patient who had a central lymph node dissection were re-operated because of the detection of metastatic lymph nodes in the follow-up. In this study, 80% cytoplasmic and 4% membranous PD-L1 staining was detected in cases with the expression of PD-L1 level. In this study, three patients with PTC developing on HT was detected. In the histopathological evaluation, 9 (25.7%) of the patients diagnosed with PTC were defined as classic, 10 (28.6%) were defined as follicular, 6 (17.1%) were defined as oncocytic, 5 (14.3%) were defined as encapsulated, 2 (5.7%) were defined as solid, 1 (2.9%) were defined as tall cell, and 2 (5.7%) were defined as Warthin-like. The tumour diameter of 18 (51.4%) of the patients was between 1 and 2 cm, 13 (37.1%) was between 2.1 and 4 cm, and 4 (11.4%) was over 4.1 cm. When evaluating the patients with PTC within the scope of thyroid CI, 10 (28.6%) of the patients had CI, while 25 (71.4%) did not. When the PTC patients were evaluated for LVI, 7 (20%) of the patients had LVI, while 28 (80%) of the patients did not. Of the patients with lymph node metastasis (LNM), seven patients underwent central lymph node dissection (CLND) and total thyroidectomy. In addition to CLND, two other patients also underwent lateral lymph node dissection (LLND). Furthermore, postoperative radioactive iodine (RAI) treatment was given to all of these patients.

Differences in the expression of PD-L1 level were determined to be statistically significant between these groups ($p<0.001$, Table 1). When PD-L1 expression was compared between the groups with the Bonferroni method, the expression of PD-L1 level was found to be significantly higher than the NG group, both in the HT group and in the PTC group ($p<0.001$). Similarly, PD-L1 expression in the PTC group was significantly higher than in the HT group ($p<0.001$). When patients with PTC were evaluated in terms of thyroid CI with PD-L1 expression, no significant difference was found ($p=0.194$). However, when patients with PTC were assessed in terms of thyroid LVI with the expression of PD-L1 level, the difference was determined to be significant ($p=0.047$, Table 2). When the significance of LVI between the groups was compared with the Bonferroni method, it was found that the significant difference between the groups was due to patients with PTC with severe PD-L1 expression. In addition, when a comparison was conducted between the tumour diameter according to the expression of PD-L1 level, it was determined that there was no significant difference between the tumour diameter according to the Kruskal-Wallis test results ($p=0.165$). Median tumour diameter was 1.8 cm in patients without LVI, and 3.3 cm in patients with LVI. In addition, median tumour diameter was 1.5 cm in patients without CI, and 4 cm in patients with CI. A significant relationship was determined between the increase in the tumour size and the CI and LVI via the Mann-Whitney U test, ($p<0.001$ and $p=0.022$, respectively, Table 3). Notably, the PD-L1 expression and LVI were observed to be more severe in patients with follicular, tall cell, and oncocytic subtype PTC ($p=0.011$ and $p=0.026$, respectively, Table 4).

Table 1. PD-L1 expression in the NG, HT, and PTC groups

	NG (n=35)	HT (n=35)	PTC (n=35)	p
PD-L1 Staining				
Weak	34 (97.1)	18 (51.4)	5 (14.3)	
Middle	1 (2.9)	17 (48.6)	22 (62.9)	**<0.001**
Severe	0 (0.0)	0 (0.0)	8 (22.9)	

PD-L1: programmed death-ligand 1, NG: nodular goiter, HT: Hashimoto's thyroiditis, PTC: papillary thyroid cancer

Table 2. Comparison of anti-PD-L1 staining score and LVI

	LVI (-) (n=28)	LVI (+) (n=7)	p
PD-L1 Staining			
Weak	5 (17.9)	0 (0.0)	
Middle	19 (67.9)	3 (42.9)	**0.047**
Severe	4 (14.3)	4 (57.1)	

PD-L1: programmed death-ligand 1, LVI: lymphovascular invasion

Table 3. Comparison of the tumour diameter with the LVI and CI

	n	Median	IQR	Min-Max	Mean Rank	p
LVI						
Negative	28	1.8	1.65	1.2 - 4.5	16.02	**0.022**
Positive	7	3.3	3.13	2.5 - 6.0	25.93	
CI						
Negative	25	1.5	0.80	1.2 - 3.4	13.34	**<0.001**
Positive	10	4	1.58	2.5 - 6.0	29.65	

LVI: lymphovascular invasion, CI: capsule invasion, IQR: interquartile range

Table 4. Comparison of PTC subgroups in terms of anti-PD-L1 staining and LVI

	Follicular (n=10)	Classic (n=9)	Oncocytic (n=6)	Tall-cell (n=1)	Encapsulated (n=5)	Solid (n=2)	Warthin-like (n=2)	p
PD-L1 Staining								
Weak	0 (0.0)	1 (11.1)	3 (50.0)	0 (0.0)	1 (20.0)	0 (0.0)	0 (0.0)	
Middle	4 (40.0)	8 (88.9)	2 (33.3)	0 (0.0)	4 (80.0)	2 (100)	2 (100)	**0.011**
Severe	6 (60.0)	0 (0.0)	1 (16.7)	1 (100)	0 (0.0)	0 (0.0)	0 (0.0)	
LVI								
Negative	5 (50.0)	9 (100)	5 (83.3)	0 (0.0)	5 (100)	2 (100)	2 (100)	**0.026**
Positive	5 (50.0)	0 (0.0)	1 (16.7)	1 (100)	0 (0.0)	0 (0.0)	0 (0.0)	

PTC: papillary thyroid cancer, PD-L1: programmed death-ligand 1, LVI: lymphovascular invasion,

DISCUSSION

It was reported that 77% of the patients who have total thyroidectomy and thyroid lobectomy due to PTC are female (15). The rate of female patients in the current sample was 72% and was compatible with the literature. In similar research, of the patients who had PTC, 70.5% did not have cervical LNM, and 74.9% did not have lateral LNM (16). In the current study, 71.4% (n=25) of the patients did not have CI, and 80% (n=28) did not have LVI. Approximately less than 1% of patients with PTC make up these variants. PTC subtypes, including tall-cell, hobnail/micropapillary, columnar cell, diffuse sclerosing and solid variants. These variants of these tumours have a worse prognosis when compared to other types of PTC (17). In the current study, the PD-L1 expression was more severe in tall cell, oncocytic, and follicular type PTCs.

It is recommended to perform bilateral total thyroidectomy in patients with PTC and/or cervical and/or LLND and total thyroidectomy in patients with LNM (18). In addition to performing total thyroidectomy, 5 of the patients also underwent CLND and 2 of the patients also underwent CLND in addition to LLND. RAI for postoperative residual metastatic tissue in patients with PTC will reduce the risk of experiencing a recurrence. However, in relapse cases, the mortality rate is as high as 33-50% (19). In this study, postoperative RAI treatment was applied to all of the patients who had LNM.

HT is a T-cell-mediated autoimmune disease that affects the thyroid. HT produces autoreactive CD4 + T-cells, CD8 + cytotoxic T-cells and immunoglobulin G (IgG) autoantibodies. The production of large amounts of IL-1β in the thyroid glands in HT plays a role in its progression by causing massive thyrocyte apoptosis. Immune-mediated apoptosis of tyrosides is guided by CD8 + cells. The receptors trigger the lymphocyte ligands on the target cells, released as soluble factors, and transmitted to the target cells. Although apoptosis is rarely seen in normal thyroid tissue, it increases significantly during HT (20). HT is a commonly seen form of chronic autoimmune thyroid disease, and the pathogenesis and prognostic effect of coexistence with PTC is a controversial condition. Some studies have reported an association between PTC and HT, which has been associated with a good prognosis, low relapse rate, and less aggressive disease (21,22). However, some other researchers have observed that the coexistence of HT does not have such a protective effect on the results of a patient with PTC (23,24). A recent meta-analysis of patients with PTC who had HT reported that female gender, tumour multifocality, but extrathyroidal extension, LNM were less common and associated with high relapse-free survival rates (25). Our main limitation is the low number of cases with papillary carcinoma that developed based on Hashimoto's thyroiditis in our study.

Also reported was that the expression of PD-L1 increased in patients with PTC developing on a prediagnosis of HT. The expression of PD-L1 was observed to increase when combined with LNM in these patients (26). In the current study, the expression of PD-L1 was very weak or completely absent in patients with NG. However, the expression of PD-L1 was observed to be higher in patients who had HT than in the NG patients. Additionally, the PD-L1 expression was determined to have increased in patients with PTC compared to the other groups of patients. Although the increased mechanism of PD-L1 expression is not fully understood, it has been reported that cytokines released in the presence of inflammation induce PD-L1 expression (27). Oncocytic changes in the follicular epithelium in HT have been reported to have an association with an increase in the expression of PD-L1. In addition, PD-L1 expression was shown to increase in the presence of lymph node metastasis in the patients who had PTC coexisting with HT (26). In the current study, HT was found as a prediagnosis in 3 patients with PTC. One of these patients had LNM in addition to a follicular variant of PTC, and the expression of the PD-L1 score was 3+. The other two patients had no LNM, and the expression of PD-L1 score was 2+.

Increased expression of PD-L1 in tumour cells in PTC was reported to show membranous staining in 6.1% of patients (13,26). In the current study, the expression of PD-L1 was slightly lower than that in previous studies, and 4% membranous PD-L1 staining was detected. In studies using cytoplasmic staining to identify PD-L1 positivity, it was reported that PD-L1 expression was seen at a rate of 66.5-82.3% (28,29). In the current study, the cytoplasmic PD-L1 expression was 80%.

Previous studies have shown that as tumour diameter increases, CI and LVI increase (30). We obtained similar results in our research. However, it was observed that PD-L1 expression did not increase significantly with the increase in tumor diameter. On the contrary, our study reveals that PD-L1 expression is higher in PTC variants (even if it is a smaller size in diameter than classical PTC) and tumours that cause LVI. Our study is valuable because it shows that anti-PD-L1 treatment will be beneficial in patients with metastatic lymph nodes despite neck dissection surgery and RAI treatment, especially in patients at risk for reoperation. PD-L1 has an essential role in determining how the PTC will be aggressive so that patients' prognosis can be predictable. Therefore, it is recommended that the suppression of PD-L1 can be used when treating PTC with a high PD-L1 expression (31).

Immunotherapy is a promising method that can be used to treat patients who have aggressive differential TC and ATC (32). PTCs occurring against a prediagnosis of HT may be resistant to standard treatment. The decision to use immunotherapy should be considered when treating metastatic PTCs. However, it should be remembered that destructive thyroiditis may occur after triggering thyroiditis, following the administration of cancer immunotherapy, monoclonal antibodies, or cancer vaccines (33,34).

CONCLUSION

In conclusion, it was found that the expression of PD-L1 increased in the patients who had PTC. The expression of PD-L1 was observed to be higher in the patients who had PTC than in the patients who had HT. No significant difference was observed between the expression of PD-L1 and the tumour diameter and CI. However, a meaningful relationship was determined between the LVI and the expression of PD-L1. The PD-L1 expression and LVI were more severe in patients with follicular, tall cell, and oncocytic subtype PTC. It is necessary to conduct more studies to investigate the effect of PD-L1 expression on patient prognosis with PTC and its impact on patients who have PTC that develops with a prediagnosis of HT.

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Clinical Results after Surgical Treatment of Spindle Cell Lipomas

İğsi Hücreli Lipomların Cerrahi Tedavisi Sonrası Klinik Sonuçlar

Tulay ZENGİNKİNET[1]
0000-0003-3056-2074
Erhan OKAY[2]
0000-0003-2443-2505
Aykut ÇELİK[2]
0000-0002-5910-0829
Ayşe Nur TOKSÖZ YILDIRIM[1]
0000-0003-1708-0003
Muhammed Beşir ÖZTÜRK[3]
0000-0002-8977-3741

[1]Department of Medical Pathology, Göztepe Prof. Dr. Süleyman Yalçın City Hospital, İstanbul, Turkey

[2]Department of Orthopedics and Traumatology, Göztepe Prof. Dr. Süleyman Yalçın City Hospital, İstanbul, Turkey

[3]Department of Plastic, Reconstructive and Aesthetic Surgery, Göztepe Prof. Dr. Süleyman Yalçın City Hospital, İstanbul, Turkey

Corresponding Author
Sorumlu Yazar
Erhan OKAY
erhanokay@yahoo.com

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ABSTRACT

Aim: Spindle cell lipoma is a rare tumor. Although located subcutaneously, they are localized in the posterior neck, shoulder, and lumbar regions. It can radiologically mimic liposarcoma. It is important to differentiate spindle cell lipoma from atypical lipomatous tumors. In this study, we aimed to present our studies on our patients with spindle cell lipoma with histopathological and clinical findings and give brief information about the differential diagnosis of this rare lipomatous lesion.

Material and Methods: A total of 29 patients (27 male, 2 female) who underwent surgical treatment for spindle cell lipoma between January 2015 and December 2020 were included in this retrospective study. All patients were presented with mass lesions, and preoperative biopsy was performed in cases with low-fat content mass lesions.

Results: The mean age of the patients was 57.0±14.6 (range, 25-79) years and the mean follow-up period was 25.0±8.6 (range, 6-47) months. No additional disease was detected in the patients. Marginal resection was preferred in 5 (17.2%) patients with lesions close to neurovascular structures. Wide resection was performed in the other 24 (82.8%) patients. CD34 was positive in all lesions. In one patient, CDK4 was positive, and the diagnosis was made as atypical spindle cell lipoma. At the last follow-up, there was no complication and recurrence in any of the cases.

Conclusion: Spindle cell lipomas should be regarded in the differential diagnosis of lipomatous tumors. Local excision with negative margins may prevent a recurrence. Correct histopathologic differentiation from liposarcoma is of paramount importance for appropriate treatment.

Keywords: Spindle; lipoma; upper back; shoulder; neck.

ÖZ

Amaç: İğsi hücreli lipom nadir görülen bir tümördür. Cilt altı yerleşimli olmakla birlikte arka boyun, omuz ve bel bölgesinde de yerleşim göstermektedirler. Radyolojik olarak liposarkomu taklit edebilmektedir. İğsi hücreli lipomanın atipik lipomatöz tümörlerden ayırımı önemlidir. Bu çalışmada, histopatolojik ve klinik bulguları olan iğsi hücreli lipomlu hastalarımıza ilişkin çalışmalarımızı sunmayı ve bu nadir gözlenen lipomatöz lezyonun ayırıcı tanıları hakkında kısaca bilgi vermeyi amaçladık.

Gereç ve Yöntemler: Bu geriye dönük çalışmaya Ocak 2015 ve Aralık 2020 tarihleri arasında iğsi hücreli lipom nedeniyle cerrahi tedavi uygulanmış olan toplam 29 hasta (27 erkek, 2 kadın) dahil edildi. Tüm hastalar kitle lezyonu ile başvurdular ve düşük yağ içerikli kitle lezyonu olan olgularda ameliyat öncesi biyopsi uygulandı.

Bulgular: Hastaların ortalama yaşı 57,0±14,6 (aralık, 25-79) yıl ve ortalama takip süresi ise 25,0±8,6 (aralık, 6-47) ay idi. Hastalarda başka herhangi bir ek hastalık saptanmadı. Nörovasküler yapılara yakın lezyonu olan 5 (%17,2) hastada marjinal rezeksiyon tercih edildi. Diğer 24 (%82,8) hastaya ise geniş rezeksiyon uygulandı. CD34 tüm lezyonlarda pozitifti. Bir hastada CDK4 pozitifti ve bu hastaya atipik iğsi hücreli lipom tanısı konuldu. Son kontrollerinde hiçbir vakada komplikasyon veya nüks saptanmadı.

Sonuç: Lipomatöz tümörlerin ayırıcı tanısında iğsi hücreli lipomlar düşünülmelidir. Negatif sınırlar ile lokal eksizyon nüksü önleyebilir. Uygun tedavi için liposarkomdan doğru histopatolojik ayrım oldukça önemlidir.

Anahtar kelimeler: İğsi; lipom; sırt; omuz; ense.

INTRODUCTION

Lipomatous tumors are common lesions of mesenchymal origin. They affect pediatric and adult patients and exhibit a broad spectrum of clinical behavior from benign to malignant. Its prevalence is 2.1 per 1000 people. Most lipomas are located at the subcutaneous tissue and present as a small mass, usually less than 2-3 cm. They can sometimes grow to a larger size and may cause diagnostic challenges. For a lipoma to be referred to as a giant, it should be at least 10 cm in the axial or coronal dimension or weigh a minimum of 1000 g. These types of lipomas can cause compression to adjacent anatomic structures (1,2).

Spindle cell lipoma, a rare benign lipomatous neoplasm, is a subtype of lipoma composed of mature adipose tissue, ropey collagen, and bland spindle cells (3-5). They usually present as a subcutaneous mass and predilection to localize at the posterior neck, shoulder, and back region. However, sometimes, they can originate from deeper soft tissues and other anatomic localizations. It is important to distinguish spindle cell lipomas from atypical lipomatous tumors, as recurrence rate and dedifferentiation with metastatic potential may occur in atypical lipomatous tumors.

In this study, we aimed to present our patient cohort with spindle cell lipoma with histopathologic and clinical findings and give brief information about the differential diagnoses of this rare entity.

MATERIAL AND METHODS

Twenty nine cases (27 male, 2 female) with spindle cell lipoma were presented to our clinic between January 2015 and December 2020. This study was approved by the clinical research ethics committee of the Istanbul Medeniyet University, Göztepe Training and Research Hospital date: 16.06.2021, number: 0321.

Inclusion criteria included patients with histopathologic diagnosis of spindle cell lipoma in our pathology institution with complete data. Exclusion criteria included patients who underwent biopsy or resection outside of our institution.

Wide or marginal resection was made for all patients. In wide resection, the lesion was resected, leaving 1 cm of margin with the biopsy tract left with the resection specimen if present. Marginal resection was preferred for lesions with proximity to neurovascular structures. Drain was used postoperatively to avoid hematoma.

Statistical Analysis

Descriptive statistics were given as mean, standard deviation and minimum-maximum values for numerical variables. Categorical variables were summarized as numbers and percentages.

RESULTS

A total of 29 patients with spindle cell lipoma were included in the study. Twenty seven (93.1%) of the patients were male and the mean age of the patients' was 57.0±14.6 (range, 25-79) years. The mean follow-up time was 25.0±8.6 (range, 6-47) months.

Anatomic localization was the temporal area in 1 (3.5%) patient, sub-mandibular area in 1 (3.5%) patient, forehead in 3 (10.3%) patients, thigh in 3 (10.3%) patients, neck in 13 (44.8%) patients, shoulder in 1 (3.5%) patient, upper back in 2 (6.9%) patients, hand in 3 (10.3%) patients, arm in 1 (3.5%) patient, and pelvis in 1 (3.5%) patient.

Wide resection was performed in 24 (82.8) patients, and marginal resection was preferred in 5 (17.2%) lesions with close proximity to the neurovascular structures. Otherwise, wide resection was performed.

No recurrence was detected during the patients' follow-up.

Histopathologically, the diagnosis was confirmed with two pathologists experienced in orthopedic oncology cases. Preoperative biopsy was performed for 8 cases with low-fat components detected with magnetic resonance imaging (MRI) to confirm the diagnoses (Figure 1). Wide or marginal resection was performed in all patients. CD34 was positive in all specimens (Figure 2 and 3). CDK4 was positive in one patient (case 2), and the diagnosis was made as atypical spindle cell lipoma.

All the patient's data, including their demographic characteristics and tumoral lesions were given in Table 1 and Table 2 below.

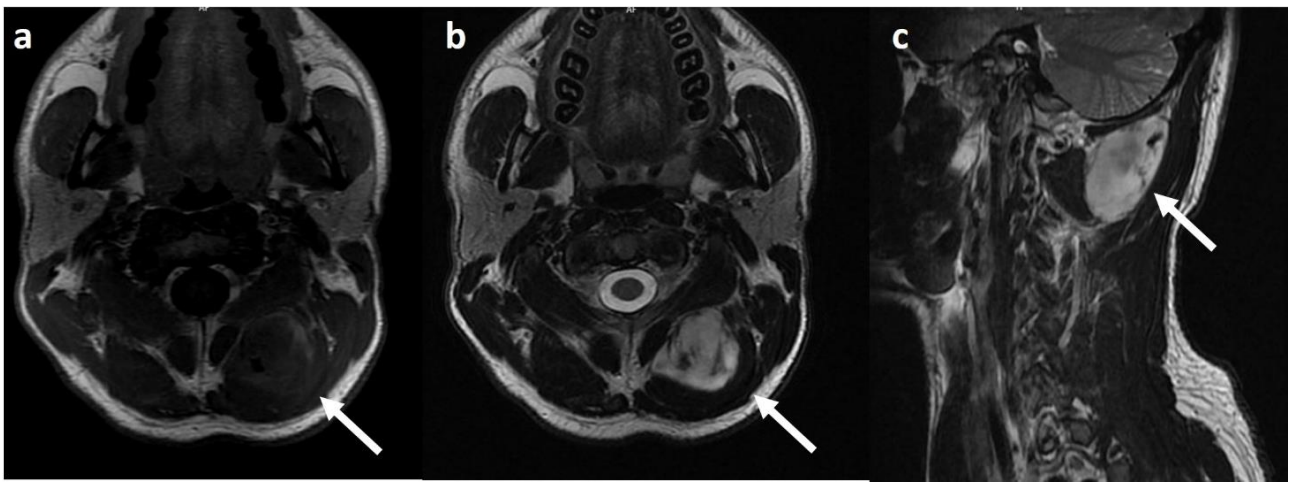

Figure 1. Magnetic resonance imaging view of spindle cell lipoma. T1 axial (a), T2 axial (b), and sagittal view (c) demonstrated well-defined calcific lesions (white arrow)

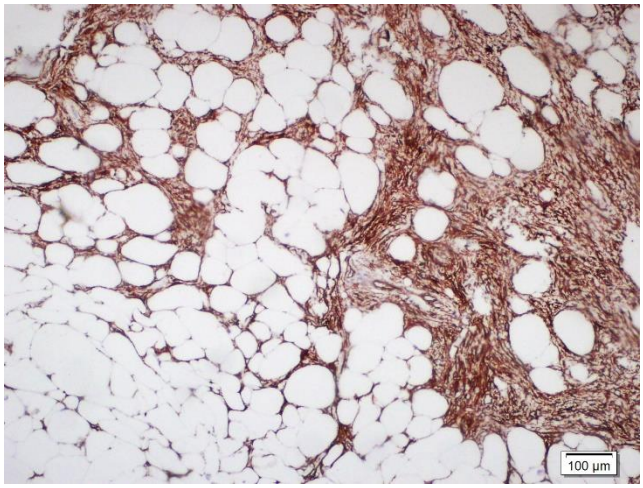

Figure 2. Immunopositivity for CD34 is a consistent feature of the spindle cell component (immunohistochemistry x200)

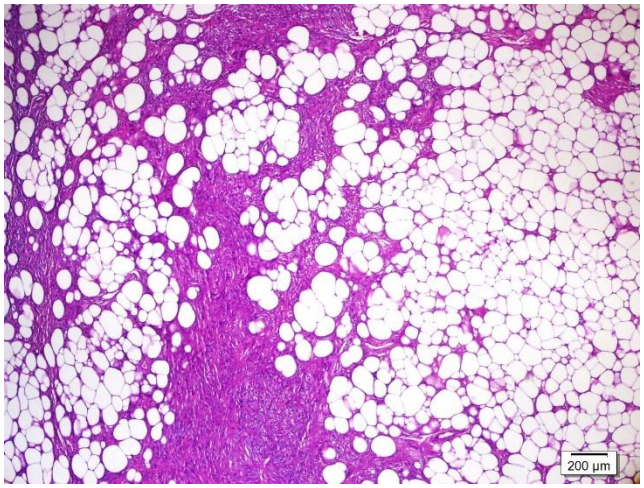

Figure 3. Spindle cells intermixed with mature adipose tissue within a collagenous stroma (Hematoxylin and eosin x100)

Table 1. Clinical data of all patients

Patient	Gender	Age	Follow-up (months)	Localization	Size (cm)
1	Male	71	24	Neck	11x8x6 cm
2	Male	55	25	Thigh	4x3.5x10.5 cm
3	Male	37	20	Forehead	0.8x0.5x0.3 cm
4	Male	61	20	Upper back	4.5x3.5x3 cm
5	Male	73	27	Shoulder	7x7x3 cm
6	Male	56	29	Neck	5.5x4x2 cm
7	Male	71	47	Forehead	1.1x0.6x0.3 cm
8	Male	34	15	Neck	1.5x1.3x0.5 cm
9	Female	44	13	Hand	4.6x2.3x2.4 cm
10	Male	63	23	Upper back	12x8.5x3 cm
11	Male	62	28	Neck	2.5x1.5x2 cm
12	Male	50	6	Hand	2x0.5x0.2 cm
13	Male	25	25	Forehead	1.7x1.3x0.5 cm
14	Male	61	20	Neck	12x10x4 cm
15	Male	57	31	Neck	3.5x2.5x1.5 cm
16	Male	61	30	Neck	3.5x3x2 cm
17	Male	37	34	Temporal area	3.5x2.5x1.2 cm
18	Male	56	39	Arm	8x8x2cm
19	Male	71	39	Thigh	16x10x2 cm
20	Male	57	31	Neck	5x5x1.5 cm
21	Male	67	24	Pelvis	11x9x2.5 cm
22	Male	69	20	Submandibular	3.5x3x1 cm
23	Male	78	21	Neck	4.5x4x2 cm
24	Male	66	32	Neck	5x4x2.5 cm
25	Male	26	19	Neck	4.5x4.5x2 cm
26	Male	79	23	Neck	4.3x3x2 cm
27	Male	58	26	Thigh	2.5x1.5x1.5 cm
28	Male	65	21	Neck	3x3x2 cm
29	Female	44	13	Hand	4.6x2.3x2.4 cm

DISCUSSION

Enzinger and Harvey first coined the term “spindle cell lipoma” in 1975 (6). It is a benign lesion in which spindle cells forms collagen, replacing the mature fat tissue. As they are benign lipomatous lesions, they have never been reported to metastasize. These tumors tend to grow slowly, usually solitary and painless. It is more prevalent in males aged 45 to 65 and are usually treated by local excision. Subcutaneous tissues of the posterior neck, shoulder or back are commonly localization of spindle cell lipoma. However, the scalp, eyelids, forehead, face, mouth, lower

Table 2. Patient demographics and clinical progression

Gender, n (%)	
Male	27(93.1)
Female	2 (6.9)
Age (years), mean±SD	57.0±14.6 (25-79)
Follow-up (months), mean±SD	25.0±8.6 (6-47)
Localization, n (%)	
Neck	13 (44.8)
Forehead	3 (10.3)
Thigh	3 (10.3)
Shoulder	1 (3.5)
Upper back	2 (6.9)
Hand	3 (10.3)
Pelvis	1 (3.5)
Submandibular	1 (3.5)
Temporal	1 (3.5)
Arm	1 (3.5)
Surgical treatment, n (%)	
Wide resection	24 (82.8)
Marginal resection	5 (17.2)

SD: standard deviation

jaw, kidney, vulva, and upper and lower extremities are less commonly involved.

In 11 (%37.9) cases of our patient cohort, the lesion was located outside the typical localization. This frequency was similar to Ud Din et al. (7), which reported 57/218 atypical lesions.

Chen et al. (3) reported 40 cases with spindle cell lipoma. There were different morphologies that were difficult to distinguish from atypical lipomas. They observed no recurrence or metastasis at the mean of 2-105 months of follow-up. Jelinek et al. (8) reviewed 27 spindle cell lipomas sized between 2 cm to 10 cm. He emphasized that the imaging features might differ with minimal fat at MRI or CT compared to conventional lipoma. Imaging might not always be adequate to distinguish these lesions from high-grade sarcoma.

We only had one patient with the diagnosis of atypical spindle cell lipoma in our patient cohort. In microscopy, mild to moderately atypical spindle cells, adipocytes, lipoblasts, pleomorphic cells, multinucleated giant cells within a myxoid, and collagenous matrix are classically observed. It has no predilection for dedifferentiation and metastasis. It is usually encountered in middle-aged males. Weak or focal expression of MDM2 or CDK4 immunoreactivity may be seen as our case displayed CDK4 positivity (9).

Creytens et al. (2) demonstrated a link between atypical spindle cell lipoma and classical spindle cell lipoma. An immunohistochemical and molecular investigation is important for differential diagnosis. Microscopically, the matrix includes collagen fibrils in spindle cell lipomas. In this matrix, mature fat is filled by fibroblast-like spindle cells. Although most spindle cell lipomas are composed of a relatively equal ratio of fat and spindle cells, either component may predominate. Most tumors are composed of spindle cells, string-like collagen fibers and mature adipocytes, surrounded by a fibrous capsule. Scattered mast cells are usually encountered. Spindle cells are often bland, without apparent atypia, pleomorphism, and nuclear mitoses, and are usually arranged in bundles between the collagen fibers.

There are histopathologically different subtypes of spindle cell lipoma: classic form, fibroblastic type, myxoid subtype, Pseudoangiomotus subtype, fat rich type, and low-fat subtype. The cut surface of resected tumoral lesion may be yellow, yellowish-white or greyish-white due to the different fat and collagen ratios. In differential diagnoses, elastofibroma dorsi, solitary fibrous tumors, atypical lipomatous tumors, fibrosarcoma-like lipomatous tumors, mammary-type myofibroblastoma, and spindle cell liposarcoma should be especially taken into consideration. Myxoid subgroups of spindle cell lipoma may resemble myxoid liposarcoma. CDK4 and/or MDM2 immunohistochemical analysis was performed in 12 patients in our patient cohort for differential diagnosis of spindle cell lipoma and atypical lipomatous tumors.

Seo et al. (10) presented a 24-year-old male with spindle cell lipoma in the right supraclavicular area. It was supposed to be a lymphoma variant due to an enlarged lymph node; however, the diagnosis was spindle cell lipoma. At the 6 month follow-up, there was no recurrence. They underlined that in this location, spindle cell lipoma is a possible diagnosis. Mizoshiri et al. (1) reported a 58-year-old Japanese male with a soft tissue mass in his left deltoid muscle. They noted that there had been five other intramuscular spindle cell lipoma cases reported so far in this location.

Fibrosarcoma-like lipomatous neoplasm displays marked cellularity comparable to spindle cell lipoma. They also have lipoblasts with a characteristic lipoblast of an ice cream cone morphology. Deyrup et al. (11) identified fibrosarcoma like lipomatous neoplasm in 12 of 26 suspected cases. They stated that fibrosarcoma-like lipomatous neoplasm must be distinguished from well-differentiated liposarcoma and myxoid LPS using histologic and molecular features.

There were 6 patients with more than 10 cm tumoral mass, referred to as giant cell lipomas in our cohort. The tumors can be especially mistaken for liposarcomas. Giant lipomas may also cause a diagnostic challenge and be confused with atypical lipomatous tumors or other liposarcomas. A definitive diagnosis of giant cell lipoma can only be made by histopathological examination. Although very rare, they may also be transformed to liposarcoma (12).

Radiologically, in spindle cell lipomas, the rate of nonlipogenic area was larger than lipogenic areas accompanied by a mixture of mature adipocytes and undifferentiated spindle cells, which is contrary to lipomas as stated by Mizoshiri et al. (1), who described an intramuscular deltoid spindle cell lipoma which reflects these distinct radiological features.

Spindle cell liposarcoma is regarded as another entity. There is hypercellularity, clustered growth, and mild to moderate cytologic atypia, none of which are detected in spindle cell lipoma. Therefore, consultation with a musculoskeletal pathologist is of utmost importance for the general pathologists when in doubt.

CONCLUSION

Overall, mature adipose tissue, ropey collagen, and bland spindle cells (conventional pattern) are common pathologic features of spindle cell lipoma. Classical localization in adult male may not always be present,

making the diagnosis difficult. Spindle cell lipoma may sometimes resemble benign and malignant soft tissue tumors; therefore, pathologists, radiologists, orthopedic, and plastic surgeons should be cautious in differential diagnoses and treatment of this rare entity.

Ethics Committee Approval: The study was approved by the Ethics Committee of İstanbul Medeniyet University Göztepe Training and Research Hospital (16.06.2021, 321).

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Evaluation of Serum S100B Levels in Male Children Younger than 6 Years Old with Autism Spectrum Disorder: A Psychiatric and Biochemical Perspective

Otizm Spektrum Bozukluğu Olan 6 Yaşından Küçük Erkekler Çocuklarda Serum S100B Düzeylerinin Değerlendirilmesi: Psikiyatrik ve Biyokimyasal Perspektif

Ayşe Nihal ERASLAN[1]
0000-0002-7655-7927
İbrahim DURUKAN[2]
0000-0002-1844-6911
Şahin BODUR[2]
0000-0002-4942-7932
Özge DEMİRCAN TULACI[3]
0000-0002-7174-2154

[1]Department of Child and Adolescent Psychiatry, Ankara Training and Research Hospital, Ankara, Turkey
[2]Department of Child and Adolescent Psychiatry, Gülhane Training and Research Hospital, Ankara, Turkey
[3]Department of Child and Adolescent Psychiatry, Balıkesir Atatürk City Hospital, Balıkesir, Turkey

Corresponding Author
Sorumlu Yazar
Ayşe Nihal ERASLAN
aysenihaleraslan@gmail.com

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ABSTRACT

Aim: Autism spectrum disorder is a neurodevelopmental disorder. The S100 calcium binding protein B (S100B) is among the markers of astrocyte activation as well as brain damage. Herein, it was aimed to evaluate S100B levels to determine whether there is a relation with the severity of autism spectrum disorder and establish possible causes of different results among the studies in the literature from a psychiatric and biochemical perspective.
Material and Methods: Twenty-five male children with autism spectrum disorder were included as the study group along with twenty-seven male children as the control group. The childhood autism rating scale and the autism behavior checklist were applied. Serum S100B protein levels were measured by enzyme-linked immunosorbent assay (ELISA).
Results: The mean serum S100B level was 1008.61±171.34 pg/mL in the study group and 1060.14±182.83 pg/mL in the control group, and no statistically significant difference was found between the groups (p=0.300). Based on the childhood autism rating scale scores, 60% (n=15) of the children with autism spectrum disorder had severe autism, whereas 40% (n=10) had mild-to-moderate autism. There was no significant difference in terms of the serum S100B levels between the groups of autism spectrum disorder severity (p=0.935) or according to the autistic regression status (p=0.667).
Conclusion: For S100B to be accepted as a reliable biomarker for autism spectrum disorder, more studies considering some factors with larger samples should be performed. Moreover, to understand the effect of biochemical methodology on the results, further studies are suggested on this subject.
Keywords: Autism spectrum disorder; autism; S100B; neuroglial cells.

ÖZ

Amaç: Otizm spektrum bozukluğu nörogelişimsel bir bozukluktur. S100 kalsiyum bağlayıcı protein B (S100B), beyin hasarının yanı sıra astrosit aktivasyonunun da belirteçleri arasındadır. Bu çalışmada, otizm spektrum bozukluğunun şiddeti ile ilişkisi olup olmadığını belirlemek için S100B düzeylerinin değerlendirilmesi ve literatürdeki çalışmalar arasında farklı sonuçların olası nedenlerinin psikiyatrik ve biyokimyasal açıdan değerlendirilmesi amaçlanmıştır.
Gereç ve Yöntemler: Çalışma grubu olarak yirmi beş otizm spektrum bozukluğu olan erkek çocuk ve kontrol grubu olarak ise yirmi yedi erkek çocuk çalışmaya dahil edildi. Çocukluk otizm derecelendirme ölçeği ve otizm davranış kontrol listesi uygulandı. Serum S100B protein seviyeleri, enzyme-linked immunosorbent assay (ELISA) ile ölçüldü.
Bulgular: Ortalama S100B serum düzeyleri çalışma grubunda 1008,61±171,34 pg/mL ve kontrol grubunda 1060,14±182,83 pg/mL idi ve gruplar arasında istatistiksel olarak anlamlı bir fark yoktu (p=0,300). Çocukluk otizm derecelendirme ölçeği puanlarına göre, otizm spektrum bozukluğu tanılı çocukların %60'ı (n=15) şiddetli otizme sahipken, %40'ı (n=10) hafif-orta otizme sahipti. S100B serum seviyeleri bakımından, otizm spektrum bozukluğu şiddet grupları arasında (p=0,935) veya otistik regresyon durumuna göre (p=0,667) anlamlı bir fark yoktu.
Sonuç: S100B'nin otizm spektrum bozukluğu için güvenilir bir biyobelirteç olarak kabul edilebilmesi için daha geniş örneklem olan ve bazı faktörleri de dikkate alan daha fazla çalışma yapılmalıdır. Ayrıca biyokimyasal metodolojinin sonuçlara etkisini anlamak için bu konuda daha fazla çalışma yapılması da önerilmektedir.
Anahtar kelimeler: Otizm spektrum bozukluğu; otizm; S100B; nöroglial hücreler.

Presented as a poster at 8th International Congress on Psychopharmacology (April 20-24, 2016; Antalya)

INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by marked delays and deviation in the area of social communication and social interaction and restricted and repetitive patterns of behavior, interests, or activities starting in early childhood (1). ASD is known as a multifactorial disorder comprising the interactions of neurological, immunological, environmental, and genetic factors (2). Identifying a biomarker for ASD has been stated to be important as it will be of diagnostic and prognostic value, reflecting the underlying situation or disease (2).

S100 calcium binding protein B (S100B) is most abundant in the glial cells of the central nervous system (CNS), predominantly in astrocytes, indicating astrocyte activation. The intracellular role of S100B includes participation in cell proliferation, differentiation, transcription, survival, and enzyme activities, whereas its extracellular role is exerted mostly through receptors for advanced glycation end-products (RAGE) and triggering intracellular signaling cascade, leading to processes such as neuroinflammation and neurodegeneration (3-5). It has been confirmed that the action of S100B is either neurotrophic/neuroprotective or neurotoxic/apoptotic depending on the concentration (3,6). In neural cultures neurotrophic effects have been demonstrated at nanomolar levels and apoptotic effects at micromolar levels (5). In the study by Kleindienst et al. (3) it was found that the astrocytic neurotrophic protein S100B is a potential candidate to increase progenitor cell proliferation following traumatic brain injury and it appears to enhance hippocampal network repair and improve cognitive recovery. Increased levels of S100B may be prior to a significant change in neurological function or neuronal cell death (7).

S100B may contribute to developmental pathogenesis in psychiatric diseases (2). Alterations in S100B levels in patients with schizophrenia and mood disorders have been reported (4). Studies investigating the relation between S100B values and ASD in children revealed intriguing results. Elevated serum S100B levels in children with ASD have been reported by Al-Ayadhi and Mostafa (7), Ayaydın et al. (8), and Golubova et al. (9), whereas elevated plasma S100B levels in children with ASD have been shown by Guloksuz et al. (10) and Tomova et al. (5). On the other hand, Esnafoglu et al. (2) reported that there was no difference in serum S100B between children with ASD and a healthy control group.

In the present study we aimed to determine whether serum S100B concentrations in ASD patients are elevated and differ in terms of clinical severity in a more homogeneous study group in terms of age range and sex. For this purpose, serum levels of S100B were measured in order to contribute to the elucidation of the etiopathogenesis of ASD. After the analysis in addition to the mentioned aim, the possible causes of different results among studies were examined from both a psychiatric and a biochemical perspective.

MATERIAL AND METHODS

Study Design

This was a two-centered cross-sectional and descriptive study that was performed at Gülhane Medical School Child and Adolescent Psychiatry Department, Dr. Sami Ulus Gynecology Obstetrics and Child Health and Diseases Training and Research Hospital Child and Adolescent Psychiatry Department, and Dr. Sami Ulus Gynecology Obstetrics and Child Health and Diseases Training and Research Hospital Pediatric Department between January 2015 and June 2015.

It was determined to include 23 individuals as appropriate for both the control and the study group when taking the α=0.05, β=0.20, the standard deviation as 43, and the difference of mean values of S100B between groups as 36 based on the study by Al-Ayadhi and Mostafa (7).

Twenty-five male with ASD were included as the study group and 27 male as the control group. All male were under 6 years old. ASD was diagnosed by two different child and adolescent psychiatrists according to DSM-5 (Diagnostic and Statistical Manual of Mental Disorders-5) criteria. The controls were recruited from among children that were presented to the general pediatrics outpatient clinic for minor physical problems.

Having a diagnosed neurological illness (such as epilepsy), chronic systemic disease (such as chronic renal failure, chronic heart disease, or autoimmune disorders), or comorbid psychiatric disorder (except intellectual disability for the ASD study group) and using any drugs in the previous month were the exclusion criteria. Based on the history obtained from the parents, none of the controls had a history of delayed development.

The study was approved by Keçiören Training and Research Hospital Ethics Committee (number 24.12.2014/B.10.4.ISM.4.06.68.49).

All participants' rights were protected. The parents or legal guardian of each participant were informed about the study and then written consent was obtained from them before the procedures in accordance with the Declaration of Helsinki.

Assessment Measures

A sociodemographic data form was used to obtain sociodemographic characteristics including age, sex, history of autistic regression, pregnancy and birth history, family history, and medical history.

Childhood Autism Rating Scale (CARS): The childhood autism rating scale (CARS) comprises 15 items to define the severity of autism. Each item is rated on a scale from 1 to 4 with a half-degree score. A total score of 30 to 36.5 indicates mild-to-moderate autism, while 37 to 60 shows severe autism. The validity and reliability of the Turkish version of CARS were confirmed previously (11-13).

Autism Behavior Checklist (ABC): The autism behavior checklist (ABC) comprises 57 items to determine the ASD symptoms via five sub-scales: Sensory, Relating, Body and Object Use, Language, and Social Self-Help skills. The sum of these scores constitutes the total ABC score and this can be between 0 and 159 points with a cut-off point of 39. The validity and reliability of the Turkish version of the ABC were confirmed previously (14,15).

The ABC and the CARS were used only in the study group. The ABC was filled out by parents with guidance from a researcher. The CARS was applied by the same researcher. The CARS and the ABC were applied on the same day that the serum sample was taken.

Measurement of Serum S100B

A blood sample was collected from all participants by venipuncture in a vacutainer tube without anticoagulant. Blood samples were taken at any time of the day. The samples were allowed to clot for about 30 minutes at room temperature and then were centrifuged. Following centrifugation, the serum part was separated and aliquoted in an Eppendorf tube and stored at -80 °C until analysis. All serum samples were analyzed on the same day with the same laboratory batch and by the same analyst. A YH-Biosearch Human S100B enzyme-linked immunosorbent assay (ELISA) kit was used with a sensitivity 5.03 ng/L to measure S100B levels according to the manufacturer's instructions. The lower detection limit is 10 ng/L and the upper is 4000 ng/L in this kit. The coefficients of variation were <10% and <12% for intra-assay and inter-assay variability, respectively. S100B levels were expressed as pg/ml.

Statistical Analysis

The statistical package for the social sciences (SPSS) version 22.0 (NY: IBM Corp., 2012) was used. Descriptive statistics were presented as mean and standard deviation, median, interquartile range (IQR), minimum-maximum, frequency distribution, and percentage. The normality of the distribution of numeric variables was tested with the Shapiro-Wilk test. The homogeneity of variances was tested using the Levene test. For variables with a normal distribution the independent samples t test was used for comparisons, whereas the Mann-Whitney U test was used for variables that did not conform to the normal distribution. Categorical variables were analyzed using Pearson's chi-square test, Fisher's exact test, and Yates' continuity correction. Spearman's correlation analysis was carried out to determine correlations and p values <0.05 were regarded as statistically significant.

RESULTS

A total of 52 male (25 with ASD, 27 controls) were included. Their sociodemographic characteristics are presented in Table 1.

The birth/pregnancy histories of the groups were similar. Descriptive statistics of their prenatal, natal, and postnatal characteristics are presented in Table 2.

Mean serum S100B levels were 1008.61±171.34 pg/ml in the ASD group and 1060.14±182.83 pg/ml in the control group. There was no statistically significant difference between the two groups in terms of serum S100B levels (p=0.300).

Table 1. Sociodemographic characteristics of the ASD and control groups

	ASD (n=25)	Control (n=27)	p
Age (months), mean±SD	48.6±10.5	44.6±15.3	0.272[a]
Fathers' age[*] (years), median (IQR) [min-max]	30 (9) [23-50]	30 (7.5) [18-43]	0.317[b]
Mothers' age[*] (years), median (IQR) [min-max]	25 (10) [17-39]	26 (7) [18-43]	0.514[b]
Consanguineous marriage, n (%)	3 (12.0)	5 (18.5)	0.705[c]
Psychiatric disorder history of family, n (%)	3 (12.0)	4 (14.8)	0.998[c]
Sibling, n (%)	23 (92.0)	20 (74.1)	0.143[c]
Psychiatric disorder history of sibling (n=43)[**], n (%)	1[#] (4.3)	1[#] (5.0)	0.999[c]
Birth order, n (%)			
First	6 (24.0)	13 (48.2)	
Second	15 (60.0)	10 (37.0)	0.173[d]
Third	4 (16.0)	4 (14.8)	

ASD: autism spectrum disorder, SD: standard deviation, IQR: interquartile range, [*]: age at when the child was born, [**]: there were 43 (23 vs 20) children with at least one sibling in groups, [#]: in both groups, the sibling has attention deficit hyperactivity disorder, [a]: independent samples t test, [b]: Mann-Whitney U test, [c]: Fisher's exact test, [d]: Pearson chi-square test

Table 2. Prenatal, natal, and postnatal characteristics of the ASD and control groups

	ASD (n=25)	Control (n=27)	p
Prenatal disease history, n (%)	6 (24.0)	3 (11.1)	0.284[a]
Prenatal drug exposure, n (%)	3 (12.0)	1 (3.7)	0.341[a]
Prenatal cigarette smoking exposure, n (%)	1 (4.0)	0 (0.0)	0.481[a]
Gestational age, n (%)			
Term	23 (92.0)	24 (88.9)	0.998[a]
Preterm	2 (8.0)	3 (11.1)	
Birth pattern, n (%)			
Normal spontaneous vaginal delivery	9 (36.0)	8 (29.6)	0.847[b]
Cesarean section	16 (64.0)	19 (70.4)	
Birth weight (gram), mean±SD	3376.8±711.4	3259.1±592.3	0.519[c]
Birth complication history, n (%)	2 (8.0)	4 (14.8)	0.670[a]
Postnatal jaundice history, n (%)	4 (16.0)	3 (11.1)	0.698[a]
Postnatal disease history, n (%)	3 (12.0)	2 (7.4)	0.662[a]

ASD: autism spectrum disorder, SD: standard deviation, a: Fisher's exact test, b: Yates' continuity correction, c: Independent samples t test

The ABC-subscales and total scores and the CARS scores of the ASD group (n=25) are shown in Table 3. Based on the CARS scores, 60% of the children (n=15) with ASD had severe autism, whereas 40% (n=10) had mild-to-moderate autism.

Mean serum S100B levels were 1007.09±199.48 pg/ml in mild-to-moderate autism and 1009.62±157.31 pg/ml in severe autism according to CARS scores. There was no significant difference between the mild-to-moderate and severe autism groups in terms of serum S100B levels (p=0.935).

In the ASD group, no correlation was found between serum S100B levels and CARS or ABC-total or -subscale scores (Table 4).

Mean serum S100B levels were 1026.80±170.54 pg/ml in male who had autistic regression and 998.38±176.50 pg/ml in those who did not have autistic regression. There was no significant difference between serum S100B levels and autistic regression status in the ASD group (p=0.667).

Table 3. CARS and ABC scores in the ASD group (n=25)

	Mean±SD	Median (min-max)
CARS	38.48±6.31	39 (30-50)
ABC-Total	58.80±27.15	54 (18-132)
ABC-Sensory	7.84±5.07	7 (0-25)
ABC-Relating	17.36±7.29	17 (4-29)
ABC-Body and Object Use	9.80±8.10	8 (0-34)
ABC-Language	12.88±5.97	11 (4-28)
ABC-Social and Self-Help	10.92±5.27	11 (3-19)

ASD: autism spectrum disorder, CARS: childhood autism rating scale, ABC: autism behavior checklist, SD: Standard deviation, min-max: minimum-maximum

Table 4. The correlation of CARS, ABC, and serum S100B levels in the ASD group (n=25)

	S100B	
	r_s	p
CARS	-0.080	0.705
ABC-Total	-0.100	0.635
ABC-Sensory	-0.044	0.835
ABC-Relating	-0.094	0.656
ABC-Body and Object Use	0.022	0.917
ABC-Language	0.019	0.930
ABC-Social and Self-Help	-0.304	0.140

ASD: autism spectrum disorder, CARS: childhood autism rating scale, ABC: autism behavior checklist, r_s: Spearman's rho

DISCUSSION

In the present study, serum S100B levels were evaluated. The normal range values of S100B remain unclear. There are studies in the literature to determine the normal range of S100B in a non-diseased group, as well as studies to compare S100B levels between two groups including a study group with a disorder and a control group. Therefore, it can be concluded that our knowledge about S100B values is limited to the studies that have been conducted so far. Our results will be discussed in the light of these previous studies. To understand and interpret the results in the literature, it is useful to know that 1 ng/L equals 1 pg/ml and 1 µg/L equals 1000 pg/ml.

In the meta-analysis study by Zheng et al. (16), it was stated that peripheral blood S100B levels might have potential as a useful biomarker for ASD. In our study there was no difference in serum S100B levels between the study and control groups. This result is consistent with the study conducted by Esnafoglu et al. (2) that reported no difference between their study group including children with ASD and a control group. However, in other studies higher S100B levels were reported in children with ASD compared to controls. In the study by Tomova et al. (5), higher plasma S100B levels were reported in children diagnosed with autism compared to non-autistic controls. Similar results have also been reported by Ayaydin et al. (8) and by Golubova et al. (9) with serum S100B levels in the ASD group being higher than those in the control group. It appears that there is inconsistency between the studies' results. Both to use S100B as a biomarker for ASD and to elucidate ASD pathogenesis, it should be considered why such results were obtained. In our discussion part we attempt to explain and interpret these issues. In the meta-analysis study by Zheng et al. (16), no difference was found between the ASD and control groups in terms of S100B levels in China and Turkey. The authors claimed this might have been related to genetic factors, environmental factors, lifestyle, and economic conditions.

As mentioned above, although the normal range of S100B is not fully clarified yet, in our study we found serum S100B levels in both the study and control groups much higher than those in most other studies conducted in serum to determine S100B levels (2,7,8). Mean serum S100B levels in our study were 1008.61±171.34 pg/ml in the ASD group and 1060.14±182.83 pg/ml in the control group. In the study by Astrand et al. (17), the venous serum S100B protein reference value was 0.15 µg/L (equal to 150 pg/ml) in neurologically healthy children aged 3-14 years. In the study by Castellani et al. (18) including children from 3 to 18 years with no recent history of head injury, serum S100B averaged 0.10 µg/L (equal to 100 pg/ml) and the upper reference level was determined as 0.16 µg/L (equal to 160 pg/ml). In the study by Bouvier et al. (19), it was aimed to determine serum S100B levels of healthy children under 3 years old. After some analyses, mean S100B of 0.11 µg/L (equal to 110 pg/ml) was proposed. In the same study it was also reported that the standard deviation is 0.03, range is 0.07-0.20, and 95th percentile is 0.18. It is helpful to keep in mind that these three studies used an electrochemiluminescence immunoassay (17-19). Although high levels were found in the ASD group, the control group also had high levels in our study. When looking at the studies conducted in ASD groups, as stated in the meta-analysis by Zheng et al. (16), different results and ranges stand out. To give some examples of the serum S100B level in children with ASD, it was reported to be 3.33±4.19 pg/ml by Esnafoglu et al. (2), 207.97±52.6 pg/ml by Al-Ayadhi and Mostafa (7), and 49.13±1.57 pg/ml by Ayaydin et al. (8). Although Ayaydin et al. (8) found higher S100B protein levels in children with ASD compared to the healthy group, the mean value might be considered not so high compared to the studies to determine normal ranges (17,18). The study conducted by

Esnafoglu et al. (2) might be regarded in the same way so that even though no differences were found in terms of S100B values, the mean value of 3.33±4.19 pg/ml seems much lower compared to other studies aimed to determine S100B and ASD relations. In line with all these studies conducted to determine the S100B value and its associations, just comparing the results of two groups appears insufficient to determine the S100B range values or the pathogenesis of disorders. Moreover, just finding higher levels in patient groups compared to control groups in any disorder seems insufficient to consider S100B a reliable biomarker.

Putting aside some issues mentioned before, it is still possible to say that our S100B values are much higher than those of most other studies. In the rest of our discussion section, we attempt to establish why the results obtained were so high. In the literature, S100B values have been reported to decrease with age (17,19). Our study was conducted in male younger than 6 years old. To exemplify some other studies' age ranges, it was 5-12 years in the study by Al-Ayadhi and Mostafa (7) and it was 3-12 years in the study by Ayaydin et al. (8). The higher values of S100B in our study may have been related to the fact that the ages of the patients were lower than in those studies. Bouvier et al. (19) explained the high concentrations of S100B under 3 years of age by the possible permeability of the blood brain barrier and cerebral circulation, increased protein turnover in neuronal cells, and low renal excretion of S100B. In the same study another explanation for the higher concentrations of S100B found in neonates and children than in adults was that it could reflect the dynamic central neurodevelopmental processes in which S100B plays a role. Our study was conducted in only male participants, whereas the others included both sexes (7,8). The association of S100B with sex is not clearly depicted. Astrand et al. (17) found a difference between the sexes only in serum capillary S100B levels at 1 and 2 years of age in neurologically healthy children, whereas they found no difference in serum S100B levels in the 3-14 age group or in venous samples between the two sexes aged 3-14 years. However, another study revealed that the correlation of S100B levels between capillary and venous samples was low (20). Castellani et al. (18) and Bouvier et al. (19) found no significant difference between the sexes in children in terms of S100B values. As a meta-analysis has revealed, high levels of heterogeneity were detected in studies conducted for investigation of the association of S100B with ASD (16). While interpreting all the results, this should be taken into consideration. In the meta-analysis study by Zheng et al. (16), it was found that individuals with ASD who had higher peripheral blood S100B levels were detected in cross-sectional studies but not in case-control studies. This situation indicated that the results might have been affected by the study design.

Besides these issues, there might be some other factors potentially capable of affecting S100B levels. S100B can be measured by several methods, such as immunoradiometric assay, immunoluminometric assay, mass spectroscopy, western blot, enzyme linked immunosorbent assay, electrochemiluminescence, and quantitative polymerase chain reaction (21). Müller et al. (22) reported that the choice of analytical method might influence the result of S100B serum analysis. In the same study it was revealed that different methods for the determination of S100B concentrations have not been found interchangeable. It has been suggested that when planning and interpreting the results of clinical studies, all these factors must be taken into consideration (22). As a result, it may be surmised that the different measurement methods between ours and other studies might have caused differences in S100B levels. In the meta-analysis study by Zheng et al. (16), a wide range of mean S100B values reported across studies was found. According to the authors these results might have been related to the studies included, which used different measurement units and different analytical technologies.

Skogstrand et al. (23) have reported that some biomarkers like BDNF, S100B, VEGF-A, and IL-18 may be affected by being frozen. Moreover, increased fragility to a freeze-thaw cycle has been reported especially in samples stored for more than 15 years. Müller et al. (22) claimed that the duration of sample storage may influence the result of S100B serum analysis. They found serum S100B concentrations increased in most samples stored 6 years before, while 13% of the reanalyzed samples showed decreased concentrations. However, Ikeda and Umemura (24) showed that serum S100B values measured from the same frozen sample after 2 months and after 9 months were almost the same. In our study the analysis of serum S100B values was performed within one year at the latest of the samples being frozen. Hence, the time that passed between sample collection and sample analysis is assumed not to have affected the S100B values in our study. Raabe et al. (25) noted that S100B can be measured in arterial or venous serum, is not affected by hemolysis, and remains stable over hours without the need for immediate centrifugation and freezing of the sample; moreover, no special technique is required for sampling or analysis.

In our study, the samples were collected at any time of the day. Ikeda and Umemura (24) showed that serum S100B protein levels were not affected by the circadian rhythm. Likewise, Morera-fumero et al. (6) found no circadian rhythm in S100B levels. In the same study different S100B values were seen due to the season, whether it is summer or winter, with higher values in summer than in winter. Although similar results in terms of higher S100B values in summer were shown by Henriksson AE. (26), the similarity was not statistically significant. The summer/winter difference has been explained by the seasonal temperature changes or the influence of physical activities based on the previous studies. In our study serum samples were collected between January and June. In Turkey, January is a winter month whereas June is a summer month. Thus it might be presumed that seasonal changes were involved that might have affected S100B values when conducting our study. In another study, a day/night variation in S100B levels has been shown with serum S100B levels at 12:00 (midday) being higher than those at 00:00 (midnight) in schizophrenia patients at the time of admission to the inpatient clinic. This day/night difference was not seen in the healthy control group or at discharge time in the schizophrenia patients either (27). It has been documented that both ADHD and healthy control groups had higher serum S100B concentrations in the mornings than in the evenings (28). Considering all, it may

be concluded that whether this is related to a psychiatric disorder pathophysiology or S100B circadian rhythm remains unclear. In the light of these studies it is assumed that the technical issues mentioned above did not affect our S100B values, although it is unclear whether some of them might have been affected. As mentioned before, the usefulness of some features for interpreting S100B results is still unclear. Thus, for using S100B values in the health system reliably, it is suggested that the reasons for previous studies' different results need to be researched further. In future studies, it must be revealed more clearly whether biochemical materials and methods, circadian rhythm, seasonal factor, sex factor, age factor, etc. affect S100B results. Therefore, future research might be designed considering and minimizing all these factors for better and clearer results.

Regarding S100B levels in terms of severity, there are some studies investigating the S100B and ASD relationship. Guloksuz et al. (10) found higher plasma S100B values in severe ASD than in mild-to-moderate ASD based on CARS scores but also showed that S100B concentrations were no longer significantly different between children with severe ASD and those with less severe ASD after controlling for age, sex, and BMI. In the study conducted by Golubova et al. (9), serum S100B levels were found higher in children with severe ASD. On the other hand, although Al-Ayadhi and Mostafa (7) revealed a positive correlation between CARS score and serum S100B levels in ASD patients it was not statistically significant. Shaker et al. (29) revealed that autism patients had higher serum S100B levels compared to healthy controls but no significant correlation between S100B levels and severity of autism was found. It is important to keep in mind that in their study a different assessment tool (Gilliam Autism Rating Scale) was used to determine autism severity. In our study there was no statistically significant relationship between serum S100B levels and autism severity based on CARS scores. Moreover, no statistically significant relationship was found between serum S100B levels and ABC total and subscores either. To elucidate the relation between ASD symptom severity and S100B values, more studies are needed.

A subset of children experience a period of apparently normal development for the first one to two years of life, followed by an abrupt or gradual loss of previously acquired skills, a phenomenon termed regression. The phenomenon of developmental regression, or the loss of a previously established skill, has been discussed in the autism literature (30). In our study, 36% (n=9) of the ASD subjects had autistic regression. According to the results of our study no statistically significant difference was found between ABC-subscale scores, ABC-total scores, CARS scores, and serum S100B levels in terms of autistic regression status. The absence of a statistically significant difference between the autistic regression status and serum S100B levels in our study group may indicate the lack of more CNS injury in individuals with autistic regression. However, the absence of a statistically significant difference in serum S100B levels between the study group and the control group may be considered to refute this theory.

There were some limitations of the present study. It can be presumed that the limited sample size, not comparing the age ranges and sex differences, and the absence of BMI evaluation were limitations of this study. Although all confirmed cases of neurological diseases were excluded, the absence of neurological and general pediatric examinations might have caused some situations in which the effects were involved. The absence of detailed questioning about the situations that might affect serum S100B protein levels prior to the study may be considered a limitation as well.

CONCLUSION

No statistically significant difference was found in serum S100B levels in ASD and no relation between S100B and ABC or CARS scores. Due to the fact that no significant difference was found between CARS scores and S100B values, it was concluded that there was no relationship between autism severity and serum S100B values. No statistically significant difference was found between serum S100B levels with regard to autistic regression status either. Performing similar studies with larger samples in the future is thought to be helpful for elucidating the etiopathogenesis of ASD. Just comparing the S100B results of two groups seems insufficient for considering S100B protein a reliable biomarker. More studies considering some factors (biochemical materials and methods, circadian rhythm, seasonal factor, sex factor, age factor, etc.) with larger samples should be performed. Moreover, to understand the effect of biochemical methodology on the results, further studies are suggested on this topic.

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Protective Effects of Myricitrin and Vitamin E on Nephropathy of Aging Mice Model Induced By D-Galactose

D-Galaktoz ile İndüklenen Farelerde Yaşlanma Modelinin Nefropatisi Üzerine Mirisitrin ve Vitamin E'nin Koruyucu Etkileri

Vahid RADMEHR[1]
0000-0002-1889-2233
Akram AHANGARPOUR[2]
0000-0002-9534-9699
Layasadat KHORSANDI[3]
0000-0002-3391-3055
Mina OMIDI[1]
0000-0002-2935-9426

[1]Student Research Committee, Ahvaz Jundishapur University of Medical Science, Ahvaz, Iran

[2]Department of Physiology, Health Research Institute, Diabetes Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

[3]Department of Anatomical Sciences, School of Medicine, Medical Basic Sciences Research Institute, Cellular and Molecular Research Center, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

Corresponding Author
Sorumlu Yazar
Akram AHANGARPOUR
akramahangarpour@gmail.com

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ABSTRACT

Aim: Aging occurs in cells and tissues due to oxidative stress in physiological conditions. D-galactose (DG) is widely used to cause aging in animal studies. In this study, the renal protective effects of myricitrin and vitamin E in the aging mice model induced by DG was evaluated.

Material and Methods: Subcutaneous DG injection was used for induction of the aging model. 72 female mice were randomly divided into six groups: All groups were received DG at 500 mg/kg/d for six weeks. In the last 28 days, the groups treated with myricitrin subcutaneously received 5, 10, and 20 mg/kg/d, and the vitamin E group received 100 mg/kg/d by gavage. Urine and plasma albumin, BUN, creatinine levels, MDA, TAC, and kidney histological changes were evaluated.

Results: Plasma albumin was significantly decreased ($p=0.001$), but a significant increase in urine albumin ($p=0.001$), BUN ($p<0.001$), and creatinine ($p=0.010$) levels was observed in the DG group when compared with the control. Also, a significant increase in MDA levels ($p=0.002$) along with a significant decrease in TAC ($p=0.012$) was observed. Histopathological changes such as congestion of erythrocytes ($p<0.001$), infiltration of inflammatory cells ($p<0.001$), and proximal tubule cell damage ($p=0.004$) significantly increased, while glomerulus diameter significantly decreased ($p=0.038$) in comparison to the control. Administration of myricitrin and vitamin E showed a significant ameliorative effect on all studied variables.

Conclusion: The improvement effects of myricitrin on DG-induced kidney damage was approximately equivalent to vitamin E. Myricitrin and vitamin E could have beneficial effects on the nephropathy of aging model.

Keywords: Aging; D-galactose; nephropathy; mice; myricitrin.

ÖZ

Amaç: Fizyolojik koşullarda oksidatif strese bağlı olarak hücre ve dokularda yaşlanma meydana gelir. D-galaktoz (DG), hayvan çalışmalarında yaşlanmaya neden olmak için yaygın olarak kullanılmaktadır. Bu çalışmada, DG ile indüklenen farelerde yaşlanma modelinde mirisitrin ve vitamin E'nin renal koruyucu etkileri değerlendirildi.

Gereç ve Yöntemler: Yaşlanma modelinin uyarılması için subkutan DG enjeksiyonu uygulandı. 72 dişi fare rastgele şekilde altı gruba ayrıldı: Tüm gruplara altı hafta boyunca 500 mg/kg/gün DG verildi. Uygulamanın son 28 günü mirisitrin ile tedavi edilen gruplara subkutan olarak 5, 10 ve 20 mg/kg/gün, E vitamini grubuna ise gavaj yoluyla 100 mg/kg/gün uygulandı. İdrar ve plazma albümini, BUN, kreatinin seviyeleri, MDA, TAC ile böbrek histolojik değişiklikleri değerlendirildi.

Bulgular: Kontrol grubu ile karşılaştırıldığında, DG grubunda plazma albumin düzeyi önemli ölçüde azalırken ($p=0.001$), idrar albümini ($p=0.001$), BUN ($p<0.001$) ve kreatinin ($p=0.010$) düzeylerinde anlamlı bir artış gözlendi. Ayrıca MDA düzeylerinde anlamlı artış ($p=0.002$) ile birlikte TAC'de de anlamlı düşüş ($p=0.012$) gözlendi. Kontrol ile karşılaştırıldığında, eritrosit konjesyonu ($p<0.001$), inflamatuar hücrelerin infiltrasyonu ($p<0.001$) ve proksimal tübül hücre hasarı ($p=0.004$) gibi histopatolojik değişiklikler önemli ölçüde artarken, glomerül çapı önemli ölçüde azaldı ($p=0.038$). Mirisitrin ve E vitamininin uygulanması, çalışılan tüm değişkenler üzerinde önemli bir iyileştirici etki göstermiştir.

Sonuç: Mirisitrinin DG'nin neden olduğu böbrek hasarı üzerindeki iyileştirme etkileri yaklaşık olarak E vitaminine eşdeğer idi. Mirisitrin ve E vitamini, yaşlanma modelinin nefropatisi üzerinde faydalı etkilere sahip olabilir.

Anahtar kelimeler: Yaşlanma; D-galaktoz; nefropati; fare; mirisitrin.

INTRODUCTION

Aging is a process that the mechanism of body organs changes with time gradually. This process can enhance the risk of damage to various parts of the body, including the kidneys (1).

Body weight loss, occurs in some experimental diabetic models such as streptozotocin administration (2) while, in the diabetic models in which, a carbohydrate such as sucrose is used to induce diabetes, body weight increases (3). Also, the body weight of mice increases in the high-fat diet (4). In contrast to these studies, one study showed that the body weight of diabetic rats did not change significantly in the high-fat diet (5). D-galactose (DG) can cause the progression of aging in the brain, kidney, and liver (6), and this aging effect is contributed to nephropathy (7). In addition, DG induces oxidative stress by increasing lipid peroxidation that causes similar symptoms to normal aging (8). Many morphological changes are observed in the kidney with aging such as glomerular and tubular destruction. In addition, the number and volume of glomeruli and kidney tubules decrease in the aging process (9). Antioxidant agents are capable to protect cells and tissues against oxidative damage. Flavonoids have strong medicinal properties such as antioxidant, anti-inflammatory, and anti-diabetic effects (10). Myricitrin is a main component of *Myricacerifera* that has antioxidant and anti-inflammatory properties (11). Myricitrin could decrease oxidative damage by decreasing MDA levels and ameliorated antioxidant enzyme activity during cell damage caused by reactive oxygen species (ROS). In addition, this antioxidant agent has protective properties against cellular apoptosis caused by oxidative stress (12). In a previous study, myricitrin improved diabetic nephropathy by enhancement of antioxidant enzyme activity and reduction of oxidative stress (13). Vitamin E (Vit E) has antioxidant and anti-inflammatory effects. Protective effects of Vit E on kidney damages have been cleared previously (14). The purpose of this study was to investigate the protective effects of myricitrin and Vit E on DG-induced kidney damages in mice.

MATERIAL AND METHODS

Animals

In our study, 72 adult female NMRI mice, 4 months old (25-30 g) were taken from Ahvaz Jundishapur University of Medical Sciences (AJUMS) animal facility. The study was performed according to the principles and guidelines of AJUMS laboratory animal care with the code of the ethics committee (IR. AJUMS.REC.1398.010). Mice were kept in a 12 hour light/12 hour dark cycle at 20±4 °C and with free access to water and rodent chow.

Sample Size

The number of animals was determined according to our previous studies with considering the values of α=0.05 and β=0.2, and with the help of Minitab software. Assuming a 35% drop, 12 mice in each group were placed (15,16).

Chemicals

Myricitrin was purchased from Ava Chem San Antonio, U.S.A (purity 98%), and DG was bought from Merck, Germany. Xylazine2% and Ketamine10% from Alfasan Co. (Netherlands), malondialdehyde (MDA) and total antioxidant capacity (TAC) kits were purchased.

Experimental Design

The design of this experiment is schematically presented in Figure 1. In the current study, after one week of acclimatization, the aging model was induced by subcutaneous (SC) injection of DG 500 mg/kg/d for six weeks (17). The animals were divided randomly into six groups, as well as, 12 animals were placed in each group:

1) Control: mice administered SC vehicle; normal saline for six weeks (0.1 ml) and drinking water by gavage from the beginning of the third week to the end of the experiment.
2) DG: mice injected SC with DG for six weeks and concomitant gavage of normal saline from the beginning of the third week to the end of the experiment.
3-5) Myricitrin+DG: DG mice that simultaneously treated by gavage with myricitrin 5, 10, and 20 mg/kg/d from the beginning of the third week to the end of the experiment.
6) DG+Vit E: DG mice that received Vit E 100 mg/kg/d by gavage from the beginning of the third week to the end of the experiment (18).

Finally, in order to urine collection, animals were placed in metabolic cages for 24 hours. The animals were anesthesiaed by the combination of ketamine and xzylazin (90/10 mg/kg, respectively) 24 hours after the last drug treatment, sacrificed, and plasma samples were obtained after cardiac puncture, blood collection, and centrifuging at 3000 rpm for 15 min. Plasma and urine samples were stored at -20 °C and used for the evaluating of BUN, albumin, and Cr levels. After removing the kidneys and washing them with normal saline, the left kidney

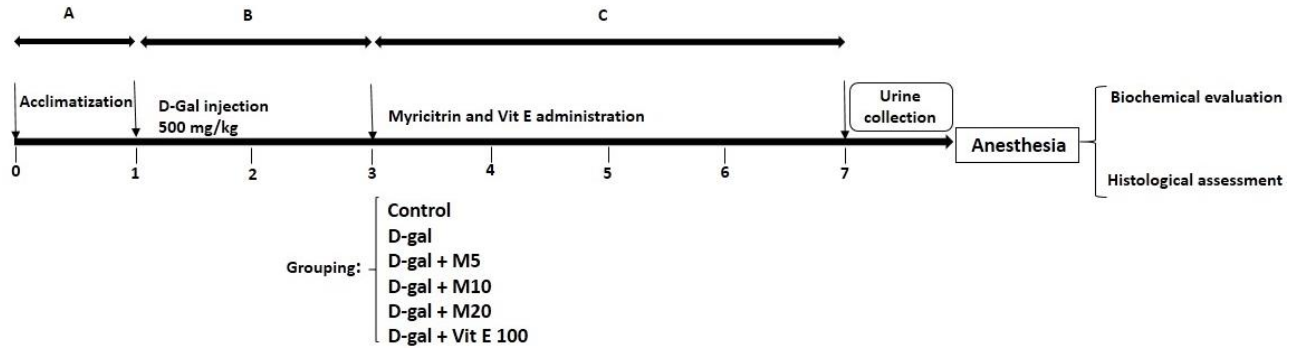

Figure 1. Diagrammatic representation of experimental protocol (time: week)

A: Acclimatization lasted a week, B: induction of aging by D-galactose injection (500 mg/kg), C: Treatment with myricitrin (5, 10 and 20 mg/kg) and Vitamin E (100 mg/kg), at the end of third week, mice divided into following groups: Control, DG: D-galactose 500 mg/kg/d, DG+M5: D-galactose 500 mg/kg/d + myricitrin 5 mg/kg, DG+M10: D-galactose 500 mg/kg/d + myricitrin 10 mg/kg, DG+M20: D-galactose 500 mg/kg/d + myricitrin 20 mg/kg, DG+Vit E 100: D-galactose 500 mg/kg/d + Vitamin E 100 mg/kg. After anesthesia, the biochemical and histological assays were evaluated

snap-frozened in liquid nitrogen, and reserved at -80 °C to evaluate MDA and CAT activity. Another one used for histological study.

Experimental Measurement

The left kidney was defrized and homogenized in ice-cold Tris-HCl buffer (0.1 M, pH 7.4, ratio 1:4 w/v), centrifuged for 15 min at 10,000 g, and supernatants were maintained at -20 °C and used for measurement of kidney MDA and CAT activity by commercial assay kits (ZellBio GmbH, Germany). Also, the concentration of albumin, BUN, and Cr were measured by auto analyzer (BT3000, Italy) devices and biochemical assay kits (Pars Azmoon, Iran).

Histological Assessment

The kidney washed and fixed in 10% neutral formalin solution, dehydrated through a series of graded alcohol, placed in paraffin, cut into 5 μm sections using a microtome (Leica RM 2125, Leica Microsystems Nussloch GmbH, Germany) and stained with hematoxylin and eosin (H&E) to evaluate tissue changes such as red blood cells (RBC) congestion, inflammation, and proximal tubular cell damage under a digital research microscope (BMZ-04-DZ, Behin Pajouhesh ENG. CO., Iran).

Statistical Analysis

The data were presented as mean±standard deviation and analyzed by GraphPad Prism 9 for windows (GraphPad Software, San Diego, CA). The Shapiro-Wilk test was used to examine normality and the Levene test for homogeneity of variance. One-way analysis of variance (ANOVA) was conducted for differences among the groups followed by post hoc Tukey's HSD test. Significant difference was set at $p<0.05$.

RESULTS

Effect of Myricitrin and Vitamin E on Body Weight

As shown in Figure 2, body weight was significantly higher in DG compare to myricitrin treated groups ($p<0.001$) and Vit E received group ($p<0.001$). Kidney weight in DG ($p<0.001$) and M5 ($p=0.020$) groups was significantly lower than control. Kidney weight in M5 ($p=0.002$), M10, M20, and Vit E groups were significantly higher ($p<0.001$) than DG. The percentage of the kidney to body weight ratio was markedly reduced in the DG compared to the control ($p<0.001$). Myrycitrin receiving mice and Vit E group significantly improved it ($p<0.001$).

Effect of Myricitrin and Vitamin E on Kidney Function

Urine albumin had a significant difference between DG and control groups ($p=0.001$). Administration of M5 ($p=0.050$), M10 ($p=0.010$), M20 ($p=0.009$), and vitamin E ($p=0.006$), showed a significant decrease in urine albumin levels in comparison to the DG group. Also, the plasma albumin level of DG was markedly lower than control ($p=0.001$) and the ameliorative effect of M5, M10 groups ($p=0.002$), M20 ($p=0.005$), and Vit E ($p=0.003$) was observed. There was a significant increase of BUN in the DG ($p<0.001$) compared to the control, and administration of M10 ($p=0.010$) and Vit E ($p=0.020$) significantly decreased it. Decreased levels of BUN in M5 and M20 groups were not significant compared to the DG. Plasma levels of Cr increased in the DG group compared to control ($p=0.010$), and it was decreased in M5, M10, Vit E groups ($p=0.040$), and M20 ($p=0.010$, Table 1).

Effect of Myricitrin and Vitamin E on Antioxidant Activity in the Kidney

A dramatic increase effect of DG on lipid peroxidation was observed through increasing of MDA levels in DG mice ($p=0.002$). Also, DG had a decreasing effect on TAC

Table 1. Effect of different doses of myricitrin and Vit E on urine albumin and plasma level of albumin, BUN and creatinine

	Control	DG	DG+M5	DG+M10	DG+M20	DG+Vit E	p
Urine Alb (mg/24h)	155.3±18.78	232.7±21.74**	180.0±19.48$^{\#}$	169.9±17.3$^{\#}$	168.6±24.82$^{\#\#}$	165.5±26.96$^{\#\#}$	**0.001**
Plasma Alb (g/dl)	2.92±0.08	2.65±0.1**	2.90±0.08$^{\#\#}$	2.90±0.06$^{\#\#}$	2.88±0.05$^{\#\#}$	2.90±0.04$^{\#\#}$	**<0.001**
BUN (mg/dl)	52.5±3.07	70.6±2.96***	59.6±5.03	56.7±2.6$^{\#}$	59.8±2.69	57.5±9.52$^{\#}$	**0.002**
Creatinine (mg/dl)	0.22±0.04	0.37±0.06*	0.24±0.04$^{\#}$	0.25±0.05$^{\#}$	0.23±0.05$^{\#}$	0.25±0.04$^{\#}$	**0.008**

DG: D-galactose 500 mg/kg/d, DG+M5: D-galactose 500 mg/kg/d + myricitrin 5 mg/kg, DG+M10: D-galactose 500 mg/kg/d + myricitrin 10 mg/kg, DG+M20: D-galactose 500 mg/kg/d + myricitrin 20 mg/kg, DG+Vit E: D-galactose 500 mg/kg/d + Vitamin E 100 mg/kg, Alb: albümin, *: different from Control, $^{\#}$: different from DG, 1 symbol $p<0.050$, 2 symbols $p<0.010$, 3 symbols $p<0.001$

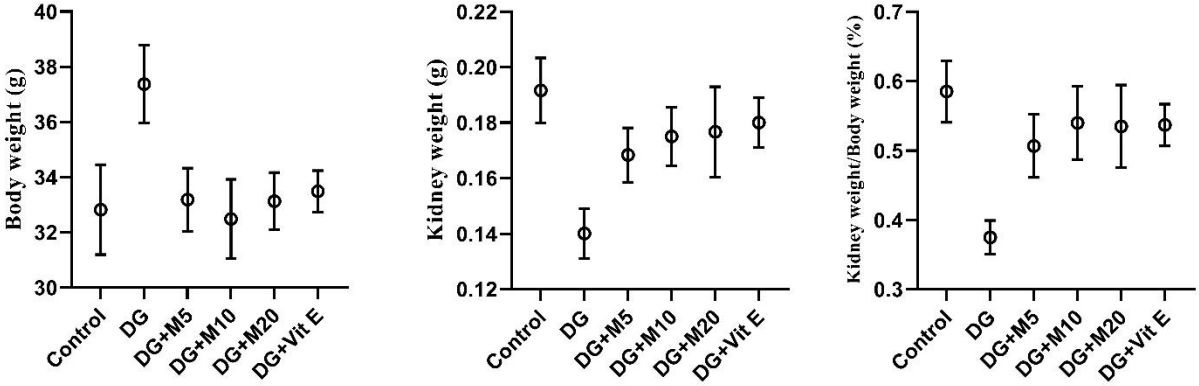

Figure 2. The effect of myricitrin and vitamin E on the body weight, kidney weight, and kidney to body weight ratio

DG: D-galactose 500 mg/kg/d, DG+M5: D-galactose 500 mg/kg/d + myricitrin 5 mg/kg, DG+M10: D-galactose 500 mg/kg/d + myricitrin 10 mg/kg, DG+M20: D-galactose 500 mg/kg/d + myricitrin 20 mg/kg, DG+Vit E: D-galactose 500 mg/kg/d + Vitamin E 100 mg/kg

level, as observed in DG animals (p=0.012). Myricitrin and Vit E remarkably decreased lipid peroxidation of kidney tissue through decreasing MDA and increasing TAC levels. The effect of M5 on reducing MDA (p=0.008) and increasing TAC (p=0.035) was more pronounced than M20 group (Table 2).

Effect of Myricitrin and Vitamin E on Renal Histopathology

As shown in Table 3 and Figure 3, the natural appearance of the kidney decreased in DG mice, and treatment with myricitrin and Vit E improved it. Glomerulus diameter markedly decreased in DG (p=0.038), M5 (p=0.044), and M10 (p=0.047) in comparison with the control group, but M20 and Vit E improved these changes (p=0.049). Furthermore, proximal tubule damage (brush border loss, tubular dilation, and vasodilatation) was increased in the DG, M5, and M10 groups (p=0.009), and it was effectively improved by the M10 (p=0.046), M20 and Vit E (p=0.008). Inflammatory cell infiltration has occurred in groups that received DG and administration of myricitrin and Vit E had a preventive effect. Furthermore, it was revealed that accumulation of RBCs had a remarkable increase in the DG group (p<0.001), and treatment with M10 (p=0.048), M 20, and Vit E (p<0.001) effectively improved this parameter.

DISCUSSION

The present study indicated that the renal dysfunction induced by DG improved by the administration of myricitrin and Vit E. In agreement with this result, it was shown that DG promotes aging alterations (15). Also, we found that DG can induce nephropathy through increases in urine albumin, BUN, plasma Cr, and a decrease of plasma albumin level. Increased renal biomarkers may be due to acute nephropathy that is related to tubular dysfunction (19). Endogenous plasma creatinine levels indicate changes in kidney function caused by diet and diabetes in mice (20). It is widely known that BUN and Cr are two important factors in the evaluation of renal function (21). Also, an increase in BUN level is a predictable factor during renal damage (22). Plasma Cr is a more specific factor than urea level to evaluate kidney function; because Cr has all the features needed for a perfect filtration indicator. It was revealed that elevation of urea and plasma Cr along with a reduction of plasma albumin level occurs in nephropathy (23). The increase of BUN and Cr levels are related to glomerular filtration dysfunction caused by DG. The present study indicated that myricitrin could ameliorate albumin, BUN, and Cr levels by improvement of the filtration function.

It was considered that DG promotes oxidative damage in the kidney of rodents and causes the excessive generation of ROS and diminishes endogenous antioxidant activity (24). Thereby, inhibition of oxidative stress provides a therapeutic strategy against renal injury. Previously, the protective effects of Vit E on kidney function was investigated. It was shown that vit E improves the antioxidant defense system by suppression of free radicals (25). Myricitrin eliminates the overproduction of ROS during kidney injury in rats (26). Also, a previous study revealed that myricitrin and Vit E had a preventive effect against DG induced lipid

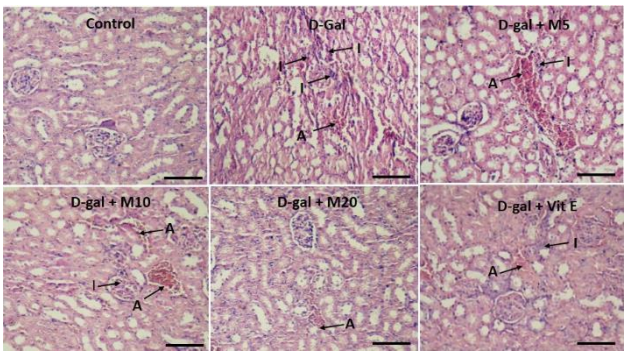

Figure 3. Effect of myricitrin and vitamin E on renal histology (scale bar: 50 µm)

DG: D-galactose 500 mg/kg/d, DG+M5: D-galactose 500 mg/kg/d + myricitrin 5 mg/kg, DG+M10: D-galactose 500 mg/kg/d + myricitrin 10 mg/kg, DG+M20: D-galactose 500 mg/kg/d + myricitrin 20 mg/kg, DG+Vit E: D-galactose 500 mg/kg/d + Vitamin E 100 mg/kg, I: inflammation; A: accumulation of red blood cells

Table 2. Effect of different doses of myricitrin and Vit E on MDA and TAC level in the kidney

	Control	DG	DG+M5	DG+M10	DG+M20	DG+Vit E	p
MDA (µM/g tissue)	6.08±0.7	8.85±0.6**	5.32±0.5$^{\#\#\#}$	7.12±0.5$^{\#}$	7.60±0.4$^{\$\$}$	6.83±0.4$^{\#}$	**0.004**
TAC (mM/g tissue)	0.77±0.02	0.5±0.04*	0.83±0.08$^{\#\#}$	0.71±0.03$^{\#}$	0.63±0.07$^{\$}$	0.76±0.10$^{\#}$	**0.034**

DG: D-galactose 500 mg/kg/d, DG+M5: D-galactose 500 mg/kg/d + myricitrin 5 mg/kg, DG+M10: D-galactose 500 mg/kg/d + myricitrin 10 mg/kg, DG+M20: D-galactose 500 mg/kg/d + myricitrin 20 mg/kg, DG+Vit E: D-galactose 500 mg/kg/d + Vitamin E 100 mg/kg, MDA: malondialdehyde, TAC: total antioxidant capacity, *: different from Control, $^{\#}$: different from DG, $^{\$}$: different from M5, 1 symbol p<0.050, 2 symbols p<0.010, 3 symbols p<0.001

Table 3. Effect of different doses of myricitrin and Vit E on kidney histology

	Control	DG	DG+M5	DG+M10	DG+M20	DG+Vit E	p
Glomerulus diameter (µm)	239.3±16.3	190.2±12.1*	195.4±9.3*	210.5±13.8*	225.7±12.9$^{\#}$	223.8±13.4$^{\#}$	**0.025**
Proximal tubule damage (%)	0.1±0.04	0.6±0.06**	0.53±0.11**	0.33±0.07$^{**\#}$	0.16±0.05$^{\#\#}$	0.17±0.04$^{\#\#}$	**0.004**
Inflammatory cell infiltration	0.09±0.01	2.1±0.31***	0.83±0.23$^{***\#}$	0.52±0.18$^{***\#\#}$	0.12±0.04$^{*\#\#\#}$	0.11±0.03$^{*\#\#\#}$	**<0.001**
Accumulation of RBCs	0.08±0.02	2.3±0.19***	1.82±0.25***	1.12±0.29$^{***\#}$	0.52±0.09$^{**\#\#\#}$	0.58±0.1$^{**\#\#\#}$	**<0.001**

DG: D-galactose 500 mg/kg/d, DG+M5: D-galactose 500 mg/kg/d + myricitrin 5 mg/kg, DG+M10: D-galactose 500 mg/kg/d + myricitrin 10 mg/kg, DG+M20: D-galactose 500 mg/kg/d + myricitrin 20 mg/kg, DG+Vit E: D-galactose 500 mg/kg/d + Vitamin E 100 mg/kg, RBCs: red blood cells, *: different from Control, $^{\#}$: different from DG, 1 symbol p<0.050, 2 symbols p<0.010, 3 symbols p<0.001

peroxidation in the reproductive system (15). In addition, a report verified that DG increased lipid peroxidation (MDA) and reduced SOD in the testis of DG-induced aging mice model (27). In the present study, myricitrin and Vit E restored the antioxidant defense system through enhancement of the antioxidant enzyme activity and reduction of MDA in the kidney of DG treated mice. It seems that myricitrin at a low dose has a better function than Vit E.

Hypoalbuminemia routinely occurs in nephropathy (28). Albuminuria is one of the main symptoms of kidney damage (29). Structural dysfunction of the glomerular filtration barrier causes passes of serum protein into the urine and eventually causes albuminuria. It seems that, reduction of plasma albumin in DG mice is associated with renal dysfunction in our study. In agreement with our study, it has been reported that proximal damage is also involved in the reduction of serum albumin due to oxidative stress induced by DG (30). In another study, it was shown that increased proximal damage of the glomerulus in the DG receiving mice decreased the functional capacity of the nephrons (31). Decreased plasma albumin levels can also be related to decreased reabsorption of albumin by damaged tubules. This may be the cause of albuminuria in the DG group. Myricitrin and Vit E improved tubular damages and glomerular degradation that might be the cause of albuminuria due to DG.

In carbohydrate-induced diabetic models such as sucrose, body weight enhances due to the accumulation of fat mass in the abdominal area (3,32). Therefore, it is concluded that the ratio of organ weight to body weight decreases in these models. In the current study, due to the carbohydrate nature of DG, body weight increased and the percentage of the kidney weight to body weight ratio markedly decreased in the DG group. Administration of myricitrin and Vit E could improve this ratio near to the normal range. Furthermore, the diameter of the glomerulus decreased in the DG group, whereas this reduced effect is markedly lower in mice that also received myricitrin or vitamin E. It has been reported that the total number of glomeruli in the kidney reduces with age (33). Thus, a reduction in the percentage of the kidney weight to body weight in the DG group may be related to a decrease in the diameter of the glomerulus and loss of glomeruli.

DG can cause infiltration of inflammatory cells in the kidney tissue. Flavonoids have anti-inflammatory effects (34). An increase in inflammatory cells in kidney tissue shows that DG disrupts the function of enzymes and proteins in the interstitial tissue of the kidney, imbalances the antioxidant defense system, generates ROS, and eventually causes an inflammatory response (35). Therefore, a decrease in inflammatory cell number in mice that received myricitrin and Vit E is associated with their anti-inflammatory activities. It was reported that Vit E diminishes the peroxidation of unsaturated membrane lipids through the scavenging of oxygen (36). In this study, Vit E has improved histological alterations and plasma levels of albumin, BUN, and creatinine in comparison to the DG group. So, we found that Vit E is a protective component for kidney tissue against peroxidative damage. Myricitrin at low doses exerted antioxidant properties by reducing MDA level.

CONCLUSION

In brief, our study showed that myricitrin, given its high antioxidant properties, may be a candidate for the prevention or treatment of aging-relative disorders approximately equivalent to Vit E.

Ethics Committee Approval: The study was approved by the Ethics Committee of Ahvaz Jundishapur University of Medical Science (07.05.2019, IR.AJUMS.REC.1398.010).

Conflict of Interest: None declared by the authors.

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Author Contributions: Idea/Concept: AA; Design: VR, MO; Data Collection/Processing: VR, LK, MO; Analysis/Interpretation: AA; Literature Review: VR, AA; Drafting/Writing: AA; Critical Review: VR, MO.

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Protective and Therapeutic Effects of Beta Glucan Administration on Experimental Ulcerative Colitis Model Induced by TNBS

TNBS ile Oluşturulan Deneysel Ülseratif Kolit Modelinde Beta Glukan Uygulamasının Koruyucu ve Terapötik Etkileri

Deren TOKMAK[1]
0000-0002-7953-8684
Ferhat ŞİRİNYILDIZ[2]
0000-0001-8800-9787
Rauf Onur EK[3]
0000-0003-3923-0156

[1]Department of Physiology, Aydın Adnan Menderes University Health Science Institute, Aydın, Turkey
[2]Department of Physiology, Aydın Adnan Menderes University Faculty of Medicine, Aydın, Turkey
[3]Department of Physiology, İstinye University Faculty of Medicine, İstanbul, Turkey

Corresponding Author
Sorumlu Yazar
Ferhat ŞİRİNYILDIZ
ferhat.sirinyildiz@adu.edu.tr

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ABSTRACT

Aim: In this study, it was aimed to investigate the effects of beta glucan (BG) on the experimental colitis model created by using trinitrobenzene sulfonic acid (TNBS).
Material and Methods: Thirty-two Wistar Albino rats were divided equally into four groups as sham control, TNBS, TNBS-BG3, and TNBS-BG10 groups. While saline was administrated to sham group, TNBS was administered intrarectally to the TNBS groups under anesthesia. BG was administered at a dose of 100 mg/kg by oral gavage, intragastrically, for 3 days (TNBS+3) to the TNBS-BG3 group and for 10 days (7+TNBS+3) to the TNBS-BG10 group. At the end of the study, macroscopic, histological and biochemical tests were applied to the colon tissues taken.
Results: It was determined by histopathological scoring and biochemical results that BG administration caused positive effects on colon damage due to colitis. Malondialdehyde level and myeloperoxidase activity were found to be significantly higher in the TNBS group compared to the other groups ($p=0.003$ and $p<0.001$, respectively). Antioxidant levels increased in BG treated groups compared to TNBS group. While this increase was statistically significant among glutathione levels ($p<0.001$), it was not statistically significant in catalase enzyme activity ($p=0.218$). BG administration reduced the increase in lipid peroxidation and leukocyte infiltration level in the colon tissue. Positive changes due to the prophylactic effect of BG were determined in histological and biochemical results.
Conclusion: BG administration has been found to show anti-inflammatory and antioxidant properties, and BG has a treatment potential in reducing colon tissue damage due to TNBS-induced colitis.
Keywords: Anti-inflammatory; antioxidant; beta glucan; ulcerative colitis.

ÖZ

Amaç: Bu çalışmada trinitrobenzen sülfonik asit (TNBS) kullanılarak oluşturulan deneysel kolit modeli üzerinde beta glukan (BG)'ın etkilerinin araştırılması amaçlanmıştır.
Gereç ve Yöntemler: Otuz iki Wistar Albino sıçan eşit olarak sham kontrol, TNBS, TNBS-BG3 ve TNBS-BG10 gruplarına ayrılmıştır. Sham grubuna serum fizyolojik uygulanırken, TNBS gruplarına anestezi altında intrarektal yol ile TNBS uygulanmıştır. BG 100 mg/kg dozunda, TNBS-BG3 grubuna 3 gün (TNBS+3) süreyle, TNBS-BG10 grubuna ise 10 gün (7+TNBS+3) süreyle, intragastrik şekilde oral gavaj yolu ile verilmiştir. Çalışmanın sonunda alınan kolon dokularına makroskobik, histolojik ve biyokimyasal testler uygulanmıştır.
Bulgular: BG uygulamasının kolite bağlı kolon hasarında olumlu etkilere neden olduğu, histopatolojik skorlama ve biyokimyasal sonuçlar ile tespit edilmiştir. Malondialdehid seviyesi ve miyeloperoksidaz aktivitesinin diğer gruplar ile karşılaştırıldığında TNBS grubunda, anlamlı derecede yüksek olduğu tespit edilmiştir (sırasıyla $p=0,003$ ve $p<0.001$). BG tedavisi uygulanan gruplarda, antioksidan düzeyi TNBS grubuna göre artış göstermiştir. Bu artış glutatyon düzeyleri arasında istatistiksel olarak anlamlı iken ($p<0.001$), katalaz enzim aktivitesinde istatistiksel olarak anlamlılık ortaya çıkmamıştır ($p=0.218$). BG uygulaması, kolon dokusundaki lipit peroksidasyon ve lökosit infiltrasyon düzeyindeki artışı azaltmıştır. Histolojik ve biyokimyasal sonuçlarda BG'nin profilaktik etkisine bağlı olarak olumlu değişiklikler tespit edilmiştir.
Sonuç: BG uygulamasının anti-inflamatuar ve antioksidan özellikler gösterdiği tespit edilmiştir ve TNBS ile indüklenen kolit sonucu oluşan kolon doku hasarını azaltmada BG tedavi edici potansiyel taşımaktadır.
Anahtar kelimeler: Anti-inflamatuar; antioksidan; beta glukan; ülseratif kolit.

INTRODUCTION

Inflammatory bowel disease (IBD) is a group of diseases of unknown etiology, caused by genetic, environmental and immunological causes. IBD is a disease that affects the gastrointestinal system with chronic, widespread, recurrent gastrointestinal inflammation and has a clinical course with intestinal and extra-intestinal involvement (1). IBD gathers ulcerative colitis and Crohn's disease, although these two diseases have similar clinical course and treatment methods; complications differ from each other in that they are localized to different parts of the intestine (2). In the pathogenesis of IBD, the effect of reactive oxygen species (ROS) on the increase of tissue damage in many inflammatory disease groups, including colitis, has been found (3,4). Mucosal damage in IBD causes leukocyte migration to the intestinal mucosa. As a result of neutrophil and macrophage infiltration, oxidative damage in the mucosa increases and the increase in leukocyte migration together with the release of proinflammatory cytokines turns into a vicious circle (5). In ulcerative colitis, inflammation generally starts from the rectal mucosa and progresses uninterruptedly proximally and is observed as a disease that keeps the mucosa without bypassing (6). Environmental factors, genetic predisposition, changes in intestinal microflora and immunological response are thought to play a role in the etiopathogenesis of IBD (7). ROS contribute to the destruction of the mucosal barrier by disrupting the protective layer in the colonic epithelial tissue. It causes leukocyte infiltration and passage of bacterial toxins into the lamina propria (8).

Although oxidative activities continue in the body, antioxidant capacity, which is one of the body's defense mechanisms, protects tissues from ROS under physiological conditions. However, when the production rates of oxygen radicals exceed the capacity of the endogenous antioxidant defense mechanisms, they can destroy the tissue. Endogenous mechanisms for defense have many radical scavengers and enzymes, including catalase (CAT), glutathione (GSH), superoxide dismutase and peroxidases (8-11). Free oxygen radicals cause lipid membrane peroxidation, damage to protein and DNA, and damage to cells and tissues. Neutrophil migration in inflamed tissue facilitates the emergence of strong cytotoxic oxidants with the effect of myeloperoxidase (MPO) enzyme (12).

Beta glucan (BG) is a polysaccharide found in baker's yeast, cereals and fungi, and is involved in the structure of the cell wall (13). When BG is taken orally, it is absorbed from the gastrointestinal tract and plays a role in stimulating the immune system by binding to receptors on macrophages, neutrophils, and natural killer cells (14,15). It is known that BG has antioxidant properties and increases the level of antioxidant enzymes and reduces oxidant substances (16). It has been understood that BGs stimulate collagen tissue synthesis by increasing NF-1 activity in fibroblasts and play a role in wound healing by increasing the amount of procollagen mRNA and type 1 and type 3 collagen levels (17).

In this study, the antioxidant and anti-inflammatory effects of BG given by gastric gavage were investigated in an experimental colitis model created with trinitrobenzene sulphonic acid (TNBS), by measuring histopathological evaluations, and malondialdehyde (MDA), MPO, CAT, and GSH levels in the colon tissue by ELISA method.

MATERIAL AND METHODS

The studies were carried out using 32 Wistar Albino female rats aged between 8 and 10 weeks, after the approval which was obtained from the Experimental Research Ethics Committee of Aydin Adnan Menderes University, dated 14.08.2015 and numbered 64583101/2015/094. The rats were randomly divided into 4 equal experimental groups as sham group, TNBS group, TNBS-BG3 group, and TNBS-BG10 group. Except for the rats in the sham group, all subjects were exposed to experimental colitis.

2,4,6-trinitrobenzene sulphonic acid, purchased from Sigma company (Sigma, La Verpaillere, France) and prepared by dissolving 25 mg in 37% ethanol, BG is Imuneks brand (Imuneks brand (Sigma, La Verpaillere, France) isolated from *Saccharomyces cerevisiae*). Mustafa Nevzat İlaç San. A.Ş., Istanbul, Turkey) was prepared by dissolving the preparation in drinking water.

Induction of Colitis

Animals that will cause colitis with TNBS were fasted 24 hours before the application and their colons were emptied by triggering the defecation reflex. Intrarectal administrations were given under ketamine (75 mg/kg) and xylazine (8 mg/kg) anesthesia, by entering 0.8 ml of TNBS through a polyethylene cannula 8 cm from the anal orifice. Animals in the sham group were given physiological saline instead of TNBS.

Experimental Groups

The groups were organized as given below.

Sham Control Group: Saline was administered rectally under ketamine and xylazine anesthesia through the cannula. The rats were sacrificed 3 days after this treatment.

TNBS Group: Experimental colitis was formed by rectally giving 25 mg of TNBS dissolved in 0.8 ml 37% ethanol through a cannula under ketamine and xylazine anesthesia. The rats were sacrificed 3 days after this treatment.

TNBS-BG3 Group: BG at a dose of 100 mg/kg was given intragastrically via an oral gavage needle for 3 days from TNBS administration to sacrification. After 3 days of treatment, the rats were sacrificed.

TNBS-BG10 Group: BG at a dose of 100 mg/kg was given intragastrically via an oral gavage needle for 7 days before TNBS administration and for 3 days until sacrification.

At the end of the experiment, the rats were sacrificed by the cervical dislocation method while under ketamine xylazine anesthesia. Columns approximately 10 cm long were cut open longitudinally, the tissue was washed with physiological saline and made ready for histopathological and biochemical examination.

Histopathological Evaluation

Histopathological evaluation was performed using standard tissue fixation and sectioning procedures. Colon tissue taken from rats was kept in 10% neutral formaldehyde. The fixation time was 24 hours, and the temperature was 4 °C. Colon tissues were soaked in ethanol for dehydration and cleared in xylene. Paraffin blocks were used for embedding the tissues and 5 µm thick random sections were taken using a microtome (Leica model RM 2135, Leica, Germany). Hematoxylin-eosin procedure was used for staining these sections and the stained sections were examined under a light microscope.

Screenshots were captured using an Olympus DP20 digital camera connected to an Olympus BX51 microscope.

Changes in histopathological parameters were scored appropriately. The degree of change in the columns was evaluated on a scale of 0 to 3 (18): These scores mean: 0, no change; 1, slight damage; 2, moderate damage; 3, severe damage. The evaluated parameters with respect to the change were inflammatory cell infiltration, submucosal edema, mucosal hemorrhage, and damage/necrosis. Scoring of tissue samples was done by a blinded observer.

Biochemical Evaluation

A 2-cm segment of the colon was separated and used for biochemical evaluations. These tissues were homogenized in pH 7.0 50 mM phosphate buffer at 0-4 °C (w/v=1/10). Analyzes were performed by ELISA method using kits from BioVision Incorporated, California, USA.

MDA Activity: Homogenized tissue samples and Biovision Lipid Peroxidation Colorimetric/Fluorometric Detection Kit were used for MDA level.

MPO Activity: Homogenized tissue samples and Biovision Myeloperoxidase Activity Colorimetric Detection Kit were used for MPO activity.

GSH Level: Homogenized tissue samples and Biovision Glutathione Fluorometric Detection Kit were used for GSH level.

CAT Activity: Homogenized tissue samples and Biovision Catalase Activity Colorimetric/Fluorometric Detection Kit were used for CAT activity.

Statistical Analysis

All statistical analyses were performed via IBM SPSS Statistics 22.0 (IBM Corp. Released 2013. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). The distributions of biochemical measurements (GSH, MDA, CAT and MPO) were evaluated by the Shapiro-Wilk's test and skewness/kurtosis statistics. Homogeneity of the variances among groups for these measurements were examined by Levene's test. All these measurements were provided by mean±standard deviation (mean±SD). The four groups were compared with respect to the biochemical measurements by ANOVA. Tukey's HSD test was performed, if necessary. A p-value <0.05 was accepted as statistically significant.

RESULTS

Histopathological Results

The histopathological results are given in Table 1. No damage was detected in the sham control group (Figure 1). In the colitis group, intense mucosal damage and necrosis, moderate inflammation, edema, and hemorrhage were detected (Figure 2). Moderate mucosal damage, inflammation, and hemorrhage were seen in the TNBS-BG3 group (Figure 3). Although there was no necrosis in the TNBS-BG10 group, mild edema and inflammation were detected (Figure 4).

Biochemical Results

The results of the biochemical measurements are given in Table 2. Accordingly, GSH level ($p<0.001$), MDA level ($p=0.003$) and MPO activity ($p<0.001$) showed statistically significant difference between groups, while CAT activity was found to be similar between groups ($p=0.218$). The post-hoc test revealed that the mean GSH level was significantly higher in sham group compared to all other groups (all p values <0.001) and in TNBS-BG10 group compared to TNBS group ($p=0.018$). The mean MDA level and mean MPO activity in sham group ($p=0.008$ and $p<0.001$, respectively), and also in TNBS-BG10 group ($p=0.005$ and $p=0.014$, respectively) were significantly lower than those in TNBS group. There was also a significant difference between sham group and TNBS-BG3 group, indicating lower MPO activity in sham group ($p=0.037$).

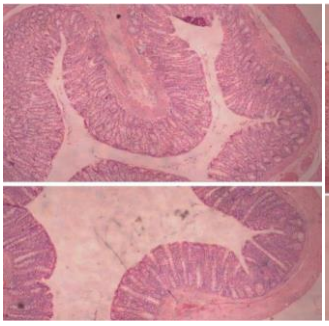

Figure 1. Histopathological features of the tissue of the sham control group (10X-40X)

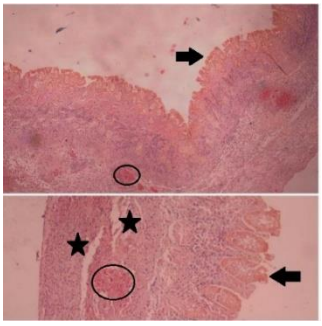

Figure 2. Histopathological features of the tissue of the TNBS group (10X-40X)

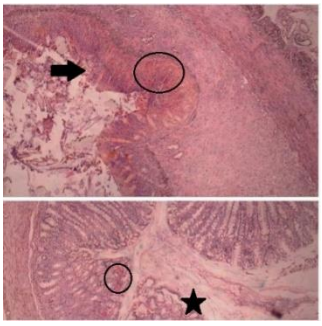

Figure 3. Histopathological features of the tissue of the TNBS-BG3 group (10X-40X)

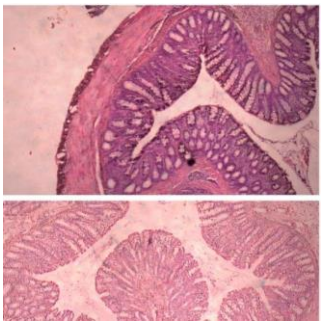

Figure 4. Histopathological features of the tissue of the TNBS-BG10 group (10X-40X)

Table 1. The histological (microscopic) scores for each parameter in groups

	Sham Control	TNBS	TNBS-BG3	TNBS-BG10
Mucosal Damage / Necrosis	0	3	2	0
Inflammation	0	2	2	1
Edema	0	2	1	1
Hemorrhage	0	2	2	0

TNBS: trinitrobenzene sulfonic acid, BG: beta glucan

Table 2. Comparison of the biochemical measurements between groups

	Sham Control	TNBS	TNBS-BG3	TNBS-BG10	p
GSH (nmol/mg), mean±SD	16.575±2.629	9.304±1.085	11.463±1.885	12.238±1.411	**<0.001**
MDA (nmol/g), mean±SD	239.538±19.715	265.600±12.762	252.263±11.951	238.350±13.748	**0.003**
CAT (mU/ml), mean±SD	4.540±0.664	4.116±0.956	3.918±0.740	3.762±0.635	0.218
MPO (U/g), mean±SD	10.131±1.427	13.683±1.176	12.129±1.605	11.393±1.333	**<0.001**

TNBS: trinitrobenzene sulfonic acid, BG: beta glucan, GSH: glutathione, MDA: malondialdehyde, CAT: catalase, MPO: myeloperoxidase, SD: standard deviation, Tukey HSD post hoc test results; GSH; Sham vs TNBS: p<0.001, Sham vs TNBS-BG3: p<0.001, Sham vs TNBS-BG10: p<0.001, TNBS vs TNBS-BG3: p=0.113, TNBS vs TNBS-BG10: p=0.018, TNBS-BG3 vs TNBS-BG10: p=0.835; MDA; Sham vs TNBS: p=0.008, Sham vs TNBS-BG3: p=0.336, Sham vs TNBS-BG10: p=0.999, TNBS vs TNBS-BG3: p=0.397, TNBS vs TNBS-BG10: p=0.005, TNBS-BG3 vs TNBS-BG10: p=0.263; MPO; Sham vs TNBS: p<0.001, Sham vs TNBS-BG3: p=0.037, Sham vs TNBS-BG10: p=0.290, TNBS vs TNBS-BG3: p=0.140, TNBS vs TNBS-BG10: p=0.014, TNBS-BG3 vs TNBS-BG10: p=0.718

DISCUSSION

In this study, we found that the mean GSH and CAT levels were significantly higher in sham group compared to others. Also, MPO and MDA levels were significantly lower in sham group compared to others. This indicates that oxidative damage was observed in the biochemical results of the TNBS-treated groups. Furthermore, histological examination results support the biochemistry results. Oxidative damage and histological tissue damage decreased with BG administration.

Jurjus et al. (19) reported that the inflammatory process and wound development in TNBS colitis may be due to disruption of the intestinal mucosal barrier depending on the dose of the chemical applied. Likewise, Millar et al. (20) reported that widespread mucosal edema, necrosis and ulcerations were observed in the TNBS colitis model. In addition, according to current studies, Xue et al. (21) used the chemical Oxazolone to induce experimental ulcerative colitis. Shanmugam et al. (22) used indomethacin, and Motawea et al. (23) and Guazelli et al. (24) used acetic acid for induction of colitis. Their results support the results of TNBS-induced ulcerative colitis and our results. Significant histopathological changes were detected in all experimental groups in which colitis was induced by TNBS colitis model used in our study. Among the TNBS-treated groups, necrosis and hemorrhage were not observed in the mucosa in the TNBS-BG10 group, and inflammation was observed to be more limited than in the other TNBS-treated groups.

Nosál'ová et al. (25) reported that N-acetyl cysteine treatment showed antioxidant properties in acetic acid-induced colitis model. Ademoğlu et al. (26), on the other hand, investigated the antioxidant effects of vitamin E and selenium on ulcerative colitis in their experimental study and concluded that MDA decreased significantly in the experimental group given vitamin E and selenium together. Ek et al. (27) determined that caffeic acid phenethyl ester (CAPE) administered to rats with colitis with TNBS caused a decrease in MPO levels in the treatment group. Ige et al. (28) in their study in which they associated the effects of ulcerative colitis induced by acetic acid with maize consumed in the diet, showed that while the MPO values of the colitis group increased significantly compared to the control group, the MPO levels of the rats fed a diet supplemented with maize at an appropriate rate approached the values of the control group. Motawea et al. (23), in their study investigating the effects of oleuropein against acetic acid-induced ulcerative colitis, found that MDA and MPO levels, which increased significantly in the colitis group, decreased in the oleuropein-administered groups and approached the results of the control group. With our study findings, it was observed that BG reduced lipid peroxidation with its antioxidant property, and the lowest MDA level was found in the TNBS-BG10 group. In addition, a statistically significant increase was found in the colon tissue MPO activity values in the colitis group, which was applied TNBS, and it was observed that the values approached the control group with the treatment.

Karabeyoglu et al. (29) examined the effect of ethyl pyruvate in the experimental colitis model created in rats and studied the glutathione levels in the colon tissue to investigate the antioxidant property of ethyl pyruvate. GSH levels in the treatment group were found to be significantly higher than in the plain colitis group. Shanmugam et al. (22) in their study investigating the effects of *Passiflora subpeltata* Ortega on experimental colitis, found that the GSH levels, which decreased significantly in the colitis group, increased with the administration of *Passiflora subpeltata* Ortega depending on the dose they applied. Kuralay et al. (30) reported that CAT activity did not change with colitis in acetic acid-induced colitis model. In the study conducted by Yildiz et al. (31), it was found that there was no significant difference in CAT levels between the colitis group and the treatment group in which resveratrol was applied in the colitis model induced by TNBS. In our study, no statistically significant difference was found between the groups in terms of CAT levels. The inability to detect a change in CAT level is due to the more effective functioning of other cellular antioxidant systems. Şener et al. (32) investigated the antioxidant properties of BG and its protective role against oxidative organ damage in the heart, brain, kidney and liver in rats in which they caused sepsis as a result of cecal ligation and piercing and observed that it reduced TNF-α levels in the organs after sepsis. Kayali et al. (33) stated that β-glucan reduces oxidative damage in experimentally induced spinal cord injury, Şener et al. (34) investigated the protective and therapeutic effects of β-glucan on bladder and kidney tissues in their study of nicotine-induced oxidative damage. They found that the events increase the activities of antioxidant enzymes. Tatli-Seven et al. (35) investigated the effects of BG against lead acetate-induced hepatic damage and reproductive damage and determined that it showed protective and healing properties against different tissues and organs.

CONCLUSION

In the results obtained in our study, the antioxidant effect of β-glucan was found to be compatible with the literature. BG increased the level of glutathione, which is an indicator

of antioxidant parameter, decreased the levels of MDA and MPO, and caused improvement in histopathological scores. In this way, it has been shown by our study that BG can be a therapeutic agent. Treatment with BG has the potential to reduce colon tissue damage in TNBS-induced colitis in rats. Therefore, with these features, BG can be considered as a new and effective adjunctive therapy in the prevention and treatment of IBD.

Ethics Committee Approval: The study was approved by the Ethics Committee of Aydin Adnan Menderes University (14.08.2015, 64583101/2015/094).

Conflict of Interest: None declared by the authors.

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Evaluation of the Risk Factors for Mechanical Failure of Intertrochanteric Femoral Fracture Treated with Intramedullary Nailing

İntramedüller Çivileme ile Tedavi Edilen İntertrokanterik Femur Kırıklarında Mekanik Yetmezliğe Etki Eden Risk Faktörlerinin Değerlendirilmesi

Samet ERİNÇ
0000-0002-6494-6415
Mustafa Hacı ÖZDEMİR
0000-0001-6189-3605

Department of Orthopedics and Traumatology, Şişli Hamidiye Etfal Research and Training Hospital, İstanbul, Turkey

Corresponding Author
Sorumlu Yazar
Samet ERİNÇ
sameterinc@gmail.com

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ABSTRACT

Aim: The aim of this study was to evaluate the radiological and clinical risk factors predisposing the proximal lag screw to mechanical failure in patients with pertrochanteric femur fractures treated with intramedullary nailing.

Material and Methods: All intertrochanteric fracture cases were evaluated retrospectively and 298 patients (24 had mechanical failure) were included in this study. The patients were compared in terms of demographic data, Singh index, reduction quality according to the Baumgaertner scale, proximal lag screw position according to Cleveland-Bosworth quadrants and the Parker ratio, and the calcar femorale restoration and tip-apex distance.

Results: There was no statistically significant difference in terms of gender (p=0.745), age (p=0.848), American Society of Anesthesiology scores (p=0.725), body mass index (p=0.648) and Singh index (p=0.119) between the two groups. There were statistically significant differences between the two groups in terms of the following variables; number of patients with unstable fracture (p<0.001), poor reduction quality (p<0.001), calcar femorale discontinuity (p<0.001), center-center/center-inferior lag screw position (p<0.001), and Parker ratio on the lateral view (p=0.002). The center-center/center-inferior lag screw position, good reduction quality and calcar femorale restoration were found to be parameters predicting superior outcomes according to logistic regression analyses.

Conclusion: From the results of this study, it was concluded that although the preoperative reduction of the fracture and tip-apex distance are mandatory to prevent failure of the proximal lag screw, posteromedial discontinuity and lag screw position have a vital role in the treatment of interochanteric femur fracture fixed with intramedullary nailing.

Keywords: Intertrochanteric femoral fracture; intramedullary nailing; proximal femoral nailing.

ÖZ

Amaç: Bu çalışmanın amacı intramedüller çivileme ile tedavi edilen pertrokanterik femur kırıklı hastalarda proksimal çektirme vidasının mekanik yetmezliğine sebep olabilecek radyolojik ve klinik risk faktörlerini değerlendirmektir.

Gereç ve Yöntemler: Tüm intertrokanterik kırık vakaları retrospektif olarak değerlendirildi ve 24'ünde mekanik yetmezliği olan 298 hasta bu çalışmaya dahil edildi. Hastalar, demografik veriler, Singh indeksi, Baumgartner skalasına göre redüksiyon kalitesi, Cleveland-Bosworth kadranlarına ve Parker oranı göre proksimal çektirme vida pozisyonu, femoral kalkarın restorasyonu ve tip-apeks mesafesi açısından karşılaştırıldı.

Bulgular: İki grup arasında cinsiyet (p=0.745), yaş (p=0.848), Amerikan Anesteziyoloji Derneği (American Society of Anesthesiology) skorları (p=0.725), vücut kitle indeksi (p=0.648) ve Singh indeksi (p=0.119) açısından istatistiksel olarak anlamlı bir farklılık yoktu. Aşağıdaki değişkenler açısından iki grup arasında istatistiksel olarak anlamlı farklılıklar vardı; stabil olmayan kırığı olan hasta sayısı (p<0,001), kötü redüksiyon kalitesi (p<0,001), femoral kalkar restorasyonu (p<0,001), merkez-merkez/merkez-alt çektirme vidası pozisyonu (p<0,001) ve lateral görünümde Parker oranı (p=0,002). Lojistik regresyon analizine göre merkez-merkez/merkez-alt çektirme vidası pozisyonu, iyi redüksiyon kalitesi ve femoral kalkar restorasyonunun üstün sonuçları öngören parametreler olduğu saptandı.

Sonuç: Bu çalışmanın sonuçlarından; proksimal çektirme vidasının mekanik yetmezliğinin önlenebilmesi için kırığın redüksiyon kalitesine ve uç-apeks mesafesine dikkat edilmesinin zorunlu olması ile birlikte, intramedüller çivileme ile sabitlenen interochanterik femur kırığı tedavisinde posteromedial kalkar restorasyonunun ve çektirme vidasının femur başı içindeki konumunun ciddi bir role sahip olduğu sonucuna varıldı.

Anahtar kelimeler: Intertrokanterik femoral kırık; intrameduller çivileme; proksimal femoral çivileme.

INTRODUCTION

Osteoporotic hip fractures are one of the major health problems in the growing elderly population worldwide. In the 2000s, the estimated number of osteoporotic fractures throughout the world was 9 million, of which 1.9 million were hip fractures (1). Pertrochanteric femur fractures, which comprise half of all hip fractures, occur between the extracapsular segment of the femoral neck and the distal segment of the lesser trochanter (2). The high mortality and morbidity rates of patients with hip fractures necessitate the successful treatment of these types of fractures.

Selection of the fixation method and device depends on the fracture behavior, which may be stable or unstable. There is a general tendency to use an intramedullary nail for the treatment of an unstable intertrochanteric femur fracture. Intramedullary nails prevent the mechanical failure of unstable fractures which have a severely comminuted medial wall and/or a reverse oblique fracture line. Other mechanical advantages of the nails are the provision of a buttress effect when the lateral wall is missing and resistance to medialization of the proximal fragment. Many prospective randomized trials have shown better preservation of the reduction with intramedullary nails (3-5). However, proper reduction and correct application of the proximal femoral nail have a vital role in preventing complications. Mechanical failure after proximal femoral nailing remains a major complication with rates of 4 - 14% (6,7). The most evaluated risk factors are tip-apex distance, reduction quality, and position of the proximal lag screw in the femoral head (8,9).

The aim of this study was to evaluate the radiological and clinical risk factors predisposing the proximal lag screw to mechanical failure in patients with pertrochanteric femur fractures treated with intramedullary nailing.

MATERIAL AND METHODS

Approval for this retrospective study was granted by the Institutional Ethics Committee Board (11.02.2020/1431, Şişli Hamidiye Etfal Training and Research Hospital). A total of 586 consecutive patients were identified who were admitted to our clinic with a pertrochanteric femur fracture between January 2013 and January 2019. Exclusion criteria were age >60 years, fracture fixation other than cephalomedullary nailing, pathological fracture, multiple traumas, high energy trauma, mortality in the early postoperative period, and less than 6 months of regular follow-up examinations. After the application of exclusion criteria, 298 patients were found to be eligible for this study. Demographic data, American Society of Anesthesiology (ASA) scores, and Singh index values were collected from the hospital database.

Both pre-operative and post-operative radiographs were evaluated. The follow-up anteroposterior (AP) and lateral radiographs taken on postoperative day 1, then at 1, 3, 6, and 12 months were also evaluated. If there was no regular follow-up after 6 months, the last radiological examination was evaluated. The evaluations were made by two experienced orthopedic surgeons at the same time with consensus.

Reduction and internal fixation were performed in the supine position under fluoroscopic examination. No patient received an open reduction, and all fractures was reduced by closed fracture reduction techniques. For fixation, the proximal femoral nail system (Tasarimmed PN-1, Istanbul, Turkey) was used, which has lengths of 170, 200, and 230 mm. All patients received the standard postoperative protocol of protected weight-bearing with a walker or walking stick immediately postoperatively, and gradual progression to full weight-bearing within 3 months.

The Arbeitsgemeinschaft für Osteosynthesefragen / Orthopaedic Trauma Association (AO/OTA) classification systems were used to classify the fractures. According to the Fracture and Dislocation Classification Compendium (2018), if the lateral wall thickness is <20.5 mm, which is measured from 3 cm below the innominate tubercle of the greater trochanter and angled 135° upward to the fracture line (Figure 1), the fracture was considered an AO A2 type fracture (10). Reduction quality and posteromedial support were also evaluated. Reduction quality was classified as good if both of the following criteria were met; neck-shaft angle of 1250 - 1450 on the AP view, <200 angulation on the lateral view and displacement of <4 mm (11). Posteromedial support was determined according to bone to bone continuity on the postoperative x-ray, loss of bone stock due to displaced trochanter minor fracture was determined as lack of posteromedial support (12, Figure 2). The tip-apex distance of the proximal lag screw was measured as described by Baumgaertner et al. (9) and proximal lag screw placement was analyzed according to Cleveland-Bosworth quadrants and Parker ratio (13, Figure 3).

The patients were separated into two groups; patients with screw cut-out as the failure group and patients with uneventful healing group as the control group. All the radiological parameters (described above), demographic data, Singh index for osteoporosis evaluation and ASA scores were examined in the analyses and compared between the two groups.

Statistical Analyses

Data obtained in the study were analyzed statistically using SPSS v.15.0 software. Mean, standard deviation, median, minimum, maximum values, frequency and percentage were used for descriptive statistics. Kolmogorov-Smirnov test was performed to examine normal distribution. Comparisons of two independent groups were made with the Mann-Whitney U test since the numerical variables did not meet the normal distribution. Categorical variables were compared with the Pearson chi-square and Fisher's exact test. Mechanical failure was used as a dependent variable in the multivariate logistic regression analyses. The independent covariates were reduction quality (good or poor), tip-apex distance, the presence of posteromedial continuity, lag screw location (at the center-center and inferior-center or not) and Singh index. The odds ratio and 95% confidence intervals were calculated. Statistical significance level was accepted as $p<0.05$.

RESULTS

Of the 298 patients, cut-out of the proximal lag screw was observed in 24 (8%) patients. Cut-out was observed within 3 months postoperatively, especially immediately after full weight-bearing on the fractured side. There was no statistically significant difference in terms of gender, age, ASA scores, body mass index (BMI), and Singh index

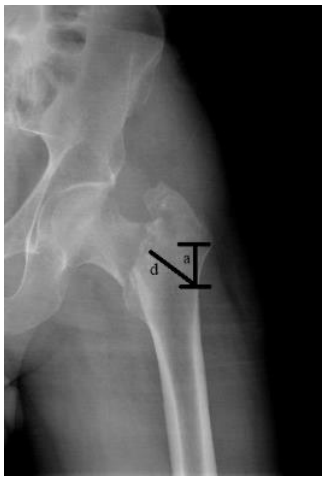

Figure 1. Measurement of lateral wall thickness according to Arbeitsgemeinschaft für Osteosynthesefragen / Orthopaedic Trauma Association Fracture and Dislocation Classification Compendium (2018) (a=3 cm, d=lateral wall thickness)

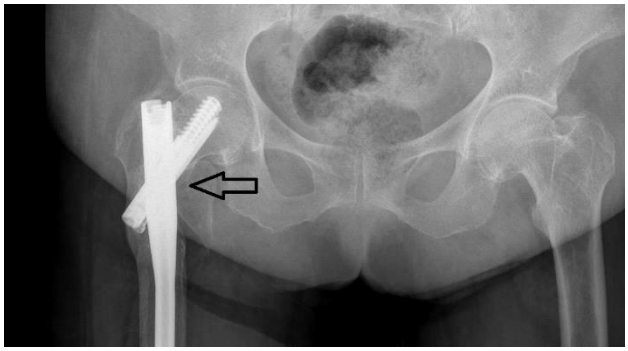

Figure 2. Lack of posteromedial support after fracture reduction

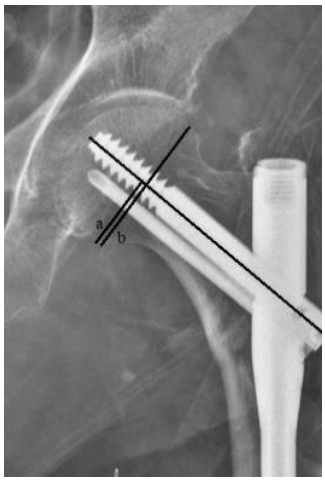

Figure 3. Measurement of the Parker ratio on the anteroposterior view (a/b x 100)

between two groups (Table 1). There were 14 (58.4%) female and 10 (41.6%) male patients in the failure group and 141 (51.5%) female, and 133 (48.5%) male patients in the control group (p=0.745). The median age was 76.1 years in the failure group and 76.6 years in the control group (p=0.848). The median Singh index score was 3 in both groups (p=0.119).

AO type 31-A1 fracture was determined in 128 (46.7%) patients in the control group, and in 3 (12.5%) patients in the failure group. AO type 31-A2 fracture, which implies lateral wall thickness <20.5 mm was seen in 17 (70.8%) patients in the failure group, and in 141 (51.5%) patients in the control group. AO type 31-A3 fracture was determined in 4 (16.7%) patients in the failure group, and in 5 (1.8%) patients in the control group. There were statistically significant differences in the fracture types of the patients between the two groups (p<0.001, Table 1).

The median tip-apex distance was 15.5 (12 - 26) mm in the failure group and 14.4 (8 - 28) mm in the control group, and the difference was statistically significant (p=0.004). According to Cleveland-Bosworth quadrants, the lag screws were in the center-center / center-inferior (zone 5 and 8) of the femoral head in 12 (50.0%) patients in the failure group (Table 2), and in 215 (78.5%) patients in the control group (Table 3). There was a statistically significant difference between the two groups in terms of the distribution of the proximal lag screws according to Cleveland-Bosworth quadrants (p<0.001, Table 1). The median Parker ratio was statistically significantly higher in the failure group than in the control group on lateral views (0.51 vs. 0.48, p=0.002), but there was no statistically significant difference between the two groups on the AP views (0.48 vs. 0.49, p=0.489).

When the reduction quality was assessed, poor reduction was seen in 9 (37.5%) patients in the failure group and in 19 (6.9%) patients in the control group, and the difference between the groups was statistically significant (p<0.001). The posteromedial cortical continuity was also evaluated with reduction quality and cortical discontinuity was found to be statistically higher in the failure group than in the control group (p<0.001, Table 1).

Multivariate logistic regression demonstrated that peripheral location of the lag screw (OR: 10.935; %95 CI: 2.864-41.761, p<0.001), reduction quality (OR: 6.544; %95 CI: 1.274-33.608, p=0.024), and posteromedial continuity (OR: 14.836; %95 CI: 2.925-75.239, p=0.001), were the most important factors in the mechanical failure. According to the univariate analyses, the number of patients with unstable fracture and tip-apex distance was statistically higher in the failure group than in the control group, although no statistically significant difference was found in the multivariate analyses (Table 4).

DISCUSSION

The results of this study demonstrated that the peripheral location of the lag screw, posteromedial discontinuity and reduction quality were the most important factors in the cut-out risk of proximal lag screw. Mechanical failure was seen in 24 (8%) patients, which was consistent with previous studies (14,15). In the literature, it has been strongly advocated that the risk of mechanical failure increases with peripheral location of the femoral lag screw with higher tip-apex distance, although the risk decreases in patients with a proximal lag screw supported by the calcar femorale and located at the center-center (AP-lateral views) or inferior-center part of the femoral head (16,17). One of the earlier studies on proximal femoral nails stated that the major factor influencing cut-out was lag screw position rather than tip-apex distance and the second distal quarter of the femoral head regarding middle central-neck axis was demonstrated as the 'safe zone' (14). In a cadaveric study, it was demonstrated that the inferior-center position of the lag screw provides better angular and rotational stability due to stronger support of the calcar femorale and posteromedial cortex than the other positions of the lag

screw (17). In the current study, 50% (n=12) of the patients in the failure group had a lag screw in the center-center or inferior-center position, while in the control group the rate of lag screw in the center-center or inferior-center position was 78.5% (n=215). Furthermore, the Parker ratio on the lateral view was statistically significantly higher in the failure group than in the control group which implied more posterior positioning of the lag screw in the failure group (0.51 vs 0.48). Many studies have suggested that the posterior location of the proximal lag screw on the

Table 1. Comparison of demographic and clinical characteristics

	Failure (n=24)	Control (n=274)	p
Age, median (IQR) [min-max]	76.1 (10.7) [70-86]	76.6 (15.2) [41-109]	0.848
Gender, n (%)			
Male	10 (41.6%)	133 (48.5%)	0.745
Female	14 (58.4%)	141 (51.5%)	
AO Type, n (%)			
A1	3 (12.5%)	128 (46.7%)	
A2	17 (70.8%)	141 (51.5%)	**<0.001**
A3	4 (16.7%)	5 (1.8%)	
Tip-Apex Distance, median (IQR) [min-max]	15.5 (7) [12-26]	14.4 (4) [8-28]	**0.004**
Screw Placement, n (%)			
1	0 (0.0%)	3 (1.1%)	
2	3 (12.5%)	9 (3.3%)	
3	3 (12.5%)	3 (1.1%)	
4	2 (8.4%)	15 (5.5%)	
5	11 (45.8%)	172 (62.8%)	**<0.001**
6	3 (12.5%)	18 (6.6%)	
7	1 (4.2%)	5 (1.8%)	
8	1 (4.2%)	43 (15.7%)	
9	0 (0.0%)	6 (2.2%)	
Parker Ratio (Lateral), median (IQR) [min-max]	0.51 (0.09) [0.41-0.72]	0.48 (0.10) [0.21-0.72]	**0.002**
Parker Ratio (AP), median (IQR) [min-max]	0.48 (0.06) [0.12-0.72]	0.49 (0.05) [0.20-0.69]	0.489
Reduction, n (%)			
Good	15 (62.5%)	255 (93.1%)	**<0.001**
Poor	9 (37.5%)	19 (6.9%)	
PMC, n (%)			
Positive	16 (66.7%)	268 (97.8%)	**<0.001**
Negative	8 (33.3%)	6 (2.2%)	
ASA, median (IQR) [min-max]	3 (1) [2-4]	3 (1) [1-4]	0.725
ASA, n (%)			
1	0 (0.0%)	3 (1.1%)	
2	6 (25.0%)	88 (32.1%)	0.701
3	14 (58.3%)	126 (46.0%)	
4	4 (16.7%)	57 (20.8%)	
Singh Index, median (IQR) [min-max]	3 (1) [2-4]	3 (2) [2-5]	0.119
Body Mass Index, median (IQR) [min-max]	23 (3) [19-27]	24 (4) [15-28]	0.648
Length of Nail, n (%)			
170	2 (8.3%)	0 (0.0%)	
200	1 (4.2%)	66 (24.1%)	0.102
230	21 (87.5%)	208 (75.9%)	

AO: Arbeitsgemeinschaft für Osteosynthesefragen, AP: anteroposterior, PMC: Posteriomedial continuity, ASA: American Society of Anesthesiology, IQR: interquartile range

Table 2. The distribution of failed proximal lag screws according to Cleveland-Bosworth quadrants

	Superior		
Posterior	Zone 1 0 (0.0%)	Zone 2 3 (12.5%)	Zone 3 3 (12.5%)
	Zone 4 2 (8.4%)	Zone 5 11 (45.8%)	Zone 6 3 (12.5%)
	Zone 7 1 (4.2%)	Zone 8 1 (4.2%)	Zone 9 0 (0.0%)
	Inferior		Anterior

Table 3. The distribution of the proximal lag screws in the control group according to Cleveland-Bosworth quadrants

	Superior		
Posterior	Zone 1 3 (1.1%)	Zone 2 9 (3.3%)	Zone 3 3 (1.1%)
	Zone 4 15 (5.5%)	Zone 5 172 (62.8%)	Zone 6 18 (6.6%)
	Zone 7 5 (1.8%)	Zone 8 43 (15.7%)	Zone 9 6 (2.2%)
	Inferior		Anterior

Table 4. Results of the logistic regression analysis

	OR (%95 CI)	p
Gender (Female)	3.814 (0.961-15.138	0.057
Screw Position (Peripheral)	10.935 (2.864-41.761)	**<0.001**
AO Type (A2)	2.579 (0.674-9.860)	0.166
AO Type (A3)	10.024 (1.018-98.696)	0.068
Reduction (Good)	6.544 (1.274-33.608)	**0.024**
PMC (Negative)	14.836 (2.925-75.239)	**0.001**
Tip-Apex Distance	1.117 (0.984-1.268)	0.086
Singh Index	1.179 (0.566-2.458)	0.660

OR: odds ratio, CI: confidence interval, AO: Arbeitsgemeinschaft für Osteosynthesefragen, PMC: Posteriomedial continuity

lateral view with >0.5 Parker ratio was associated with higher tip-apex distance and an increased risk of mechanical failure, especially in unstable intertrochanteric femoral fractures (18,19).

The importance of the tip-apex distance and proximal lag screw position in the femoral head have been demonstrated since the earliest reports of clinical and radiological outcomes of the intertrochanteric femoral fracture surgery (15). Baumgaertner et al. (9) described the tip-apex distance, which has become one of the most important factors in the treatment of intertrochanteric femoral fractures. It is generally accepted that a tip-apex distance >20-25 mm increases the risk of mechanical failure. In this study, although the median tip-apex distance was <25 mm in both groups, it was statistically significantly higher in the failure group. A more peripheral and posterior position of the proximal lag screw may have caused the higher tip-apex distance in the failure group (11).

Under axial loading, the calcar femorale redistributes the stress, and consequently, disruption of the calcar femorale results in collapse and varus displacement with axial loading of the femur (20). In 1949, Evans EM. (21) first emphasized the importance of providing posteromedial cortical continuity, otherwise, an unstable fracture will collapse into varus and rotational instability will occur after fixation of the femur. Many previous studies have reported that medial calcar discontinuity may lead to poor bone healing and mechanical failure. Furthermore, establishment of the medial calcar support has been linked to superior outcomes in the treatment of unstable intertrochanteric femoral fractures (22). Liang et al. (23) demonstrated that the highest stress occurred at the calcar femorale under axial loading. In another study, loss of posteromedial support was demonstrated as an independent factor for mechanical failure in unstable intertrochanteric fractures. Recently, fixation of the lesser tubercle has been advocated in patients with medial calcar discontinuity (24,25). In the current study, good reduction and posteromedial continuity were correlated with uneventful healing of the intertrochanteric femoral fracture. Loss of posteromedial continuity was found in 33.3% (n=8) of the patients in the failure group, although the rate was 2.2% (n=6) in the patients with uneventful healing and the poor reduction was observed in 9 (37.5%) patients in the failure group, while in 19 (6.9%) patients in the control group.

Lateral wall integrity and a superolateral buttress are also essential to provide mechanical stability in intertrochanteric fractures. An intact lateral wall prevents the excessive collapse and medialization of the distal fragment. (26,27). Tan et al. (28) conducted a study on the morphology and pitfalls of an unstable intertrochanteric femur fracture variant which was not well recognized in the existing classification systems. It was reported that the lack of a superolateral buttress was the main contributory factor to mechanical failure rather than medial calcar buttress. Another study by Tawari et al. (29) suggested that the existing fracture classifications, which classify fractures according to posteromedial comminution and the direction of the fracture line, are insufficient. Han et al. (30) proposed a comprehensive radiological examination including AP, lateral, and traction internal rotation views. It was suggested that 3D-CT should be obtained before any intervention in cases of highly comminuted fracture, and the type of lateral wall fracture according to the CT scan should be accepted for the classification of the fracture. In 2018, the Fracture and Dislocation Classification Compendium revised the AO classification of intertrochanteric fractures and emphasized the lateral wall thickness. The revised system separates pertrochanteric fractures as A1 or A2 according to the severity of the greater trochanteric fragmentation and lateral wall thickness. In this study, 70.8% (n=17) of the patients had type A2, and 16.7% (n=4) had type A3 fracture in the failure group, whereas these rates were 51.5% (n=141) and 1.8% (n=5) respectively in the control group. It was assumed that insufficiency in the lateral wall and entry point bone stock may cause lateralization of the nail and incorrect peripheral lag screw placement. In addition, lack of trochanteric stability and lateral wall integrity may lead to malreduction of the fracture intraoperatively or loss of reduction postoperatively (31). Yuan et al. (32) suggested using a supporting mini locking plate for fixation of the trochanter major fragment in the intramedullary nailing of pertrochanteric fractures. Kulkarni et al. (33) evaluated 154 patients with an unstable intertrochanteric fracture and suggested lateral wall reconstruction with a cerclage wire in patients with fragmented trochanter major and lateral wall. The mechanical failure rate was 7.8% in that study, but no cut-out was found in the patients with reconstructed lateral wall.

Osteoporosis has also been evaluated as a factor predicting mechanical failure. Akan et al. (34) found lower Harris hip scores after intramedullary femoral nailing surgery for intertrochanteric fracture in patients with high-grade osteoporosis (Singh index III-IV) compared to those with low-grade osteoporosis (Singh index III-IV). Barrios et al. (35) suggested that osteoporosis is one of the major factors for mechanical failure after intramedullary nailing surgery. In the current study, no statistically significant difference was found between the two groups in respect of osteoporosis.

The major limitation of this study was that although the patient follow-up examination findings were recorded prospectively, the study was conducted as a retrospective, non-controlled study. In addition, bone mineral density, which may be associated with bone fragility, was not examined and the sample size in the subgroups was small.

CONCLUSION

From the results of this study, it was concluded that although the preoperative reduction of the fracture and tip-apex distance are mandatory to prevent failure of the proximal lag screw, posteromedial discontinuity and lag screw position have a vital role in the treatment of interochanteric femur fractures fixed with intramedullary nailing. Furthermore, it can be emphasized that a lack of mechanical support at the entry point of the intramedullary nail and a severely comminuted pertrochanteric area should be considered important factors in the treatment of pertrochanteric femur fractures with intramedullary nailing.

Ethics Committee Approval: The study was approved by the Ethics Committee of Şişli Hamidiye Etfal Training and Research Hospital (11.02.2020, 1431).

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Investigation of Factors Affecting the Stone-Free Rate in Elderly Patients with Urinary Stones After Shock Wave Lithotripsy

İdrar Taşı Olan Yaşlı Hastalarda Şok Dalgası Litotripsi Sonrası Taşsızlık Oranını Etkileyen Faktörlerin Araştırılması

Alpaslan YÜKSEL
0000-0003-0076-4812
Dursun BABA
0000-0002-4779-6777
Yusuf ŞENOĞLU
0000-0002-3072-9252
Arda Taşkın TAŞKIRAN
0000-0003-4556-3475

Department of Urology, Düzce University Faculty of Medicine, Düzce, Turkey

Corresponding Author
Sorumlu Yazar
Alpaslan YÜKSEL
dralpyuksel@gmail.com

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ABSTRACT

Aim: Urinary stone disease is an important disease seen in all age groups, including elderly patients. It can cause kidney failure and urinary infection problems. Shock wave therapy, which is the minimal invasive among the different treatment alternatives, is preferred for kidney and proximal ureteral stones smaller than 2 cm. Studies on the efficacy and safety of this treatment in elderly patients are limited. This study aimed to investigate the factors affecting the stone-free rate (SFR) in elderly patients with urinary stones after shock wave lithotripsy.

Material and Methods: The data of 120 patients in the Urology department of Duzce University Faculty of Medicine between 2010 and 2018 over 65 years old who underwent extracorporeal shock wave lithotripsy (ESWL) for urinary system stone disease were evaluated retrospectively. The data obtained from these patients included sex, age, location of the stone (upper, middle, or lower calyx, renal pelvis, ureter), number of shock waves, stone size, and the need for the auxiliary procedure were analyzed.

Results: Of the 120 patients, 82 (68.3%) were male and 38 (31.7%) were female. Comorbidity was present in 49 patients. An overall SFR of 65.8% (n=79) was found. The highest SFR was found in middle calyx stones with 79.3% (23 of the 29 patients). Post-ESWL auxiliary procedures were needed in 36 (30%) patients. Two patients developed subcapsular renal hematoma and pyelonephritis.

Conclusion: ESWL is an appropriate even the first option for elder male with favorable stone size. Furthermore, ESWL caused acceptable morbidity in older patients.

Keywords: comorbidities; elderly; extracorporeal shock wave lithotripsy; urolithiasis.

ÖZ

Amaç: Üriner taş hastalığı, yaşlı hastalar da dahil olmak üzere tüm yaş gruplarında görülen önemli bir hastalıktır. Böbrek yetmezliği ve idrar yolu enfeksiyonu sorunlarına neden olabilir. Farklı tedavi alternatifleri arasında minimal invaziv olan şok dalga tedavisi böbrek ve proksimal üreter taşlarında 2 cm'den küçük olanlarda tercih edilmektedir. Yaşlı hastalarda bu tedavinin etkinliği ve güvenliği ile ilgili çalışmalar sınırlıdır. Bu çalışmada, üriner taşları olan yaşlı hastalarda şok dalga litotripsi sonrası taşsızlık oranını (stone-free rate, SFR) etkileyen faktörlerin araştırılması amaçlanmıştır.

Gereç ve Yöntemler: 2010 ve 2018 yılları arasında Düzce Üniversitesi Tıp Fakültesi Üroloji Anabilim Dalı'nda üriner sistem taş hastalığı nedeniyle ekstrakorporeal şok dalga litotripsi (extracorporeal shock wave lithotripsy, ESWL) uygulanan 65 yaş üstü 120 hastanın verileri retrospektif olarak değerlendirildi. Bu hastalardan elde edilen cinsiyet, yaş, taşın bulunduğu yer (üst, orta veya alt kaliks, renal pelvis, üreter), şok dalgalarının sayısı, taş boyutu ve yardımcı prosedüre duyulan ihtiyacı içeren veriler analiz edildi.

Bulgular: 120 hastanın 82'si (%68,3) erkek, 38'i (%31,7) kadındı. 49 hastada komorbidite mevcuttu. Toplam SFR %65,8 (n=79) olarak bulundu. En yüksek SFR %79,3 (29 hastanın 23'ü) ile orta kaliks taşlarında bulundu. 36 (%30) hastada ESWL sonrası yardımcı prosedürlere ihtiyaç duyuldu. İki hastada subkapsüler renal hematom ve piyelonefrit gelişti.

Sonuç: ESWL, uygun taş boyutuna sahip yaşlı erkekler için bile ilk seçenektir. Ayrıca, ESWL yaşlı hastalarda kabul edilebilir morbiditeye neden olmuştur.

Anahtar kelimeler: komorbidite; yaşlı; ekstrakorporal şok dalga tedavisi; urolitiyazis.

INTRODUCTION

Urinary system stone disease is an important disease whose incidence has increased significantly in recent years and is thought to affect approximately 11% of the world population (1). Urinary system stone disease can be seen in different age groups, including the elderly patients (2). It is predicted that the number of individuals over the age of 65 will double in 2050. The ratio of urinary stone disease in elderly patients will increase in proportion to this number (3). The handicap of elderly individuals is that they are more fragile due to comorbidity and are prone to physical and psychological weakness (4). Although urinary system stone disease mainly affects adults aged 20-60 years old, elderly patients over the age of >65 constitute 10% to 20% of all stone patients (5-7). Elderly patients have an increased risk of kidney stone formation due to slowing metabolism, taking drugs and vitamin supplements that change their metabolic profiles, and decreasing water consumption (8).

Medical expulsive therapy (MET) is recommended for patients presenting with stones smaller than 5 mm in size with oral fluid intake and non-steroidal anti-inflammatory drugs (NSAIDs). Calcium channel blockers or α-adrenergic antagonists that provide ureteral smooth muscle relaxation are added to the treatment. However, compared with younger patients, elderly patients with stones are more likely to fail MET therapy and require surgical intervention (9).

There are different alternatives in the treatment of urinary system stone disease, and the scale of the treatment ranges from minimally invasive methods to invasive methods. Percutaneous nephrolithotomy is the standard treatment for patients with kidney stones >2 cm, while ureteroscopy or extracorporeal shock wave lithotripsy (ESWL) is recommended for smaller stones (10). Each treatment alternative has its advantages and disadvantages, especially in the elderly population. ESWL is the preferred method, especially in renal and ureteral stones smaller than 2 cm (11). While the current stone treatment guidelines guide in managing adult patients, they are not very clear in elderly patients (12). Data on the efficacy of ESWL treatment in elderly patients with stones are limited in the literature. We aimed to research the factors related to the stone-free rate (SFR) in elderly patients with urinary stones after shock wave lithotripsy.

MATERIAL AND METHODS

In this study, the records of 120 patients over 65 years old who received ESWL treatment in the Urology department of Duzce University Faculty of Medicine between 2010 and 2018 were retrospectively reviewed. During this period, 1706 patients underwent ESWL and 7% were over 65 years old. Medical history, physical examination, urinalysis, urine culture and creatinine values of the patients were recorded. The data obtained from these patients included sex, age, location of the stone (ureter, lower calyx, middle calyx, upper calyx or renal pelvis), number of shock waves, size of the stone, and the need for the auxiliary procedure were analyzed.

The stones of the patients were evaluated with intravenous pyelography (IVP) or computed tomography (CT) of the abdomen. All patients were required to have stones of less than 20 millimeters in the kidney or proximal ureter, radiopaque on X-ray imaging, and no contraindications for ESWL. Patients with suspected ureteral stenosis, coagulopathies and dysfunctional kidneys were excluded from the study.

Comorbidities of the patients were recorded. Anticoagulant drugs of the patients were discontinued seven days before and 60 mg of enoxaparin sodium was started. The same protocol was applied to all patients with anticoagulant medication. Half an hour before the procedure, i.m. 75 mg of diclofenac sodium was administered to all patients for analgesia. PCK Stonelith-V3 brand ESWL device was applied to all patients in each session. The average number of shocks applied per stone in one session was 2800±500; the average voltage was 18.4±1.2 kV. One week after each ESWL session, the form of the stone was evaluated by direct X-ray or ultrasound. The absence of any stones or presence of ≤4 mm fragments was considered stone-free, which means ESWL success and the presence of >4 mm fragments of stone were considered ESWL failure. Re-treatment was performed if >4 mm fragments of stone were observed. If there was no response after repetitive sessions, the case was considered as ESWL failure. Follow-up was done by kidney, ureter, and bladder X-ray and renal ultrasound every week until ESWL success. All data were collected and analyzed after the 2-month.

The study protocol was approved by the institutional ethics committee, Non-interventional Clinical Research Ethics Committee of Duzce University (06.10.2015, 51).

Statistical Analysis

Normality assumption was examined with the Kolmogorov-Smirnov test. Comparisons of continuous variables between groups were done with the Independent samples t-test or Mann-Whitney U test, according to the normality assumption. Categorical data were analyzed with Pearson chi-square or Fisher's exact test, as appropriate. Descriptive statistics were given as mean±standard deviation or median, interquartile range, and minimum-maximum values for numerical variables, while categorical variables were presented as number and percentage. IBM SPSS v.22 statistical package was used for statistical analyses and a p-value of 0.05 was considered as statistical significance level.

RESULTS

A total of 120 patients over 65 years of age were included in this study. The mean age of the patients was 66.77±2.04 years, with a maximum of 79 years. Of the 120 patients, 82 (68.3%) were male and 38 (31.7%) were female. All patients had only one stone. A total of 46 (38.3%) patients had past histories of urolithiasis. Total of 56 comorbidities following were present in 49 patients; arterial hypertension (HT) in 32 (26.7%), diabetes mellitus (DM) in 15 (12.5%), coronary artery disease (CAD) in 5 (4.2%), and the other comorbidities in 4 (3.3%) were recorded. The details of the patients are summarized in Table 1.

The mean length of the stone sizes was 10.17±3.38 mm, and the longest stone size was 21 mm. A SFR of 65.8% (n=79) was found. The highest SFR with a rate of 79.3% (23 of the 29 patients) was found in middle calyx stones (Table 2).

A comparison of demographic and clinical characteristics of the patients with and without successful treatment was given in Table 3. There was no statistically significant difference in SFR of the patients, in terms of gender (p=0.684), comorbidity (p=0.495), smoking (p=0.659), stone history (p=0.911), family history (p=0.763), creatinine level of ≥1.2 (p=0.683), stone side (p=0.760) and stone location (p=0.228).

While there was no statistically significant difference was found between the patients with successful and unsuccessful treatment in terms of age (p=0.284) and creatinine level (p=0.874), stone size (p=0.022) and the number of ESWL sequences (p=0.008) were higher in patients with unsuccessful treatment (Table 4).

Auxiliary procedures were needed in 36 (30%) of the patients in this study. Ureterorenoscopy was performed in 23 (63.9%) patients, percutaneous nephrolithotomy was performed in 9 (25%) and double J stent was placed in 4 (11.1%) patients. Despite successful ESWL treatment, a double J stent was placed in 2 patients and ureterorenoscopy was performed in 2 patients due to ureteral obstruction.

Subcapsular renal hematoma and pyelonephritis developed in 2 patients in terms of major complications. The patients were hospitalized and no surgical intervention was needed in follow-up.

Table1. Characteristics of the patients and the stone (n=120)

Age (years), mean±SD (min-max)	66.77±2.04 (65-79)
Gender, n (%)	
Male	82 (68.3)
Female	38 (31.7)
Comorbidities, n (%)	
HT	32 (26.7)
DM	15 (12.5)
CAD	5 (4.2)
Other	4 (3.3)
Smoking, n (%)	35 (29.2)
Stone history, n (%)	46 (38.3)
Family history, n (%)	16 (13.3)
Creatinine ≥1.2, n (%)	29 (24.2)
Side, n (%)	
Left	65 (54.2)
Right	55 (45.8)
Location, n (%)	
Upper	6 (5.0)
Middle	29 (24.2)
Lower	20 (16.7)
Pelvis	17 (14.2)
Proximal	48 (40.0)
Stone size (mm), mean±SD (min-max)	10.17±3.38 (4-21)

HT: hypertension, DM: diabetes mellitus, CAD: coroner artery disease, SD: standard deviation

Table 2. The relationship between stone location and stone-free rate, n (%)

Stone location	Total	Stone-free rate
Upper calyx	6 (5.0%)	3 (50.0%)
Middle calyx	29 (24.2%)	23 (79.3%)
Lower calyx	20 (16.7%)	15 (75.0%)
Kidney pelvis	17 (%14.2)	9 (%52.9)
Proximal ureter	48 (%40.0)	29 (%60.4)
Total	120 (%100)	79 (%65.8)

Table 3. Comparison of stone free rates according to the demographic and clinical characteristics of the patients

Characteristics	n	SFR	p
Gender, n (%)			
Male	82	53 (64.6)	0.684
Female	38	26 (68.4)	
Comorbidity, n (%)			
No	71	45 (63.4)	0.495
Yes	49	34 (69.4)	
DM, n (%)			
No	105	69 (65.7)	0.942
Yes	15	10 (66.7)	
HT, n (%)			
No	88	58 (65.9)	0.977
Yes	32	21 (65.6)	
CAD, n (%)			
No	115	77 (67.0)	0.337
Yes	5	2 (40.0)	
Other, n (%)			
No	116	75 (64.7)	0.298
Yes	4	4 (100)	
Smoking, n (%)			
No	85	57 (67.1)	0.659
Yes	35	22 (62.9)	
Stone history, n (%)			
No	74	49 (66.2)	0.911
Yes	46	30 (65.2)	
Family History, n (%)			
No	104	69 (66.3)	0.763
Yes	16	10 (62.5)	
Creatinine, n (%)			
<1.2	91	59 (64.8)	0.683
≥1.2	29	20 (69.0)	
Side, n (%)			
Left	65	42 (64.6)	0.760
Right	55	37 (67.3)	
Location, n (%)			
Upper	6	3 (50.0)	
Middle	29	23 (79.3)	
Lower	20	15 (75.0)	0.228
Pelvis	17	9 (52.9)	
Proximal	48	29 (60.4)	

SFR: stone free rate, HT: hypertension, DM: diabetes mellitus, CAD: coroner artery disease

Table 4. Comparison of age, creatinine, stone size and number of ESWL sequence of the patients with and without successful treatment

	SFR (-) (n=41)	SFR (+) (n=79)	p
Age (years)	66.49±1.52	66.91±2.27	0.284
Creatinine (mg/dL)	0.91±0.28	0.92±0.33	0.874
Stone size (mm)	11.15±3.13	9.66±3.42	**0.022**
Number of ESWL	3 (3) [1-8]	2 (1) [1-8]	**0.008**

SFR: stone free rate, ESWL: extracorporeal shock wave lithotripsy

DISCUSSION

Since ESWL is a less invasive procedure and usually does not require anesthesia, it has been considered a suitable treatment alternative for elderly patients (1). In many studies, the incidence of urolithiasis in elderly patients has been reported to be between 7.1 and 23.1% (13,14). Elderly patients constituted 7% of all patients who underwent ESWL in our study. In the study of Polat et al. (13) this rate was found to be 13.6%. Ureteral stone location in this study had the worst SFR, but kidney stones had satisfactory SFR. When the SFR success was evaluated according to the stone localization, the highest success was obtained in the middle calyx stone group (79.3%), and the lowest success was obtained in the upper calyx stones (50%) in the present study. In some studies, SFR after ESWL treatment was higher in younger patients than older patients. An early report evaluating prognostic factors for treatment outcome of kidney stones found that patients over 60 years of age had the lowest SFR of all age groups (15). It was thought that the reason for this might be physiological changes due to senility with a decrease in renal parenchyma thickness and in GFR (14). In some studies, SFR rates after ESWL applied in elderly patients have been reported between 52.1% and 63.5% (16,17). In our study, this rate was 65.8% and correlated with these results (10,11).

When the factors related to SFR such as age, creatinine value, stone size and the number of sessions were compared, the effect of stone size and the number of sessions on SFR was statistically significant. In the study of Abdel-Khalek et al. (18) stone site, stone size and the existence of a ureteral stent had a significant effect on the success rate. In their study, 28 (3%) patients required auxiliary procedures to relieve obstruction and remove residual fragments. Auxiliary procedures were needed in 36 (30%) of the patients after ESWL in our study. This difference is due to the wide age scale (range 5 to 75 years) of the patients in the study of Abdel-Khalek et al. (18).

No procedure was interrupted due to any serious major complications. Mild subcapsular hematoma and pyelonephritis were detected in two patients. They were hospitalized and followed up with appropriate treatment and discharged without any problems. In a study by Polat et al. (13), subcapsular hematoma was found in 3 (1.2%) patients and pyelonephritis was found in one (0.4%) patient. Comorbidity was high in this age group expectedly. HT (26.7%), DM (12.5%) and CAD (4.2%) were the most common diseases. In the study of Lamacchia et al. (1), comorbidity rates including HT (66.7%), CAD (24%) and DM (18%) were found to be higher. The difference is due to the fact that the patients in this study were over 70 years of age. The relatively small number of patients and the retrospective design are the main limitations of this study. However, it should be considered that elderly patients receiving ESWL treatment constitutes a small part of the population.

CONCLUSION

In conclusion, stone size and the number of sessions was statistically significant on SFR in elderly patients. ESWL treatment should be considered an appropriate even the first option for elder male with favorable stone size in kidney and proximal ureteral stones. Furthermore, ESWL caused acceptable morbidity and high efficacy in older patients.

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Evaluation of Sense of Smell in Onychomycosis Patients Receiving Terbinafine Treatment

Terbinafin Tedavisi Alan Onikomikoz Hastalarında Koku Duyusunun Değerlendirilmesi

Mualla POLAT[1]
0000-0001-9226-5134
Tekden KARAPINAR[2]
0000-0003-3795-0731
Belgin KÜÇÜKYANGÖZ[1]
0000-0003-3877-918X
Yusuf Özgür BİÇER[3]
0000-0002-4646-7250
Özge UZUN[4]
0000-0003-1380-350X
Tuna SEZER[5]
0000-0002-6861-2869

[1]Department of Dermatology, Bolu Abant İzzet Baysal University, Faculty of Medicine, Bolu, Turkey

[2]Dermatology Clinic, Bingöl State Hospital, Bingöl, Turkey

[3]Department of Otorhinolaryngology, Bolu Abant İzzet Baysal University, Faculty of Medicine, Bolu, Turkey

[4]Department of Pharmacology, Arel University Faculty of Medicine, İstanbul, Turkey

[5]Dermatology Clinic, İzzet Baysal State Hospital, Bolu, Turkey

Corresponding Author
Sorumlu Yazar
Mualla POLAT
polatmualla@gmail.com

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ABSTRACT

Aim: The most common side effects of terbinafine are gastrointestinal symptoms. Loss of taste and smell side effects are presented in a small number of case reports. We aimed to measure the effect of terbinafine on the sense of smell and the degree of change in the sense of smell in this study.
Material and Methods: Odor identification test (OIT), odor threshold test (OTT), and odor discrimination test (ODT) were applied to the patients treated with terbinafine and the control group. It was applied with "Sniffin Sticks" test pens. Smell test was performed on the patient group just before the start of terbinafine treatment and at the end of 3 months of treatment.
Results: There was no statistical difference between the patient and control groups in terms of age (p=0.991) and gender (p=0.811). There was no statistical difference when the initial odor tests of the patient group and the control group were compared. The OIT value of the patient group was 10.15±1.43 at the beginning and 10.10±1.15 at the end of the treatment (p=0.743). The OTT values were 6.23±1.08 at the beginning and 6.21±0.85 at the end of the treatment (p=0.811). The ODT values were 9.71±1.44 at the beginning and 9.69±1.34 at the end of the treatment (p=0.767). There was no statistical difference in the results of the tests performed at the beginning and end of the treatment.
Conclusion: There was no adverse effect on the sense of smell associated with the use of systemic terbinafine treatment for 3 months.
Keywords: Terbinafine; onychomycosis; Sniffin'Sticks test; odor.

ÖZ

Amaç: Terbinafinin en sık karşılaşılan yan etkileri gastrointestinal şikayetlerdir. Tat ve koku kaybı yan etkisi ise az sayıda vaka sunumunda bildirilmiştir. Bu çalışmadaki amacımız terbinafinin koku duyusu üzerine etkisini ve koku duyusundaki değişimin derecesini ölçmektir.
Gereç ve Yöntemler: Terbinafin tedavisi başlanan hastalara ve kontrol grubuna koku tanımlama testi (KTT; odor identification test, OIT), koku duyarlılık testi (KDT; odor threshold test, OTT) ve koku ayırım testi (KAT; odor discrimination test, ODT) uygulandı. Koku testi "Sniffin Sticks" test kalemleri ile yapıldı. Hasta grubuna koku testi terbinafin tedavisi başlamadan hemen önce ve 3 aylık tedavi süresinin sonunda yapıldı.
Bulgular: Hasta ve kontrol grupları arasında yaş (p=0,991) ve cinsiyet (p=0,811) açısından istatistiksel olarak anlamlı bir fark saptanmadı. Hasta grubunun başlangıç koku testleri ve kontrol grubunun koku testleri karşılaştırıldığında da aralarında istatistiksel olarak anlamlı bir fark yoktu. Hasta grubunun KTT değeri tedavi başlangıcında 10,15±1,43 ve tedavi sonunda ise 10,10±1,15 olarak saptandı (p=0,743). KDT değerleri tedavi başlangıcında 6,23±1,08 ve tedavi sonunda ise 6,21±0,85 olarak tespit edildi (p=0,811). KAT değerleri tedavi başlangıcında 9,71±1,44 ve tedavi sonunda ise 9,69±1,34 olarak saptandı (p=0,767). Tedavi başında ve sonunda yapılan test sonuçlarında istatistiksel olarak anlamlı bir fark yoktu.
Sonuç: Sistemik terbinafin tedavisinin 3 ay süreyle kullanımına bağlı olarak koku duyusu üzerinde herhangi bir yan etki tespit edilmemiştir.
Anahtar kelimeler: Terbinafin; onikomikoz; Sniffin'Sticks tes; koku.

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INTRODUCTION

Terbinafine is a highly lipophilic synthetic allylamine antifungal drug that is widely used in the treatment of cutaneous dermatophyte infections. It has been used in topical and systemic forms since 1991 (1,2). It shows its effect by inhibiting the squalene epoxidase enzyme. It is an easily tolerated, effective agent used systemically for 3 months without interruption in the treatment of onychomycosis (3). Common side effects of terbinafine include gastrointestinal side effects such as abdominal pain and nausea (2). Skin reactions and cholestatic hepatitis are among the rare side effects that can be seen. In addition, loss of taste and smell has been reported among the rare side effects (4,5).

Loss of taste and smell are often overlooked as they are not life-threatening disorders, but they are senses that significantly affect quality of life. Numerous studies have been performed on the evaluation of olfactory functions and the conditions under which these functions are affected (6-8). Two types of tests are used for odor measurement: psychophysical (subjective) tests and electrophysiological (objective) tests. Psychophysical tests include the odor threshold test (OTT), the odor discrimination test (ODT), and the odor identification test (OIT). Sniffin' Sticks is a widely used psychophysical test. It has some advantages such as long shelf life, reusability, and relatively short application time (9).

In the literature, there are some studies which measuring the effect of terbinafine on the sense of taste, but there is no large-scale study investigating the effect on the sense of smell (4,10-13). We aimed to measure the effect of terbinafine that is commonly used drug in the treatment of onychomycosis on the sense of smell and the degree of change in the sense of smell.

MATERIAL AND METHODS

This prospective study was approved by Bolu Abant İzzet Baysal University Clinical Research Ethics Committee with the decision numbered 2017/84 and dated 14.09.2017, and was carried out between March 2018 and March 2019. The Sniffin Sticks Test (Burgart Medical Technology, Wedel, Germany) is a simple, easy-to-apply, standardized, and quantitative odor test. Sniffin Sticks Test was applied to the patients who diagnosed with onychomycosis and started on 250 mg/day terbinafine treatment due to onychomycosis and to the control group. The odor test was performed just at the start of terbinafine treatment and at the end of 3 months of treatment. All participants signed a written informed consent.

Patient Selection

The study was made with a patient group that older than 18 years old and younger than 65 years of age who were diagnosed with onychomycosis and started systemic terbinafine treatment and a control group which consisting of similar age and gender healthy volunteers. Before the patients and healthy volunteers were included in the study, psychiatric diseases such as depression, schizophrenia, alcoholism; drug intaking such as metronidazole, amphotericin B, captopril, ethacrynic acid, codeine; obstructive nasal and sinus disease, upper respiratory tract infection; surgical interventions such as total laryngectomy, previous head trauma, rhinoplasty, anterior skull base surgery, old age were questioned. Patients with normal examinations were included in the study. A total of 50 patients and 50 healthy volunteers were included in the study.

Application of the Sniffin' Sticks Test

Odor Sensitivity Test, Smell Discrimination Test, and Smell Identification Test were applied with standardized odor test “Sniffin Sticks” test pens under appropriate conditions to the patients and control group (Figure 1). Sniffin' sticks odor test was applied in a quiet and well-ventilated room, holding the tip of the pens 2 cm away from the nostrils and sniffing for 3-4 seconds. The patients were warned not to eat or drink anything at least one hour before the test.

Odor Identification Test (OIT)

The Identification test is a test that measures the correct identification of odors. It was made with blue cap pens numbered 1 to 16 with black markings (Figure 1A). Sixteen common odors (orange, leather, cinnamon, mint, banana, lemon, licorice, naphtha oil, garlic, coffee, apple, clove, pineapple, rose, anise, fish) with four choices were presented. Subjects were free to sample the odors as often as necessary to make a decision. Total score ranged between 0 and 16.

Odor Threshold Test (OTT)

Threshold test shows that how intensely the patient perceives the odor. It was made with a total of 48 pens which have three different colors and labeled with red, numbered from 1 to 16 (Figure 1B). While the number 1 of the pens contain odor (n-butanol) at the highest concentration, the odor concentration decreases as the number on the pens increases. Each of the triple pens was sniffed by the patient at intervals of approximately 5 seconds. This is only one item of the three (red cap pen) contains the odor, other two (blue and green top pens) contain diluent. The patient was wanted to find the pen that smelled different from the other two. The test was completed by progressing to the lowest concentration which the patient could not smell. Total score ranged between 0 and 16.

Odor Discrimination Test (ODT)

The Discrimination test measures the ability to distinguish odors from each other. It was made with a total of 48 pencils in three different colors, labeled with green, numbered 1 to 16 (Figure 1C). Three different colored pencils of the same number were smelled to the patient at 5-second intervals. Two of the pens represented the same smell, while the green-capped pen represented a different smell. The patient was asked to know what is different between the 3 smells. Total score ranged between 0 and 16.

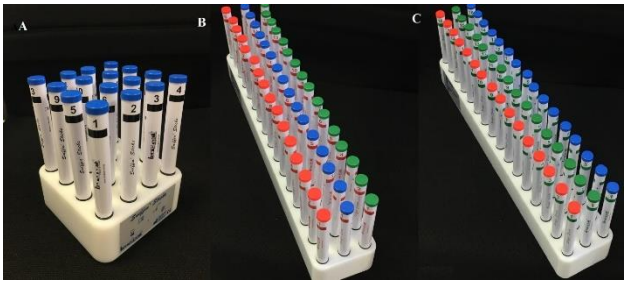

Figure 1. The Sniffin Sticks Test, **A.** Odor identification test pens, **B.** Odor threshold test pens, **C.** Odor discrimination test pens

The sum of these three scores gives the overall threshold-discrimination-identification score (TDI). Subjects with a TDI score 48-31 were considered to have a normal olfactory function (normosmia); subjects with a TDI score of 30-16 were considered to have decreased olfactory function (hyposmia); and subjects with a TDI score ≤15 were considered to have loss of olfactory function (anosmia).

Statistical Analysis

The Statistical Package for Social Sciences (SPSS, Chicago, IL) for Windows, version 22.0 program was used for statistical analysis. Descriptive statistics are given with mean and standard deviation for numerical variables, and numbers and percentages for categorical variables. Shapiro-Wilk test was used for the assumption of normality. Independent samples t test was used to compare the groups in terms of numerical variables. Pearson chi-square test was used in the analysis of categorical variables. The comparison of the measurements which before and after the treatment in the patient group was made with the Paired samples t-test. In all tests, a value of $p<0.05$ was accepted as statistical significance.

RESULTS

A total of 50 patients and 50 healthy volunteers were included in the study. One of the patients did not want to continue the test because of the nausea due to the fishy smell in the test performed after the treatment. Therefore, the study was completed with 49 patients and 50 healthy volunteers. There were 19 (38.8%) female and 30 (61.2%) male in the patient group. Healthy volunteers consisted of 20 (40.0%) females and 30 (60.0%) males. The age of the patients was between 25-64 years and the mean age was 47.15±10.88 years. The ages of the volunteers were between 23-64 years and the mean age was 47.12±9.46 years. There was no statistically significant difference between the patient and control groups in terms of age (p=0.991) and gender (0.811). In addition, no difference was observed in the education level (p=0.994), smoking (p=0.278) and alcohol using of the patient and control groups (Table 1). When the initial odor tests of the patient group and the odor tests of the control group were compared, no statistically significant difference was found in terms of OIT (p=0.874), OTT (p=0.903), and ODT (p=0.983). The OIT value of the patient group was 10.15±1.43 at the beginning and 10.10±1.15 at the end of the treatment (p=0.743). The OTT value was 6.23±1.08 at the beginning and 6.21±0.85 at the end of the treatment (p=0.811). The ODT value was 9.71±1.44 at the beginning and 9.69±1.34 at the end of the treatment (p=0.767). There was no statistically significant difference in the results of the odor test performed at the beginning and end of the treatment (Table 2).

Table 1. Characteristics of the patient and control groups

	Patient (n=49)	Control (n=50)	p
Age (year), mean±SD	47.15±10.88	47.12±9.46	0.991
Gender, n (%)			
Male	30 (61.2)	30 (60.0)	0.811
Female	19 (38.8)	20 (40.0)	
Education Status, n (%)			
Middle School	10 (20.4)	10 (20.0)	
High school	22 (44.9)	23 (46.0)	0.994
University	17 (34.7)	17 (34.0)	
Smoking, n (%)	15 (30.6)	16 (32.0)	0.278
OIT, mean±SD	10.15±1.43	10.10±1.28	0.874
OTT, mean±SD	6.23±1.08	6.20±0.94	0.903
ODT, mean±SD	9.71±1.44	9.71±1.34	0.983
TDI, mean±SD	26.06±2.21	26.04±2.05	0.960

SD: standard deviation, OIT: odor identification test, OTT: odor threshold test, ODT: odor discrimination test, TDI: threshold-discrimination-identification

Table 2. Odor test results at the beginning and end of treatment

	Before treatment	After treatment	p
OIT, mean±SD	10.15±1.43	10.10±1.15	0.743
OTT, mean±SD	6.23±1.08	6.21±0.85	0.811
ODT, mean±SD	9.71±1.44	9.69±1.34	0.767
TDI, mean±SD	26.06±2.21	26.00±2.22	0.666

SD: standard deviation, OIT: odor identification test, OTT: odor threshold test, ODT: odor discrimination test, TDI: threshold-discrimination-identification

DISCUSSION

The sense of smell can be categorized in three ways: normal (normosmic), little (hyposmic) and not perceiving at all (anosmic). There may also be disorders of the sense of smell, such as dysosmia (impaired perceived quality of smell) and phantom smell (olfactory hallucinations, phantosmia) without an obvious olfactory stimulus (14). Nasal and sinus diseases, upper respiratory tract infection, head trauma, smoking and neurodegenerative diseases are possible causes of smell disorders. Also, some anticonvulsant and antidepressant drugs cause smell and taste disorders. Dihydropyridine group calcium channel blockers, beta blockers, ciprofloxacin, diltiazem, doxycycline, enalapril, lovastatin and methotrexate are among the drugs that cause loss of smell (15).

Mechanisms that underlying drug-induced taste and/or smell alteration can be divided into two groups as primary, resulting from the direct effect of the drug and secondary, resulting from the indirect effect of the drug. Primary mechanisms include drug-receptor interaction; disruption of action potential propagation in the cell membrane of afferent and efferent neurons; altering neurotransmitter function; changes in brain regions associated with sensory coding and modulation. Limitation of access of chemicals to sensory receptors (dry mucosa, increased nasal congestion, closure of taste pores, inflammation or infections) and changes in the chemical or ionic environment in the environment of sensory receptors are secondary mechanisms (10). There was no disease that would cause loss of smell in the patients included in our study. They were also not using any other medication.

Although the sense of smell is little studied in dermatology practice, it is an important sense that directly affects the quality of life (16). Sniffin' Sticks test is widely used in Europe to evaluate the sense of smell. This test was developed by Hummel et al. (17) and Kobal et al. (18) and includes one test in the threshold range (determination of the odor detection threshold for n-butanol) and two tests above the threshold (discrimination and identification). The advantage of Sniffin' Sticks test kit is that it is reusable and easy to apply. It is emphasized that it is a modern,

standard odor test that can be used for odor testing (19). However, cultural differences complicate the application of OITs in different countries. Because odor identification is highly dependent on familiarity with the odors tested. It is observed that the Sniffin' Sticks test performs well when applied cross-culturally and Tekeli et al. (20) reported that this test will be applied to Turkish patients without any additional changes in their study. In our study, we used the Sniffin' Sticks test that suitable for Turkish society. In addition, we found no statistical difference between the test baseline values, the demographic characteristics, educational status, smoking/alcohol use of the patient group in whom terbinafine treatment was initiated and the values of the healthy control group.

Terbinafine, a drug of the allylamine group, is an effective drug against dermatophyte, cryptococcal and other fungal infections (21). Terbinafine is a drug with an oral absorption of over 70% and well tolerated. Terbinafine is a lipophilic drug that concentrates on the dermis, epidermis, and adipose tissue. It is metabolized in the liver and often excreted by the kidneys (4). Terbinafine inhibits the enzyme squalene epoxidase, which is responsible for the production of sterols necessary to protect the integrity of the cell membrane. In humans, the squalene epoxidase enzyme is involved in the biosynthesis of cholesterol. Therefore, it has been suggested that terbinafine may alter the structure or function of neurons by interfering with the cholesterol pathway (22).

Side effects were reported in 2.7% of patients using terbinafine. The most common side effects are generalized pustulosis, urticaria, gastrointestinal symptoms, loss of taste and liver toxicity. Drug-induced lupus erythematosus (often subacute cutaneous lupus erythematosus) and exacerbation of psoriasis are other rare side effects (23-25). In the literature, it is reported that the rate of taste loss due to terbinafine is 0.6-2.8%. Case reports show that taste loss occurs 4-6 weeks after drug use. Although long-term taste losses have been reported, it was reported that recovering in sense of taste occurs at 4 months after the drug was discontinued (13). Although there is a close relationship between taste and smell sensory disorders, olfactory changes have not been evaluated in patients with taste disorders (13). Terbinafine-induced loss of smell has been reported very rarely in the literature (10). Tuccori et al. (10) reported that 15 of 17 patients had taste disorders, three patients had parosmia, and two of them had parosmia without taste disorders. However, Doty et al. (13) reported that there was no change in the sense of smell in patients using terbinafine despite having taste disorders. In our study, it was determined that there was no statistically significant difference in sense of smell in patients using terbinafine.

The small number of patients and the fact that taste changes were not evaluated are the limitations of this study.

CONCLUSION

In conclusion, our study, in which we used the Sniffin' Sticks test kit, which is considered a reliable odor test, showed that systemic terbinafine treatment used for 3 months did not cause any side effects on the sense of smell and this drug can be used safely. However, the results of the study should be supported by studies with a larger number of patients.

Ethics Committee Approval: The study was approved by the Clinical Researches Ethics Committee of Bolu Abant İzzet Baysal University (14.09.2017, 84).

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The Association Between Procalcitonin, Lactate Level, and Clinical Prognosis in Patients with Crimean-Congo Hemorrhagic Fever

Kırım-Kongo Kanamalı Ateşi Hastalarında Prokalsitonin ve Laktat Düzeyinin Klinik Seyirle İlişkisi

Ferhan KERGET[1]
0000-0002-5160-4854
Buğra KERGET[2]
0000-0002-6048-1462
Sibel İBA YILMAZ[1]
0000-0002-4123-0828

[1]Depertmant of Infectious Diseases and Clinical Microbiology, Health Sciences University Erzurum Regional Education and Research Hospital, Erzurum, Turkey

[2]Department of Pulmonary Diseases, Ataturk University School of Medicine, Erzurum, Turkey

Corresponding Author
Sorumlu Yazar
Buğra KERGET
bjkerget1903@gmail.com

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ABSTRACT

Aim: Crimean-Congo hemorrhagic fever (CCHF) is a zoonotic infection characterized by fever and hemorrhage that is endemic to northeastern Turkey. This study aimed to examine the association between procalcitonin and venous blood gas parameters and clinical course and prognosis in patients with CCHF.

Material and Methods: A total of 96 CCHF patients who were followed up in the infectious diseases department between March 2020 and September 2020 were included in the study. The patients' routine laboratory tests, serum procalcitonin, and results of venous blood gas analysis were analyzed retrospectively.

Results: There were statistically significant differences in serum platelet, aspartate transaminase, alanine transaminase, creatinine kinase, lactate dehydrogenase, potassium, C-reactive protein, sedimentation, D-dimer, activated partial thromboplastin time, ferritin, procalcitonin and lactate levels, and platelet/lymphocyte ratio among the patients with mild, moderate and severe disease ($p=0.017$ for potassium, $p=0.004$ for D-dimer, and $p<0.001$ for rest of others). In receiver operating characteristic curve analysis of serum lactate for the differentiation of patients with severe disease and those with mild/moderate disease, the area under curve was 0.802 and a cut-off value of 1.9 mmol/L had 77.8% sensitivity and 76.9% specificity. For serum procalcitonin, the area under curve was 0.892 and a cut-off value of 0.61 ng/mL had 83.3% sensitivity and 89.7% specificity.

Conclusion: Serum procalcitonin and lactate level may be useful and easily obtained parameters to guide the evaluation of clinical severity and follow-up in patients with CCHF.

Keywords: Lactate; Crimean-Congo hemorrhagic fever; procalcitonin.

ÖZ

Amaç: Kırım-Kongo kanamalı ateşi (KKKA) ateş ve kanama ile seyreden Türkiye'nin Kuzeydoğusunda endemik olarak gözlenen zoonotik bir enfeksiyondur. Bu çalışmada KKKA hastalarında prokalsitonin ve venöz kan gazı parametrelerinin klinik seyir ve prognoz ile ilişkisinin incelenmesi amaçlanmıştır.

Gereç ve Yöntemler: Mart 2020 ve Eylül 2020 tarihleri arasında enfeksiyon hastalıkları kliniğinde takip edilen toplam 96 KKKA hastası çalışmaya dahil edildi. Hastaların rutin laboratuvar tetkikleri, serum prokalsitonin ve venöz kan gazı analizi bulguları geriye dönük olarak analiz edildi.

Bulgular: Hafif, orta ve ağır hastalığı olan hastalar arasındaki serum trombosit, aspartat transaminaz, alanin transaminaz, kreatinin kinaz, laktat dehidrojenaz, potasyum, C-reaktif protein, sedimantasyon, D-dimer, aktive parsiyel tromboplastin zamanı, ferritin, prokalsitonin ve laktat seviyeleri ve trombosit/lenfosit açısından istatistiksel olarak anlamlı farklılıklar vardı (potasyum için $p=0,017$; D-Dimer için $p=0,004$ ve geri kalan diğerleri için $p<0,001$). Ağır hastalığı olan ve hafif/orta hastalığı olan hastaların ayrımı için serum laktat düzeyinin alıcı işlem karakteristiği eğrisi analizinde, eğri altında kalan alan 0,802 idi ve kesim değeri 1.9 mmol/L alındığında %77,8 duyarlılığa ve %76,9 özgüllüğe sahipti. Serum prokalsitonin için eğri altında kalan alan 0,892 idi ve 0.61 ng/mL'lik bir kesim değeri alındığında %83,3 duyarlılık ve %89,7 özgüllüğe sahipti.

Sonuç: Serum prokalsitonin ve laktat düzeyi KKKA hastalarında klinik şiddetin değerlendirilmesinde ve takipte yol gösterici olması açısından yararlı ve kolay elde edilen parametreler olabilirler.

Anahtar kelimeler: Laktat; Kırım-Kongo kanamalı ateşi; prokalsitonin.

INTRODUCTION

Crimean-Congo hemorrhagic fever (CCHF) is an endemic disease in the Northern Anatolia Region of Turkey that is characterized by fever and hemorrhage and can have a severe and potentially life-threatening course (1). The CCHF virus is generally transmitted to humans through tick bites or contact with infected ticks or the bodily fluids of infected animals. The main targets of CCHF are mononuclear phagocytes, endothelial cells, and hepatocytes (2). Signs and symptoms occur as a result of the effect of the virus on target organs. Sudden-onset fever, headache, malaise, anorexia, widespread body pain, and nausea are the most common symptoms (3).

The pathogenesis of CCHF is not fully understood, though macrophage activation and hemophagocytosis are known to be the basis of the pathological process. After entering the body, the virus proliferates in local lymph nodes and tissues, then spreads via the lymph and monocytes to other parts of the body, especially the spleen, liver, lymph ganglia, lungs, adrenal glands, and endothelium (4). Secondary infection of parenchymal cells occurs by macrophage migration. Macrophage and endothelial cell activation induces inflammatory and vasoactive processes, resulting in systemic inflammatory response syndrome (SIRS) (5). Coagulation system activation and extensive fibrin accumulation in the vascular beds lead to disseminated intravascular coagulation (DIC) and multiple organ failure (MOF) (2).

Blood gas analysis is important for evaluating prognosis in SIRS and MOF. Studies have shown that low pH, high lactate, and low carboxyhemoglobin levels in venous blood gas analysis are important markers of clinical course and prognosis (6). In particular, lactate level is a serum marker frequently used in clinical practice. High serum lactate is an indicator of tissue hypoperfusion. Lactate is produced by many tissues of the human body and at high levels in muscle tissue. Under normal circumstances, lactate is rapidly eliminated by the liver and partly by the kidneys (7).

Procalcitonin level is believed to increase mostly in bacterial infections and sepsis as part of the systemic inflammatory response against infection. It is generally not expected to increase in response to viral infections. However, it has been suggested that a major increase in cytokine levels may lead to procalcitonin elevation (8).

The aim of this study was to evaluate the relationship between procalcitonin level and venous blood gas parameters and the clinical course and prognosis of CCHF.

MATERIAL AND METHODS

Study Design

This retrospective study included patients who were under follow-up for CCHF between March 2020 and September 2020. The study was designed and conducted in accordance with the ethical guidelines set forth in the Declaration of Helsinki. The study protocol was approved by the local ethics committee (02.11.2020, 20-189) and written informed consent was obtained from all patients included in the study.

Sample Size and Patients

We calculated minimum sample size as 84 patients with an effect size of 0.4, type 1 error of 0.05, and a power of 0.90 for testing three groups using GPower 3.1 software.

A total of 96 patients (58 male, 38 female) aged 18 years and older who were followed up for CCHF in the Erzurum Regional Training and Research Hospital Department of Infectious Diseases were included in the study. Patients who developed bacterial superinfection confirmed by blood, sputum, and urine cultures during CCHF follow-up and patients who were pregnant, had known malignancies, had liver or kidney failure, used anti-inflammatory drugs in the last month, or were followed up for acute coronary syndrome in the last 2 months were excluded.

Study Groups

The patients were classified as having mild, moderate, or severe disease according to the criteria defined by Swanepoel et al. (9) and the modified criteria recommended by Ergönül et al. (10), as well as clinical indicators of poor prognosis. Accordingly, there were 37 patients in the mild group, 40 patients in the moderate group, and 19 patients in the severe group. Blood, urine, and sputum cultures were performed for all patients whose procalcitonin level exceeded the upper laboratory limit.

CCHF Diagnosis

Two blood samples of at least 2 mL each were collected from every patient with suspected CCHF upon hospital admission. After 30 minutes of coagulation, the samples were centrifuged at 2000 rpm for 5 min and the serum was transferred to separate tubes. One serum sample from each patient was transported under appropriate conditions to the Erzurum Regional Public Health Laboratory, regional reference center, for serologic and virologic tests. CCHF was diagnosed based on specific anti-IgM antibody detected by immunofluorescence assay (IFA) and/or polymerase chain reaction (PCR) positivity.

Measurement of Biochemical Markers

All the serum samples were obtained within the first 2 days of the onset of their symptoms. Further, the patients' biochemical and hematological parameters were tested on admission, in the laboratory. Procalcitonin levels were tested with commercial ELISA kits (Elecsys BRAHMS PCT, Roche, Mannheim, Germany), according to the manufacturer's recommendations.

Statistical Analysis

Data analysis was performed with IBM SPSS Statistics for Windows version 22.0 (IBM Corp., Armonk, NY) and Medcalc version 16 (MedCalc Software bvba, Ostend, Belgium). Shapiro-Wilk and Kolmogorov-Smirnov tests were used to evaluate the normality assumption. The descriptive statistics were presented as frequency with percentage for categorical variables, and as median with interquartile range (IQR) for numeric data. The laboratory findings were compared among the study groups using Kruskal-Wallis test. Post hoc comparisons were done using Dunn’s post hoc test with Bonferroni correction. Spearman correlation analysis was used to evaluate the relationships between serum procalcitonin, lactate, aspartate transaminase (AST) and alanine transaminase (ALT) levels. Receiver operating characteristic (ROC) curve analysis was used to estimate the severe disease using serum procalcitonin and lactate levels. Youden index was used to find the best cut-off values. The area under curve (AUC), sensitivity and specificity with 95% confidence interval (CI) were calculated. A p-value less than 0.05 was considered statistically significant.

RESULTS

Of the 96 CCHF patients in this study, 58 (60.4%) were male and 38 (39.6%) were female. The median age of the study group was 50 years with a range of 18-79 years. The clinical symptoms are shown in Table 1. The most common symptoms were malaise, fever, and myalgia/arthralgia, respectively.

The patients' laboratory findings are shown in Table 2. There were statistically significant differences in serum platelet, AST, ALT, creatinine kinase (CK), lactate dehydrogenase (LDH), potassium, C-reactive protein (CRP), sedimentation, D-dimer, activated partial thromboplastin time (aPTT), ferritin, procalcitonin and lactate levels, and platelet/lymphocyte ratio (PLR) among the patients with mild, moderate and severe disease (p=0.017 for potassium, p=0.004 for D-dimer, and p<0.001 for rest of others).

PLR was significantly lower in patients with severe and moderate disease compared to those with mild disease (both p<0.001). Serum CRP level was statistically significantly higher in patients with severe disease compared to those with moderate disease and with mild disease (p=0.049 and p<0.001, respectively), and also there was statistically significant difference in serum CRP level among the patients with moderate disease and with mild disease (p=0.024). Serum D-dimer level was statistically significantly higher in patients with severe disease compared to those with mild disease (p=0.002). Ferritin level was significantly higher in patients with severe disease compared to those with moderate disease

Table 1. Clinical symptoms of the patients

Symptoms	n (%)
Malaise	96 (100)
Fever	92 (95.8)
Myalgia and/or arthralgia	80 (83.3)
Rash	54 (56.3)
Nausea	51 (53.1)
Headache	38 (39.6)
Vomiting	33 (34.4)
Abdominal pain	20 (20.8)
Hemorrhage	20 (20.8)
Diarrhea	17 (17.7)
Chest pain	13 (13.5)

Table 2. Comparison of laboratory parameters according to the disease severity

Parameters	Mild (n=37)	Moderate (n=40)	Severe (n=19)	p
Hemoglobin (g/L)	13.6 (12.6-14.8) [7.7-16.9]	14.3 (12.7-15.1) [8.1-16.7]	13.7 (12.7-14.9) [6.2-17.6]	0.691
WBC (1000 cells /μL)	2.2 (1.7-2.9) [1.15-3.97]	1.9 (1.5-2.9) [0.82-5.11]	2.6 (1.8-3.4) [0.64-3.89]	0.232
Platelets (1000 cells/μL)	94 (55-122) [19-159]	42.5 (32-69.3) [12-140]	28 (19-45) [8-114]	**<0.001**
Neutrophils (cells/μL)	910 (645-1535) [250-2390]	755 (590-977.5) [250-3780]	1030 (710-1570) [300-3110]	0.152
Leukocytes (cells/μL)	930 (700-1282) [440-2130]	875 (630-1392.5) [170-2340]	880 (520-1770) [280-2110]	0.964
NLR	1.01 (0.59-1.66) [0.17-5.43]	0.84 (0.56-1.25) [0.29-5.47]	1.07 (0.60-1.68) [0.36-11.11]	0.524
PLR	92.7 (51.4-150.7) [26.9-286.4]	59.6 (28.7-79.2) [13.7-325.0]	32.9 (21.7-54.0) [4.5-172.4]	**<0.001**
Monocytes (cells/μL)	220 (155-280) [80-710]	205 (150-305) [40-860]	260 (110-460) [10-700]	0.581
Eosinophils (cells/μL)	10 (0-30) [0-200]	10 (0-17.5) [0-120]	1 (0-20) [0-60]	0.751
AST (U/L)	81 (44.5-142.5) [28-650]	298.5 (230-405) [166-687]	844 (605-1060) [271-4223]	**<0.001**
ALT (U/L)	68 (42.5-106) [11-316]	220 (139-300) [12-546]	415 (229-481) [125-1617]	**<0.001**
CK (U/L)	200 (107.5-545) [33-1298]	401.5 (218.8-918.3) [123-3650]	1135 (461-2766) [133-7800]	**<0.001**
LDH (U/L)	353 (285.5-457.5) [214-863]	570 (460.8-750) [24-1161]	1101 (750-1840) [531-4380]	**<0.001**
Creatinine (mg/dL)	0.80 (0.72-0.99) [0.51-1.46]	0.76 (0.63-0.92) [0.31-1.47]	0.85 (0.71-1.08) [0.53-7.90]	0.184
Sodium (mmol/L)	133 (132-135) [125-140]	133 (131-134) [127-136]	131 (129-133) [126-155]	0.077
Potassium (mmol/L)	3.8 (3.4-4.1) [2.6-4.5]	3.6 (3.3-3.7) [0.9-4.2]	3.3 (3.1-3.7) [2.4-4.2]	**0.017**
CRP (mg/L)	3 (2.4-10.5) [0.2-47.6]	11 (3.0-23.8) [0.6-80.0]	41 (10.4-85.0) [1.0-183.0]	**<0.001**
Sedimentation (s)	6 (2-11) [2-92]	11 (5-22) [1-35]	33 (14-50) [2-97]	**<0.001**
Fibrinogen (mg/dl)	242 (206-294.5) [109-445]	208 (166.8-275.8) [148-574]	202 (167-293) [117-361]	0.165
D-dimer (ng/mL)	1.7 (0.9-4.4) [0.1-8.8]	2.7 (1.2-5.3) [0.2-80.0]	7.5 (1.4-44.5) [0.6-80.0]	**0.004**
PT (s)	14.5 (12.8-16.4) [11.4-23.9]	13.7 (12.6-15.0) [11.7-20.0]	13.6 (12.3-17.4) [11.5-23.6]	0.801
aPTT (s)	32.7 (30.4-35.6) [25.4-41.8]	35.9 (32.6-42.4) [27.0-82.6]	48.9 (37.7-57.6) [29.6-69.9]	**<0.001**
INR	1.08 (0.99-1.26) [0.37-1.89]	1.19 (1.04-1.41) [0.94-1.82]	1.23 (1.01-1.76) [0.85-4.30]	0.117
Ferritin (ng/mL)	76 (61.5-101) [9.6-634]	250 (150-705) [44.5-8746]	1033 (408-2000) [41-6500]	**<0.001**
Procalcitonin (ng/mL)	0.03 (0.03-0.08) [0.01-1.42]	0.14 (0.05-0.53) [0.01-2.20]	2.00 (1.30-5.20) [0.05-36.59]	**<0.001**
pH	7.40 (7.38-7.42) [7.35-7.49]	7.42 (7.38-7.45) [7.27-7.50]	7.40 (7.32-7.44) [7.25-7.49]	0.347
Lactate (mmol/L)	1.5 (1.2-1.8) [0.8-2.4]	1.7 (1.3-2.1) [1.0-3.3]	2.2 (2.0-3.2) [1.4-5.5]	**<0.001**
COHb (%)	0.6 (0.5-0.7) [0.2-2.2]	0.8 (0.6-0.9) [0.3-1.7]	0.6 (0.5-0.9) [0.2-2.0]	0.051

Descriptive statistics were given as median (interquartile range, Q1-Q3) [min-max], WBC: white blood cells, NLR: neutrophil/lymphocyte ratio, PLR: platelet/lymphocyte ratio, AST: aspartate transaminase, ALT: alanine transaminase, CK: creatinine kinase, LDH: lactate dehydrogenase, CRP: C-reactive protein, PT: prothrombin time, aPTT: activated partial thromboplastin time, INR: international normalized ratio, COHb: carboxyhemoglobin

and with mild disease (both p<0.001). Serum procalcitonin level was statistically significantly higher in patients with severe disease compared to those with moderate disease and with mild disease (p=0.001 and p<0.001, respectively), and also there was a statistically significant difference in serum procalcitonin level among the patients with moderate disease and with mild disease (p=0.005). Serum lactate level was statistically significantly higher in patients with severe disease compared to those with moderate disease and with mild disease (p=0.004 and p<0.001, respectively).

For serum procalcitonin and lactate levels in differentiating severe and moderate/mild disease, the AUC values were 0.892 and 0.802, and the best cut-off points were >0.61 and >1.9, respectively. According to these cut-off values, the sensitivity and specificity of procalcitonin were 83.3% and 89.7%, and the sensitivity and specificity of lactate were 77.8% and 76.9%, respectively (Table 3 and Figure 1).

There were statistically significantly medium positive correlations between serum procalcitonin level and serum AST (r_s=0.584, p<0.001), ALT (r_s=0.497, p<0.001) and lactate (r_s=0.441, p<0.001) levels. Serum lactate level was also positively correlated with serum AST (r_s=0.459, p<0.001) and ALT (r_s=0.446, p<0.001) levels (Table 4).

DISCUSSION

In this study, we found that procalcitonin and lactate levels were higher in patients with severe CCHF when compared to patients with mild and moderate CCHF. In the differentiation of severely ill patients from mild/moderately ill patients, a lactate cut-off value of 1.9 mmol/L had 77.8% sensitivity and 76.9% specificity, whereas a procalcitonin cut-off value of 0.61 ng/mL had 83.3% sensitivity and 89.7% specificity. Both procalcitonin and lactate levels were positively correlated with AST and ALT, which are frequently used parameters in CCHF follow-up.

As in all viral hemorrhagic fevers, the basis of CCHF pathogenesis is viral infection of various cell types, primarily endothelial cells, mononuclear cells, and hepatocytes, followed by proliferation and systematic spread (11,12). Increased endothelial permeability and consequent endothelial damage is the main pathogenetic process leading to death. This endothelial damage has been attributed to two main mechanisms. The first is a direct viral effect on the endothelial cells, and the second is damage caused indirectly by the cytokines secreted from infected tissues (9,13,14).

The most important of these cytokines are tumor necrosis factor alpha, interleukin (IL)-1, and IL-6, which are synthesized by type 1 helper T cells and are involved in monocyte activation. The activation of monocytes by cytokines impairs platelet activation and degranulation and leads to an abnormal coagulation cascade in CCHF (15). Monocyte activation is considered one of the main causes of hemophagocytic lymphohistiocytosis, which leads to cytopenia and liver dysfunction in CCHF. Serum AST, ALT, LDH, and CK are liver function indicators that are frequently used parameters in clinical follow-up and have been associated with poor prognosis (16-18).

Serum lactate level is another parameter frequently used in the clinical practice, and although it may increase secondary to tissue hypoperfusion, elevation may also occur due to causes unrelated to hypoperfusion (19). Lactate, which can be synthesized in many of the body's tissues, is eliminated primarily by the liver and to a lesser extent by the kidneys. A substantial proportion of studies evaluating the relationship between lactate level and clinical course and prognosis have observed that hypoperfusion increases lactate level due to sepsis and septic shock (20,21). Our literature search yielded no studies in which lactate level was associated with the clinical course of CCHF. In the present study, serum lactate level was found to increase in correlation with clinical severity in CCHF patients. This may be attributed

Table 3. Comparison of AUC, sensitivity and specificity of serum procalcitonin and lactate levels in differentiating severe and moderate/mild disease

	Procalcitonin	Lactate
AUC (95% CI)	0.892 (0.813-0.946)	0.802 (0.708-0.876)
Youden index	0.731	0.547
Associated cut-off	>0.61	>1.9
Sensitivity (95% CI)	83.3 (58.6-96.4)	77.8 (52.4-93.6)
Specificity (95% CI)	89.7 (80.8-95.5)	76.9 (66.0-85.7)

AUC: area under curve, CI: confidence interval

Table 4. Correlations between serum procalcitonin, AST, ALT and lactate levels

	Procalcitonin		Lactate	
	r_s	p	r_s	p
Procalcitonin			0.441	**<0.001**
Lactate	0.441	**<0.001**		
AST	0.584	**<0.001**	0.459	**<0.001**
ALT	0.497	**<0.001**	0.446	**<0.001**

AST: aspartate transaminase, ALT: alanine transaminase, r_s: Spearman's rho

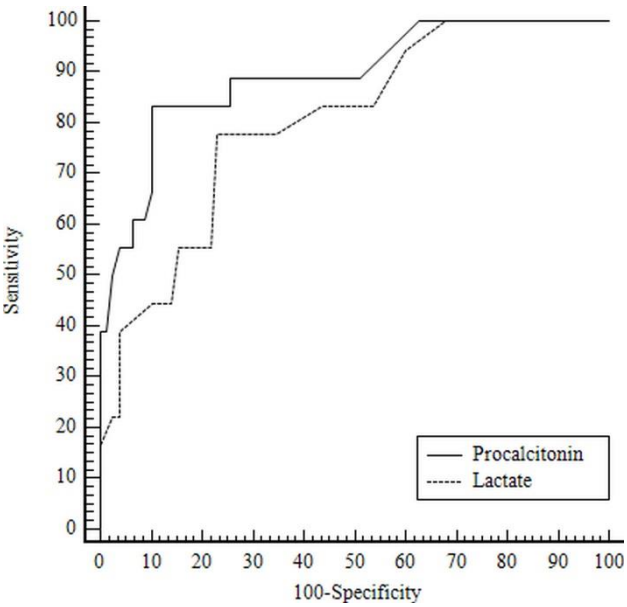

Figure 1. Receiver operating characteristic curve of serum lactate and procalcitonin levels in differentiating the severe and mild/moderate disease

to the inadequate elimination of lactate by the liver in CCHF, as liver dysfunction due to hemophagocytic lymphohistiocytosis plays an important role in the clinical course of this condition. Moreover, the increased cytokine levels seen in CCHF due to the indirect effect of the virus might have led to muscle dysfunction and higher levels of CK as well as lactate. This is corroborated by the fact that muscle and joint pain are among the most common presenting symptoms.

High serum procalcitonin level is often observed in bacterial infections rather than viral infections. However, it has been found that increased cytokine levels also increase amount of procalcitonin synthesized by thyroid C cells in viral infections, independent of bacterial superinfections (8,22,23). Procalcitonin level was reported to be higher in patients with fatal CCHF and correlated with CRP level, which is frequently used in routine practice (24). None of the patients in this study died after clinical follow-up, and in line with previous studies, we also found that procalcitonin levels were higher in severely ill patients. This level also correlated with liver function markers and serum lactate level. The lack of growth in blood, urine, and sputum cultures performed after the increase in procalcitonin levels suggest that this was due to high cytokine level.

The main limitation of our study was the inability to detect an association between lactate and procalcitonin levels and mortality. This was primarily due to the fact that during the study period, our hospital was designated as a COVID-19 pandemic hospital during the pandemic, and as a result there was a sharp decline in the number of patients we followed due to CCHF in our hospital.

CONCLUSION

In conclusion, serum procalcitonin and lactate levels may be important parameters for the early evaluation of clinical course and prognosis in patients with CCHF. Large scale studies evaluating the association between these two parameters and clinical course and mortality may provide more guidance in the course of the clinical follow-up.

Ethics Committee Approval: The study was approved by the Ethics Committee of Erzurum Regional Education and Research Hospital (02.11.2020, 20-189).

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Brain White Matter Hyperintensity Changes Associated with Vascular Cognitive Impairment and Dementia, Alzheimer's Dementia and Normal Aging

Vasküler Kognitif Bozukluk ve Demans, Alzheimer Demans ve Normal Yaşlanma ile İlişkili Beyin Beyaz Cevher Hiperintensite Değişiklikleri

Ayfer ERTEKİN
0000-0002-4313-2826

Department of Neurology, Private Siirt Hayat Hospital, Siirt, Turkey

ABSTRACT

Aim: The aim of this study was to analyze the relationship between the distribution and grading of white matter hyperintensity (WMH) obtained by brain magnetic resonance imaging and cognitive impairment associated with vascular cognitive impairment and dementia (VCID), Alzheimer's dementia (AD) and normal aging in individuals aged 65 years and older.
Material and Methods: Retrospective analysis was performed on a total of 372 patients, who met the criteria for AD, VCID and normal aging. The basic clinical criteria of DSM-V and NIA-AA were considered for AD. The DSM-V and NINDS-AIREN diagnostic criteria were used for VCID and probable vascular dementia (VaD). WMHs were graded according to the Fazekas criteria.
Results: Normal aging was detected in 58.3% (n=217) of the patients, AD in 24.7% (n=92) and VCID in 16.9% (n=63). The relationship between WMH and dementia was significant. ($p<0.001$). Periventricular hyperintensity was detected as 76.2% (n=70) in AD, 95.2% (n=60) in VCID, 40.6% (n=88) in normal aging, and deep white matter hyperintensity was detected as 63.0% (n=58) in AD, 74.5% (n=47) in VCID, 44.2% (n=96) in normal aging. In the VCID group, 52.4% (n=33) had basal ganglia lesions and 60.3% (n=38) had classical infarct areas. No relationship was found between the presence of cortical lesion and cognition.
Conclusion: This study is important in terms of showing that it would be better to focus on markers of brain damage and dementia, such as WMH rather than focusing on clinical diagnoses with mixed-type pathologies such as Alzheimer's disease or vascular cognitive impairment.
Keywords: Alzheimer's dementia; vascular cognitive impairment, brain MRI; white matter hyperintensity.

ÖZ

Amaç: Bu çalışmanın amacı, 65 yaş ve üstü bireylerde, beyin manyetik rezonans görüntüleme (MRG) ile elde edilen beyaz madde hiperintensitesinin (white matter hyperintensity, WMH) dağılımı ve derecelendirmesi ile vasküler kognitif bozukluk ve demans (VCID), Alzheimer demans (AD) ve normal yaşlanma ile ilişkili kognitif etkilenme arasındaki ilişkiyi analiz etmektir.
Gereç ve Yöntemler: AD, VCID ve normal yaşlanma kriterlerine uygun toplam 372 hasta ile geriye dönük analiz yapıldı. AD için DSM-V ve 2011 NIA-AA temel klinik kriterleri dikkate alındı. VCID ve olası vasküler demans (VaD) için DSM-V ve NINDS-AIREN tanı kriterleri kullanıldı. WMH'ler Fazekas kriterlerine göre derecelendirildi.
Bulgular: Katılımcıların %58,3'ünde (n=217) normal yaşlanma, %24,7'sinde (n=92) AD ve %16,9'unda (n=63) VCID tespit edildi. WMH ile demans arasındaki ilişki anlamlı idi ($p<0.001$). Periventriküler hiperintensite AD'de %76,2 (n=70), VCID'de %95,2 (n=60), normal yaşlanmada %40,6 (n=88) olarak tespit edildi ve derin beyaz madde hiperintensitesi ise AD'de %63,0 (n=58), VCID'de %74,5 (n=47), normal yaşlanmada %44,2 (n=96) olarak saptandı. VCID grubunda %52,4 (n=33) ile bazal ganglion lezyonları ve %60,3 (n=38) ile klasik enfarkt alanları mevcuttu. Kortikal lezyon varlığı ile kognisyon arasındaki ilişki tespit edilmedi.
Sonuç: Bu çalışma Alzheimer hastalığı veya vasküler bilişsel bozukluk gibi mix tip patolojileri olan klinik tanılara odaklanmak yerine, belki de WMH gibi beyin hasarı ve demans belirteçlerine odaklanmanın daha iyi olacağını göstermesi açısından önemlidir.
Anahtar kelimeler: Alzheimer demans; vasküler kognitif bozulma, beyin MRG; white matter hiperintensite.

Corresponding Author
Sorumlu Yazar
Ayfer ERTEKİN
ayfertekin1976@gmail.com

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INTRODUCTION

In cross-sectional and dimensional studies, there is strong evidence that white matter hyperintensities (WMHs) are clinically important markers of risk of stroke, dementia, death, depression, gait disturbance, and limitation of movement. In 22-dimensional meta-analysis studies, it has been reported that WMH is associated with a 2-fold increase in the risk of progressive cognitive impairment, dementia, and 3-fold increase in the risk of stroke (1). In the general population, the proportion of WMHs range from 11% to 21% in 64-year-old adults and rises to 94% by the age of 82. Female tend to have a higher number of WMH than male (2). In the study of Sachdev et al. (2), it was determined that 87% of the participants aged 60-70 had subcortical, 68% had periventricular WMHs, 100% of the participants aged 80-90 had subcortical and 95% had periventricular WMHs.

WMHs occur in many forms, each with a different combination of pathologies. Punctate WMHs, for example, are associated with myelin destruction, gliosis, and increased perivascular spaces, whereas large and confluent WMHs show some degree of myelin loss, axonal disruption, and more advanced pathological changes, including astrogliosis (3,4). Kim et al. (5) have suggested that fiber tract damage distribution pattern is more associated with cognitive and motor impairment when evaluated with total WMH load.

The risk of dementia is greater when vascular comorbid conditions occur. The study of Bejot et al. (6) showed that the prevalence of vascular risk factors such as hypertension (HT), diabetes, atrial fibrillation, previous myocardial infarction, and transient ischemic attack history is higher in patients with post-stroke dementia. Available data show that cerebrovascular disease (CVD) significantly increases the risk of developing dementia disorders such as Alzheimer's dementia (AD) and vascular cognitive impairment and dementia (VCID) (7).

In this study, we analyzed both the hypothesis that WMHs are associated with cognitive impairment and its severity in older individuals with AD and VCID and the distribution of WMH in individuals with normal aging without middle-to-old age cognitive impairment. In the mild cognitively impaired and/or normal aging group, the prediction was made that 'WMHs may constitute a potentially useful marker for the identification of new risk factors for dementia'. This will provide important opportunities to recognize the early stages that lead to the development of WMH, prevent (or even reverse) brain damage in the early stages, and improve cognitive, physical, stroke, and dementia outcomes.

MATERIAL AND METHODS

Study Design and Participants

This study was conducted on the data obtained from the hospital data recording system and the ministry of health e-nabız application, by retrospectively scanning a total of 372 patients aged 65 and over, who applied to the neurology outpatient clinic of Siirt Private Life Hospital between 2019 and 2021, who were eligible for AD, VCID and normal aging criteria. Consent was obtained for the study with the decision no:2021/02.04 dated 12.03.2021 and numbered 6264, at the meeting of the ethics committee of Siirt University non-interventional clinical research. The patients' registered and diagnosed sociodemographic information, systemic diseases, HT, diabetes, chronic ischemic heart disease, atrial fibrillation, hyperlipidemia, history of stroke ≥12 weeks were analyzed through the system.

Detailed biochemical blood parameters of the patients, especially B12, TSH, fT3, fT4, hemogram, liver and, kidney function tests, were examined. Diseases that may cause secondary cognitive impairment such as hypothyroidism, B12 deficiency, acute vascular damage, electrolyte imbalance (hyponatremia or hypernatremia, hypomagnesemia, hypercalcemia and hypocalcemia, hypoglycemia or hyperglycemia, anemia, uremia, and hepatic dysfunction), chronic systemic inflammatory diseases or diagnosed autoimmune disease and psychiatric disorders such as schizophrenia with neoplastic processes and which may cause cognitive impairment were excluded from the study. Anamnesis, physical and detailed neurological examination, electroencephalography (EEG), neuropsychological test results, neuroimaging details, and radiology comments were examined in detail. Cognitive evaluation is made in our clinic by applying the Mini-Mental State Examination (MMSE) test and Blessed Orientation-Memory-Concentration (BOMC) test performed by a specialist psychologist. In cognitive fields, a neuropsychological test battery was used, including memory functions (short and long-term memory), abstract thinking, judgment, aphasia, apraxia, agnosia and structural difficulty, visuospatial and structural functions.

Intima-Media Thickness (IMT) and Carotid Doppler Ultrasonography (USG) Evaluation

Detailed carotid Doppler USG reports of all patients participating in the study were examined. The standard position applied by the radiologist of our hospital was as follows; distant and proximal walls of arterial segments (common carotids, bifurcation and internal carotid arteries) are evaluated with Doppler ultrasound (7.5-10.0 MHz probe, Toshiba Aplio 300) device with patients in supine position, head turned and neck motionless. A standardized protocol is used for image analysis in B mode. The measurement of IMT measured in B-mode is from a plaque-free region with a clearly defined double line pattern. For carotid IMT measurement, a 1 cm segment is determined within the first 2 cm proximal region from the common carotid artery bulb. In this plaque-free region, measurements are made between the luminal and media-adventitia interfaces of the carotid artery, and the measurements are reported. In measurements made from bilateral common carotid arteries, an IMT value ≥1 mm is considered pathological. IMT ≥1.5 mm and above is considered as plaque. The degree of stenosis determined by gray scale and color Doppler USG is categorized as normal (no stenosis) <50% stenosis, 50-69% as stenosis, and ≥70% stenosis, total or near occlusion and thrombus.

Diagnostic Criteria for Alzheimer's Dementia (AD) and Vascular Cognitive Impairment and Dementia (VCID)

Patients were classified into three groups: AD, VCID (including multi-infarct dementia, strategic infarct dementia, and subcortical vascular encephalopathy), and normal aging.

Dementia diagnosis according to 2013 Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (DSM-V) criteria; as evidence of significant decline from the previous level of performance in one or more cognitive domains (complex attention, executive function, learning, and memory, language, motor or social cognitive domain);
A. A significant decline in cognitive function of the individual that a knowledgeable informant or clinician notices.
B.1. Cognitive performance deteriorates significantly, it is documented by another quantitative clinical assessment, preferably in the presence standardized neuropsychological tests or absence of them.
B.2. Cognitive impairments prevent independence in daily activities (need help in functional activities of daily life)
B.3. Cognitive impairments do not occur solely in the context of delirium.
B.4. Cognitive impairments are not better explained by another mental disorder. Specify:
* Without behavioral disorder: The cognitive disturbance is not accompanied by any clinically significant behavioral disturbances.
* With behavioral disorder (specify the disorder): If the cognitive disorder is accompanied by a clinically significant behavioral disorder (for example, psychotic symptoms, aggression, apathy, or other behavioral symptoms). For example, basic clinical criteria of major depressive disorder or schizophrenia (8).

Alzheimer's Dementia (AD)

AD criteria for DSM-V and 2011 National Institute on Aging and Alzheimer's Association (NIA-AA) and basic clinical criteria for probable AD were considered (9). According to DSM-V, for the diagnosis of major neurocognitive disorder due to AD, impairment in at least two cognitive domains, one of which is learning and memory is required. For mild neurocognitive impairment due to AD, insidious onset and progressive progression, as well as learning and memory deficits, are sufficient for diagnosis.

Vascular Cognitive Impairment and Dementia (VCID)

DSM-V and National Institute of Neurological Disorders and Stroke - Association Internationale pour la Recherche et l'Enseignement en Neurosciences (NINDS-AIREN) diagnostic criteria were used for VCID and probable vascular dementia (VaD). After diagnosis of mild and major neurocognitive disorder according to DSM-V diagnostic criteria, DSM-V should focus on CVD for VCID.

There should be evidence of damage supporting small vessel disease (SVD), including clinical features, significant impairment in frontal-executive function, and physical signs consistent with stroke or SVD, such as hemiparesis, pseudobulbar palsy, and visual field defects; neuroimaging evidence such as multiple lacunar infarctions or large and confluent white matter lesions. The NINDS-AIREN criteria must include at least one of the following focal neurologic symptoms: hemianopia, central facial weakness, dysarthria, motor or sensory hemisyndrome, hemiplegic gait or positive Babinski sign (10).

Normal Aging

In normal aging, individuals often retain long-standing personality traits and interests, including levels of initiative, motivation, sociability, empathy, influence, and behavior.

Vascular cognitive impairment (VCI) covers all cognitive impairments associated with CVD, from mild cognitive impairment to dementia. Simply put, VCI is a syndrome with clinical signs of a stroke or subclinical vascular brain injury and cognitive impairment affecting at least one cognitive domain. The most severe form of VCI is VaD.

Brain Magnetic Resonance Imaging (MRI) / Magnetic Resonance (MR) Angiography Evaluation and White Matter Hyperintensities (WMHs) FAZEKAS Classification

Brain MRI, MR angiography, and diffusion MRI were performed in a unit with 1.5 Tesla field strength. White matter lesions were evaluated on T2-weighted or Flair MR images. Classification was made for periventricular hyperintensity (PVH) and deep white matter hyperintensity (DWMH) according to the following criteria (11,12).

PVH was graded from 0 to 3 (11):

Grade 0, no lesion (Figure 1a); grade 1, in the form of a line or a cap (Figure 1b); grade 2, irregular hyperintensity and/or smooth halo (Figure 1c); and grade 3 lesion spreading to the periventricular region and deep white matter (Figure 1d).

DWMH was also graded from 0 to 3 (12):

Grade 0, no lesion (Figure 2a); grade 1, in the form of punctate (Figure 2b); grade 2, punctate hyperintensity prone to fusion (Figure 2c); grade 3, large confluent and enlarged punctate hyperintensity (Figure 2d).

White matter lesions were considered periventricular if the lesions were orginated from a location directly adjacent to the ventricules. If the lesions had no direct connection to the ventricules, or secondarily connected to the ventricles, they were considered to be located in the deep white matter lesions.

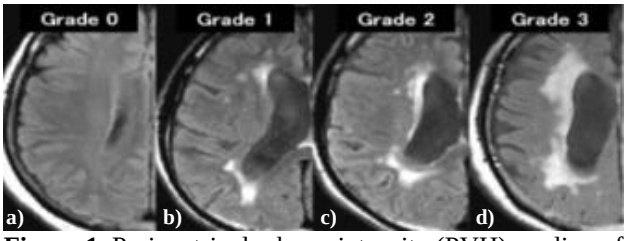

Figure 1. Periventricular hyperintensity (PVH) grading of magnetic resonance images (11), **a)** grade 0, no lesion, **b)** grade 1, in the form of a line or a cap, **c)** grade 2, irregular hyperintensity and/or smooth halo, **d)** grade 3, lesion spreading to the periventricular region and deep white matter

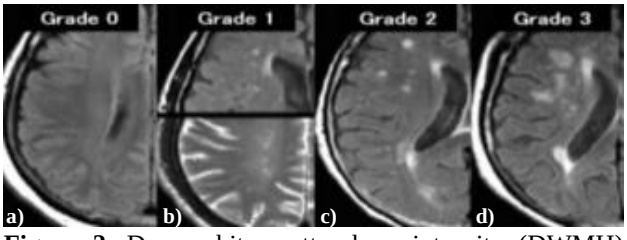

Figure 2. Deep white matter hyperintensity (DWMH) grading of magnetic resonance images (12), **a)** grade 0, no lesion, **b)** grade 1, in the form of punctate, **c)** grade 2, punctate hyperintensity prone to fusion, **d)** grade 3, large confluent and enlarged punctate hyperintensity

Radiological examinations were performed on retrospective radiology reports and images. Our hospital radiology reports are systematically transferred to the system by the radiologist on a daily basis. Brain neuroimaging reports are reported in detail such as periventricular and/or deep white matter lesions, subcortical lesions, basal ganglia lesions, incidental findings, encephalomalacia area, lacunae infarct in our radiology clinic. In our study, periventicular and deep white matter lesions were differentiated by reference to the radiology reports registered in the system, and brain MR images were opened one by one with the hospital picture archiving and communication system (PACS) and examined. WMH lesions were graded by the neurologist (author) according to references (11,12).

Statistical Analysis

Statistical analyzes of the study were performed using Statistical Package for Social Sciences for Windows (IBM SPSS version 25.0, Armonk, NY, USA) software. The normality assumption of continuous variables was tested with Kolmogorov-Smirnov and Shapiro-Wilk tests. The descriptive statistics of the variables were given as mean±standard deviation, median (25th~75th percentile), and n (%), according to the variable type and the normality assumption. Chi-square tests, Fisher-Freeman-Halton exact test, and Mann-Whitney U, One-Way Analysis of Variance (ANOVA) tests were used for univariate analyzes of the variables in the study. Duncan multiple comparison test was used to compare the groups with a statistical difference as a result of analysis of variance. Cases with a p value below 0.05 in all statistical analyzes were interpreted as statistically significant.

RESULTS

A total of 372 patients took part in the research. Females made up 53.8% (n=200) of the participants, while males made up 46.2% (n=172). The participants' mean age was 76.02±7.87 years. The normal aging group has 58.3% (n=217) of the participants, 24.7% (n=92) have AD, and 16.9% (n=63) have VCID. Table 1 shows the correlations and explanatory statistics between the groups and factors that are the focus of the study.

In terms of the age variable, there was a statistically significant difference between the groups ($p<0.001$). The mean age of the patients in the AD group (79.66±8.43) was higher than the other groups. Gender and groups have a statistically significant relationship ($p=0.011$). The proportion of female in the normal aging and VCID groups was higher than that of male, while the proportion of male in the AD group was higher than that of female. In patients with CVD ≥12 weeks, the relationship between cognitive impairment and the VCID group was statistically significant ($p<0.001$). While 89.9% (n=195) of patients in the normal aging group and 78.3% (n=72) of AD patients had no previous history of CVD, 61.9% (n=39) with VCID had a previous history of CVD ≥12 weeks. 69.8% (n=44) of those in the VCID group had HT, while 30.2% (n=19) did not ($p=0.064$). There was no relationship between atrial fibrillation and cognitive impairment in this study. A significant relationship was found between IMT and the groups ($p=0.001$). In normal aging 32.7% (n=71), 15.2% (n=14) in AD, and 15.9% (n=10) in VCID, IMT <1 mm, while 84.8% (n=78) of AD patients and 84.1% (n=53) of VCID patients, IMT ≥1 mm. The relationship between plaque and its sub formations over 1.5 mm with normal aging and cognitive impairment was not found to be statistically significant ($p=0.139$). The relationship between left internal carotid artery (ICA) stenosis and groups was statistically significant ($p=0.009$). 18.9% (n=41) in normal aging, 31.5% (n=29) in AD, and 33.3% (n=21) in VCID group had left ICA stenosis <50%. No significant correlation was found with right ICA stenosis.

PVH and the groups had a statistically significant relationship ($p<0.001$). PVH grade 0 was found in 59.4% (n=129) with normal aging, 23.9% (n=22) of patients with AD, and 4.8% (n=3) with VCID. DWMH and the groups had a statistically significant relationship ($p<0.001$). While PVH was found in 76.2% (n=70) of AD patients and 95.2% (n=60) of VCID patients, DWMH was found in 63.0% (n=58) of AD patients and 74.5% (n=47) of VCID patients. PVH 40.6% (n=88) and DWMH 44.2% (n=96) were present in normal aging, but PVH 59.4% (n=129) and DWMH 55.8% (n=121) were absent. In brain MRI scans, the number of patients with classical infarcts matching a specific arterial irrigation area was lowest in the normal aging (4.6%, n=10) group and highest in the VCID (60.3%, n=38) group ($p<0.001$). The relationship between the absence of cortical lesions and the groups was statistically significant ($p<0.001$). The proportion of patients without cortical lesions in the normal aging group was 91.7% (n=199), 71.4% (n=45) in the VCID group, and 89.1% (n=82) in the AD group. The relationship between basal ganglia lesion and the groups were statistically significant ($p<0.001$). While the rate of those without basal ganglia lesions in the normal aging group was 94.0% (n=204), this rate was 47.6% (n=30) in the VCID group. Basal ganglia lesions were prominent in the VCID group with a rate of 52.4% (n=33). The difference between the groups in terms of B12 values was not statistically significant ($p=0.707$).

A significant proportion of Alzheimer's patients had periventricular (44.6%, n=41) extensive or irregular hyperintensity (grade 2) when compared with the normal aging group (13.8%, n=30), when VCID (46.0%, n=29) was compared with the normal aging (2.3%, n=5) group, extensive PVH (grade 3) was found. Furthermore, 10.6% (n=23) DWMH was found in normal aging, 21.7% (n=20) in AD, 31.7% (n=20) grade 2 DWMH in the VCID group, and 19.0% (n=12) grade 3 DWMH in the VCID group.

DISCUSSION

White matter hyperintensities found on brain MRI were higher in AD and VCID patients than in the normal aging group, although periventricular and deep WMHs were significantly higher in the normal aging group, according to this study. Furthermore, the fact that the incidence of PVH and DWMH in brain MRI was similar in both groups suggested that periventricular and deep WMH could be part of the same disease. Moreover, contrary to what is known, the presence of white matter lesions in AD patients created a prediction for the question of what is the effect of white matter lesions on the background of AD classical neuropathology.

It was only recently discovered that neuroimaging-identified WMHs are associated with Alzheimer's disease. WMH affects the majority of adults over the age of 70 who

Table 1. Explanatory statistics and comparisons of the groups

	Normal Aging (n=217)	AD (n=92)	VCID (n=63)	p
Age (year), mean±SD	74.34±7.41	79.66±8.43	76.48±6.82	**<0.001**
Gender, n (%)				
Female	126 (58.1)	37 (40.2)	37 (58.7)	**0.011**
Male	91 (41.9)	55 (59.8)	26 (41.3)	
Hypertension, n (%)	119 (54.8)	48 (52.2)	44 (69.8)	0.064
Diabetes, n (%)	55 (25.3)	23 (25.0)	25 (39.7)	0.065
Coronary Artery Disease, n (%)	58 (26.7)	31 (33.7)	13 (20.6)	0.189
Hyperlipidemia, n (%)	61 (28.1)	25 (27.2)	13 (20.6)	0.492
Past stroke (≥12 weeks), n (%)	22 (10.1)	20 (21.7)	39 (61.9)	**<0.001**
Atrial fibrillation, n (%)	31 (14.3)	24 (26.1)	14 (22.2)	**0.036**
Intima-media thickness, n (%)				
Normal	71 (32.7)	14 (15.2)	10 (15.9)	**0.001**
Pathological	146 (67.3)	78 (84.8)	53 (84.1)	
Right ICA stenosis, n (%)				
None	148 (68.2)	62 (67.4)	39 (61.9)	0.305
<50	56 (25.8)	20 (21.7)	16 (25.4)	
50-69	9 (4.1)	7 (7.6)	6 (9.5)	
≥70	3 (1.4)	1 (1.1)	1 (1.6)	
Total/near occlusion	1 (0.5)	0 (0.0)	1 (0.3)	
Thrombus	0 (0.0)	2 (2.2)	0 (0.0)	
Left ICA stenosis, n (%)				
None	160 (73.7)	56 (60.9)	37 (58.7)	**0.009**
<50	41 (18.9)	29 (31.5)	21 (33.3)	
50-69	11 (5.1)	6 (6.5)	1 (1.6)	
≥70	5 (2.3)	0 (0.0)	3 (4.8)	
Total/near occlusion	0 (0.0)	1 (1.1)	1 (1.6)	
Plaque structure, n (%)				
None	85 (39.2)	22 (23.9)	16 (25.4)	0.139
Hypoechoic soft	15 (6.9)	7 (7.6)	5 (7.9)	
Heterogeneous hypoechoic	27 (12.4)	15 (16.3)	8 (12.7)	
Heterogeneous calcific/calcific	90 (41.5)	48 (52.2)	34 (54.0)	
PVH, n (%)				
Grade 0	129 (59.4)	22 (23.9)	3 (4.8)	**<0.001**
Grade 1	53 (24.4)	10 (10.9)	6 (9.5)	
Grade 2	30 (13.8)	41 (44.6)	25 (39.7)	
Grade 3	5 (2.3)	19 (20.7)	29 (46.0)	
DWMH, n (%)				
Grade 0	121 (55.8)	34 (37.0)	16 (25.4)	**<0.001**
Grade 1	66 (30.4)	31 (33.7)	15 (23.8)	
Grade 2	23 (10.6)	20 (21.7)	20 (31.7)	
Grade 3	7 (3.2)	7 (7.6)	12 (19.0)	
Classical infarcts, n (%)	10 (4.6)	11 (12.0)	38 (60.3)	**<0.001**
Basal ganglia lesions, n (%)	13 (6.0)	16 (17.4)	33 (52.4)	**<0.001**
Cortical lesion, n (%)	18 (8.3)	10 (10.9)	18 (28.6)	**<0.001**
B12, median (25th~75th percentile)	304.0 (222.0~384.5)	283.0 (196.2~382.0)	310.0 (200.0~494.0)	0.707

AD: Alzheimer's dementia, VCID: vascular cognitive impairment and dementia, ICA: internal carotid artery, PVH: periventricular hyperintensity, DWMH: deep white matter hyperintensity, SD: standard deviation

have been diagnosed with Alzheimer's disease (13). Alzheimer's disease is commonly referred to as a gray matter disease of the brain. However, recent neuroimaging studies have highlighted micro-and macrostructural abnormalities in white matter in the risk and progression of Alzheimer's disease, indicating that, in addition to neuronal loss, white matter degeneration and demyelination may be important pathophysiological features of the disease (14-16).

The Alzheimer's disease neuroimaging initiative cohort found a relationship between β-amyloid and WMHs in the brain in a recent study (16). The volume of WMH begins to increase 20 years before cognitive symptoms in patients with dominant AD mutations as β-amyloid and tau levels in the cerebrospinal fluid fluctuate (17). These findings further suggest that AD pathology may be related to vascular and/or nonvascular processes that result in WMHs, given that vascular disease is uncommon in patients with this young mutation (18,19). In the other hand, cerebral amyloid angiopathy (CAA) in AD is a common age-related SVD characterized by amyloid-β deposition in the walls of cortical arterioles and leptomeningeal vessels (18,19). The majority of AD brains contain histological CAA to some degree. WMHs are

caused by microvascular processes such as impaired perivascular clearance, plasma extravasation, inflammation, hypoperfusion, and endothelial dysfunction (18). In the brains of patients diagnosed with Alzheimer's disease, WMH severity was related to microinfarcts and a high burden of amyloid plaques (20). More likely, AD pathology (common in older people) and WMHs of vascular origin (even more common in older people) are often common, as noted in multiple autopsy-based studies of mixed pathologies (13). Depending on AD pathology, it is considered that some white matter lesions, especially in the late stages of the disease, occur secondary to Wallerian degeneration triggered by cortical neurodegenerative pathology (21).

Hypertension, insulin resistance, diabetes, obesity, hyperhomocysteinemia, and hyperlipidemia are all prevalent vascular risk factors in VCID and AD (22). Subclinical vascular brain injury and stroke cause VaD, which is the most severe form of VCI (7). Post-stroke dementia risk is evaluated together with age and stroke lesion burden/location (severity, previous/recurrent stroke, dysphasia), pre-morbid markers of brain susceptibility/reserve (education level, pre-morbid addiction, leukoaraiosis severity), and basic cognitive score (23,24).

There is strong evidence that middle age HT has a detrimental effect on cognitive function in old age, but the impact of old age HT on cognitive function is less clear. Observational studies have shown a cumulative effect of HT on cerebrovascular injury, but evidence from clinical trials that antihypertensive therapy improves cognitive function is inconclusive (25).

The number of patients with AD+/HT- in our study was similar to the number of patients without AD+/HT+ (single and/or dual antihypertensive drugs were used). Patients with VCID+/HT+, on the other hand, had a significantly higher rate (69.8%) than those with VCID+/HT-.

In people over the age of 65, type two diabetes mellitus (DM) is also a risk factor for cognitive impairment and dementia. Insulin resistance accelerates AD pathology (26). In a study by Abner et al. (27), it was found that DM increases brain infarctions, especially the number of lacunae, not on Alzheimer's pathology, that diabetes and infarction coexist with high levels of neuropathological changes in Alzheimer's disease compared to infarction and/or diabetes alone, and it is reported that it was associated with low MMTE scores. In our study, the incidence of DM was similar in normal aging, AD, and VCID groups, and no significant difference was found between the groups.

Increased carotid intima-media thickness (CIMT) measured by USG is a surrogate marker of atherosclerosis and a strong predictor of future vascular events. This noninvasive and simply applicable marker has been reported to be associated with cognitive impairment.

Moon et al. (28) suggest that the elderly individuals with a larger CIMT have a higher future risk of progression to MCI or dementia; therefore, they might require a more intensive monitoring for the earlier detection of cognitive dysfunction. On the other hand, carotid artery stenosis, which causes an increase in the carotid pulse-flow rate, is a major risk factor for cerebral white matter disease (29,30). It's associated with cognitive impairment and white matter damage (31). If global cerebral perfusion and heart disease induced by carotid artery stenosis/occlusion decrease below a critical threshold, they can impair cognitive function independently of brain lesions (32). This deterioration occurs with a 40-50% decrease in cerebral blood flow and suppression of brain activity and cognitive dysfunction (32). In our study, 61.9% of VCID patients had a history of stroke (≥12 weeks). In the VCID and AD patient groups, the common carotid artery IMT was significant over 1 mm, but no correlation was found between the structural formation of the plaque and cognitive impairment. It was observed that carotid stenosis was more common in AD and VCID patients compared to normal aging patients, and left carotid stenosis was more common in the AD and VCID groups, as a localization finding, compared to the right.

In recent years, the study of the causes of brain aging and dementia has shifted to cerebral white matter. Through vascular alterations such as arteriolar tortuous, reduced blood vessel fluidity, and venous collagenous, the white matter microvascular network contributes to the pathogenesis of WMH. A network of vessels "from the outside in" feeds the brain. The pial arteries, which branch off the main cerebral arteries on the brain's surface and penetrate the brain parenchyma (penetrating arteries), constitute a highly anastomotic network. The basal ganglia are supplied by penetrating vessels that originate directly from Willis and the proximal branches at the base of the brain. Unlike pial vessels and capillaries, penetrating vessels have several collateral branches, so the occlusion of a single vessel is sufficient to cause small ischemic lesions (lacuna infarctions) (33). In addition, deep subcortical white matter supplied by long penetrating arteries, whose perfusion pressure is predicted to be low, is considered to be particularly vulnerable to hemodynamic failure (34).

WMHs occur in many forms, each with a different combination of pathologies (3,4). The disruption of oligodendrocyte precursor cells (35) or an impaired perivascular ("glymphatic") space are two possible explanations. Punctate WMHs, for example, are associated with myelin destruction, gliosis, and increased perivascular spaces, whereas large and confluent WMHs show some degree of myelin loss, axonal disruption, and more advanced pathological changes, including astrogliosis (3,4). The parietal, temporal, and occipital lobes may be affected first, followed by the frontal lobe (36).

Dementia is associated with different vascular diseases (37). The neuropathology of VCID is heterogeneous and complex (38). The SVD occurs in the basal ganglia arteries and spreads to the peripheral white matter and leptomeningeal arteries, as well as the thalamic, cerebellar, and brainstem vessels, however, the neocortical vessels usually survive (39). Furthermore, pathogenic factors linked to oxidative stress and inflammation in cerebral vessels are considered to relate SVD pathology to neurovascular dysfunction and VCID (40). The number and size of macroscopic infarctions or large vessel disease appear to be related to an increased risk of dementia in clinical-pathological studies (41). As previously stated, studies in WMH have concentrated on demyelination

and axonal loss, and the changes have been termed "ischemic" (42,43). When demyelination and axonal destruction occur, the changes are permanent. Despite the fact that certain studies differentiate PVH from DWMH (3,44), imaging studies show that periventricular and deep WMH are probably mostly part of a continuous disease (44,45). A recent very large study in 2699 patients with stroke at 11 stroke centers in China created statistical maps of the WMH distribution and showed that periventricular and deep WMH is a continuation of a continuous pathology, and any significant difference in the distribution in some patients only reflects an earlier stage of disease (46). WMHs in brain MRI were shown to be considerably higher in the AD and VCID groups than in the normal aging group and were associated with cognitive impairment in this research. We found that periventricular and deep white matter lesions in brain MRI were seen at similar rates in dementia subtypes, and there was no significant difference in WMH distribution between VCID and AD groups. As in the literature example mentioned above (46), our study results suggested that PVH could not be evaluated differently from DWMH, and perhaps they could be lesions of different stages, intertwined with each other. We found that especially infarct areas matching the irrigation area of the great artery and strategically located lacunae and infarct areas with basal ganglia involvement were more common in VCID, and there was no relationship between cortical lesions and WMH and cognitive impairment. This study provides strong evidence that WMHs predict dementia risk and reveals vascular risk factors in their intrinsic dynamic.

CONCLUSION

Clinical investigations suggest that instead of focusing on mixed-type diseases like Alzheimer's disease or VCI it could be better to concentrate on markers that induce brain damage and dementia, like WMH. Currently, considering WMHs that are predicted to be of vascular origin and associated risk factors, it is certain that targeting vascular health throughout the life course as a prevention strategy will be effective in minimizing cognitive impairment in the middle and older age group and preventing and/or slowing the progression to dementia in the normal aging group.

Ethics Committee Approval: The study was approved by the Non-interventional Clinical Researches Ethics Committee of Siirt University (26.03.2021, 02.04).

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Bilateral Superior Cervical Ganglionectomy and Melatonin Levels in Rat Subarachnoid Hemorrhage Model: Simple Precautions May Preserve Melatonin Levels

Sıçan Subaraknoid Kanama Modelinde Bilateral Superior Servikal Ganglionektomi ve Melatonin Seviyeleri: Basit Önlemler Melatonin Düzeylerini Koruyabilir

Güven KILIÇ[1]
0000-0001-5050-7908
Murat KAYABAŞ[2]
0000-0002-5768-2925
Seçkin Emre CANCAN[3]
0000-0002-3260-7419

[1]Department of Neurosurgery, Düzce University Faculty of Medicine, Düzce, Turkey

[2]Department of Neurosurgery, Kafkas University Faculty of Medicine, Kars, Turkey

[3]Neurosurgery Clinics, İzzet Baysal State Hospital, Bolu, Turkey

Corresponding Author
Sorumlu Yazar
Seçkin Emre CANCAN
drsecancan@yahoo.co.uk

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ABSTRACT

Aim: Subarachnoid hemorrhage (SAH) is a serious disease, and it is thought that melatonin may have positive effects after SAH. Bilateral resection or blockage of superior cervical ganglions has constant effects on melatonin levels. Animal models with bilateral superior cervical ganglionectomy (SCG) show the role of superior cervical ganglion on melatonin and give clues about simple precautions which may help to prevent unfavorable outcomes in SAH patients. The aim of this study is to examine how melatonin levels change in SAH and SCG models.

Material and Methods: Forty-two Sprague Dawley male rats weighing 200-250 g were used in the study and randomly divided into six groups. Arterial blood samples were collected 24 hours after the procedure in all groups. Serum melatonin levels of the groups were studied.

Results: A significant difference in blood melatonin levels was observed between SAH and SCG groups, and against the control group. There was no significant difference between the melatonin levels in SCG group and SAH+SCG group (p=0.983). The mean blood melatonin level of the SAH group was higher than the SCG (p<0.001), SAH+SCG (p<0.001) and control groups (p=0.001). The mean blood melatonin levels of SAH+SCG and SCG groups were lower than the mean blood melatonin levels of the other groups and also the SAH group (p<0.001).

Conclusion: Bilateral SCG significantly inhibited the abrupt increase of serum melatonin levels after SAH model in rats. Future studies aiming to address melatonin's complex outcomes should take into account that minor exogenous factors may affect serum melatonin levels.

Keywords: Superior cervical ganglion; subarachnoid hemorrhage; melatonin.

ÖZ

Amaç: Subaraknoid kanama (subarachnoid hemorrhage, SAH) ciddi bir hastalıktır ve SAH sonrası melatoninin olumlu etkileri olabileceği düşünülmektedir. Superior servikal ganglionların bilateral rezeksiyonu veya blokajı melatonin seviyeleri üzerinde sabit etkilere sahiptir. Bilateral superior servikal ganglionektomili (superior cervical ganglionectomy, SCG) hayvan modelleri, superior servikal ganglionun melatonin üzerindeki rolünü göstermekte ve SAH hastalarında olumsuz sonuçların önlenmesine yardımcı olabilecek basit önlemler hakkında ipuçları vermektedir. Bu çalışmanın amacı SAH ve SCG modellerinde melatonin düzeylerinin nasıl değiştiğinin incelenmesidir.

Gereç ve Yöntemler: Çalışmada 200-250 g ağırlığında kırk iki adet Sprague Dawley erkek sıçan kullanıldı ve rastgele şekilde altı gruba ayrıldı. Tüm gruplarda işlemden 24 saat sonra arteriyel kan örnekleri alındı. Grupların serum melatonin düzeyleri çalışıldı.

Bulgular: SAH ve SCG grupları arasında ve kontrol grubuna karşı kan melatonin düzeylerinde anlamlı farklılık gözlendi. SCG grubu ve SAH+SCG grubu melatonin değerleri arasında anlamlı bir fark yoktu (p=0,983). SAH grubunun ortalama kan melatonin düzeyi, SCG (p<0,001), SAH+SCG (p<0.001) ve kontrol (p=0.001) gruplarından daha yüksekti. SAH+SCG ve SCG gruplarının ortalama kan melatonin düzeyleri, diğer grupların ve ayrıca SAH grubunun da (p<0,001) ortalama kan melatonin düzeylerinden daha düşüktü.

Sonuç: Bilateral SCG, sıçanlarda SAH modelinden sonra serum melatonin düzeylerinin ani artışını önemli ölçüde inhibe etti. Melatoninin karmaşık sonuçlarını ele almayı amaçlayan gelecekteki çalışmalar, minör eksojen faktörlerin serum melatonin düzeylerini etkileyebileceğini hesaba katmalı ve göz önünde bulundurmalıdır.

Anahtar kelimeler: Superior servikal ganglion; subaraknoid kanama; melatonin.

INTRODUCTION

Subarachnoid hemorrhage (SAH) is a serious disease that develops spontaneously or due to trauma, and it is thought that melatonin may have positive effects on SAH (1-3). Melatonin secretion in the pineal gland is regulated by light stimulation, and superior cervical ganglion has an important place in this regulation (4-6). Whether traumatic or spontaneous SAH can be defined as extravasation of blood into subarachnoid space of brain (7,8). Trauma which is the leading cause of SAH, is followed by other pathophysiological reasons in the list such as aneurysmatic SAH and peri-mesencephalic SAH; e.g. vascular malformation, intracranial dissection, sickle cell disease with intracerebral hemorrhage, pituitary apoplexy, cerebral amyloid angiopathy, central nervous system tumor, cocaine use, and cerebral venous thrombosis (9).

Although a remarkable decline in the incidence of aneurysmatic SAH worldwide is seen decade by decade it is not to lose its importance since almost 45% of cases end up with mortality in the first month after the incident and nearly 30% of the survivals will face off disabilities at some degree (7,10). Various papers in the literature studied effects of melatonin in early and delayed ischemia and vasospasm after SAH (11-15).

Melatonin as an endogenous antioxidant, seems to have an important role in maintaining favorable outcomes in SAH survivors (12,16,17). Therefore, safe methods to achieve the goal to increase this endogenous melatonin should be put in charge.

Supplying sympathetic innervation to head and neck, superior cervical ganglion plays an important role in regulating basilar and middle cerebral arteries (18,19). Melatonin which is a derivative of serotonin, is synthesized in pinealocytes and this procedure is regulated by ambient light through a neural pathway from the retina, ending in sympathetic neurons of the superior cervical ganglion (6,20). Bilateral resection or blockage of superior cervical ganglions has constant effects on melatonin levels (21-24). Thus, blockage of superior cervical ganglion leaves ajar a door to pass into the world of CVS by its roles in melatonin synthesis and sympathetic nervous system.

Horner's Syndrome like outcomes of cervical hyperextension, due to stretching of the cervical sympathetic chain or ischemia, have been reported in the literature which may implicate that superior cervical ganglia can be affected by the position of the patient or pressure applied by ties used to stabilize the endotracheal intubation tubes in the intensive care units (25).

Melatonin has shown to have neuroprotective effects and reduce SAH induced oxidative stress (16,26). So not only the effects of vasospasm also neuroprotection makes melatonin a remarkable molecule in management of SAH. Therefore, precautions to protect endogenous melatonin levels become important in daily practice regarding SAH patients.

Patient's head and neck position during and after the surgery, and the method to stabilize the intubation tube become very important in preserving melatonin levels induced after SAH. Performing bilateral superior cervical ganglionectomy (SCG) in animal models shows the role of superior cervical ganglion in biological mechanisms of melatonin and gives us clues about simple precautions which may help to prevent unfavorable outcomes in SAH patients.

The aim of this study is to examine how melatonin levels change in SAH and SCG models by comparing blood melatonin levels of control, sham, SAH and SCG groups.

MATERIAL AND METHODS

All procedures in this study were performed after the approval of the Animal Research and Ethics Committee of Kafkas University (date: 17/05/2018 and 25/10/2018, number: 2018/052 and 2018/081) and in accordance with the rules and procedures determined by the committee.

A total of 42 Sprague Dawley male rats weighing 200-250 g were used in the study. All animals were kept in the same physical environment, day and night, during the experiment and randomly divided into 6 groups. The average temperature of the room where the rats were kept was between 22-24 °C and with no existing windows. Free access to food and water (ad libitum) was provided under a 12-hour light/dark cycle (light off at 07:00 AM and 7:00 PM). The rats were randomized into six groups with n=7 in each group as follows: Group 1: Control group, Group 2: Sham group for subarachnoid hemorrhage (SAH Sham), Group 3: Sham group for superior cervical ganglionectomy (SCG Sham), Group 4: SAH group, Group 5: SCG group, and Group 6: SAH+SCG group.

Anesthesia

Ketamine 35 mg/kg (Ketolar Panke Davis-Apothecary, Turkey) and xylazine 5-10 mg/kg (Rompun-Bayer, Turkey) mixture was given by intraperitoneal injection. Anesthesia was determined as the rats to be unresponsive to pain and continue spontaneous respiration during the experiment; additional doses were given if necessary.

Superior Cervical Ganglionectomy (SCG)

After the rats were fixed to the dissection table under general anesthesia, the neck area was shaved and disinfected properly. Approximately 3-3.5 cm of vertical incision was performed from the intermandibular area to the presternal area, after which the skin was pulled with the retractors and deepened through the right and left mandibular salivary glands. Subsequently, the skin was pulled up and out, the layers of thin tissue between the mandibular glands and the muscles here were cut off, and the deep cervical fascia was dissected with blunt and sharp dissections. Superior cervical ganglion was identified at carotid bifurcation. To prevent external carotid artery trauma, the external carotid artery was removed using the surrounding connective tissue and then the superior cervical ganglion was visualized, subsequently superior cervical ganglion was removed by small traction maneuvers. The same procedure was repeated for the contralateral side and at the end of the procedure the skin was closed with 4/0 prolene sutures.

Subarachnoid Hemorrhage (SAH)

After general anesthesia, rats were shaved between inion and atlas. After cleaning the area with povidone-iodine solution, approximately 2 cm long skin incision between inion and atlas was made. The occipital muscles were dissected with blunt dissection from the bone, after which posterior cervical muscle was dissected and atlantooccipital membrane was revealed. 0.1 ml non-heparinized blood

was collected from the tail artery and injected into cisterna magna within 30s following the discharge of an equal amount of cerebrospinal fluid (CSF).

To prevent blood from leaking back, the insulin shot was briefly held at the point where it entered the membrane. After this procedure, rats were kept in the Trendelenburg position for 15-20 minutes, to achieve the blood to reach into the prepontine cistern.

Measurement of Serum Melatonin Level

Arterial blood samples up to 0.2 ml were collected from the tail 24 hours after the procedure (09:00 in the morning) in all groups and rats were sacrificed with deep anesthesia. After clotting in the blood samples, they were centrifuged for 20 minutes at 3000 rpm and the supernatant (serum) was aliquoted and stored at -80 °C until the measurement. Commercial ELISA kit (MyBioSource, Melatonin (MLT) ELISA Kit, USA, Catalog No: MBS020998) was used for serum melatonin measurements.

Statistical Analysis

Obtained data were analyzed with IBM SPSS Statistics 22 program. Shapiro-Wilk test was performed to evaluate normal distribution of blood melatonin levels, and skewness and kurtosis values were also studied. The Levene test was used to analyze homogeneity of variance. A one-way ANOVA test was performed to find the difference between groups in terms of blood melatonin levels. Post-hoc Tukey test was performed to determine significant differences between groups. Blood melatonin level for each group were presented with mean and standard deviation. A p value of <0.05 was considered as statistically significant.

RESULTS

The comparison of blood melatonin levels between groups were presented in Table 1. There was a significant difference in terms of melatonin levels between the groups ($F_{(5,36)}$=18.779, η2=0.723, p<0.001). Post-hoc Tukey test was used to find out the significance regarding the differences in blood melatonin levels between the groups. A significant difference in terms of the blood melatonin levels was observed between SAH and SCG groups, and also against the control group. According to the post hoc test result, there was no significant difference between the mean melatonin levels in SCG group and SAH+SCG group (101.02±18.22 vs. 111.28±17.04, respectively, p=0.983).

When control and sham groups compared, mean blood melatonin levels were as follows and no significant difference was observed between control, SAH sham and SCG sham groups; 160.72±25.63 in control group, 167.04±28.47 in SAH sham (occipital incision) group, and 170.85±34.07 in SCG sham (cervical incision) group (p=0.998 for control vs. SAH sham, p=0.984 for control vs. SCG sham, and p=0.999 for SAH sham vs. SCG sham).

The mean blood melatonin level of the SAH group (228.98±39.23) was higher than the mean blood melatonin level of control group (p=0.001), SAH sham group (p=0.003), SCG sham group (p=0.006), SCG group (p<0.001) and SAH+SCG group (p<0.001), and all these differences were found as statistically significant.

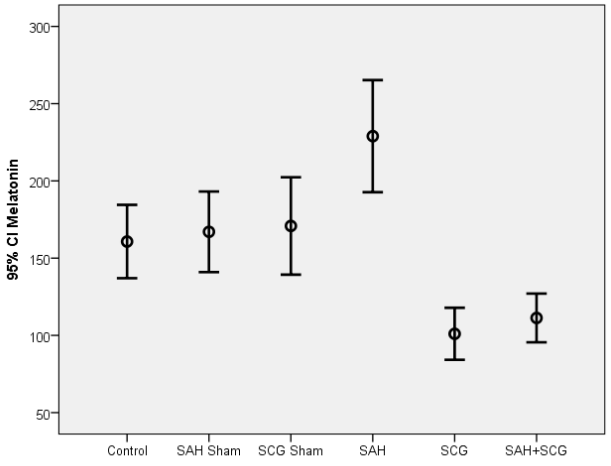
The mean blood melatonin levels of SAH+SCG and SCG groups were lower than the mean blood melatonin levels of the other groups (p=0.026 for SAH+SCG vs. control, p=0.009 for SAH+SCG vs. SAH sham, p=0.004 for SAH+SCG vs. SCG sham, p=0.004 for SCG vs. control, p=0.001 for SCG vs. SAH sham, p=0.001 for SCG vs. SCG sham); and all these differences were significant (Figure 1, Table 1).

These results indicated that blood melatonin levels were increased significantly after SAH, decreased in rats which undergone SCG, and blood melatonin levels did not increase after SAH in rats with SCG.

DISCUSSION

With the obtained results from this study, we observed that bilateral SCG reduces melatonin production. This blockage eventually will decrease the effects of melatonin on vasospasm and further neuroprotective effects of melatonin should not be expected to occur (12,13,16,26-28).

Sole increase in melatonin levels seen in the SAH group shows the successful modelling of SAH and also is parallel to the findings in the literature; prominent increase in melatonin levels is in correlation with the severity of SAH as a natural response to the event (29). Thus, inhibiting the increase in melatonin levels by bilateral SCG, prohibits this natural response causing a significant lack for melatonin's protective effects after SAH.



SAH: subarachnoid hemorrhage, SCG: superior cervical ganglionectomy

Figure 1. Mean blood melatonin levels in groups

Table 1. Comparison of blood melatonin levels in groups

	Control	SAH Sham	SCG Sham	SAH	SCG	SAH+SCG	p
Melatonin	160.72±25.63	167.04±28.47	170.85±34.07	228.98±39.23	101.02±18.22	111.28±17.04	**<0.001**

SAH: subarachnoid hemorrhage, SCG: superior cervical ganglionectomy, post hoc test results; Control vs SAH Sham: p=0.998, Control vs SCG Sham: p=0.984, Control vs SAH: p=0.001, Control vs SCG: p=0.004, Control vs SAH+SCG: p=0.026, SAH sham vs SCG Sham: p=0.999, SAH sham vs SAH: p=0.003, SAH sham vs SCG: p=0.001, SAH sham vs SAH+SCG: p=0.009, SCG sham vs SAH: p=0.006, SCG sham vs SCG: p=0.001, SCG sham vs SAH+SCG: p=0.004, SAH vs SCG: p<0.001, SAH vs SAH+SCG: p<0.001, SCG vs SAH+SCG: p=0.983

Previously mentioned cases in the literature, such as Horner's Syndrome like outcomes of cervical hyperextension, due to stretching of the cervical sympathetic chain or ischemia; highlights the importance of intraoperative positioning and also postoperative patient care in intensive care units (25). Possible compression or damage to the superior cervical ganglion may end up with decreased levels of melatonin. Interventions like central venous catheterization (CVC) into internal jugular vein (IJV) may also result in damage to the superior cervical ganglion (30).

As underlined by Aulinas A. (6) in 2019, other possible production sites of melatonin seem not to contribute much in secretion of the hormone after pinealectomy; in parallel to this statement we also report no significant change in rats which undergone bilateral SCG. Therefore, simple precautions during the treatment period of SAH patients may become more important to keep melatonin levels at a preserved level. The effect of light on melatonin secretion should be kept in mind, so the ambient luminescence in the intensive care units becomes an unnoticed additive factor in an avalanche covering the total war of saving neuroprotection after SAH. Even though debatable effects of exogenous factors can be questioned, choice of analgesic medication can also be a part of this struggle; for example, ibuprofen can reduce melatonin secretion. Inadequate sedation in patients may result in agitation of the intubated patient which leads intensive care unit (ICU) staff to be more rigid to keep safe the intubation tubes and to knot the stabilizers more tightly on the neck; at this point one should be more alert to keep superior cervical ganglia away from pressure.

Studies in the literature like Zhan et al. (29) have detailed inclusion and exclusion criteria regarding SAH patients and their melatonin levels. A significant correlation between high serum melatonin levels and poor outcome is concluded in this study, but it should not be ignored that severity of SAH itself can be in correlation to increased melatonin levels and poor outcomes; and also, perioperative interventions and ICU precautions can theoretically affect melatonin levels in this group of patients.

Not only in topic of SAH, also numerous papers in the literature studied spinal cord injury-melatonin correlation in animal models and human subjects (31-36). For the future designs of the studies both in animal models and humans, exogenous factors and superior cervical ganglia injuries should be considered in evaluating serum melatonin levels since bilateral SCG significantly reduces melatonin response of the body.

CONCLUSION

With this plain study design, we presented that bilateral SCG significantly inhibits the abrupt increase of serum melatonin levels after SAH model in rats. A second study is in our scope to evaluate pinealocytes' changes and responses to bilateral SCG. Further studies are needed to unveil the effects of melatonin after both spinal traumas and SAH. Also, future studies aiming to address melatonin's complex outcomes should take into account that minor exogenous factors may affect serum melatonin levels.

Ethics Committee Approval: The study was approved by the Animal Experiments Ethics Committee of Kafkas University (17.05.2018, 052; 25.10.2018, 081).

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The Effect of Inhaler Aromatherapy on Pain and Anxiety in Patients undergoing Shock Wave Lithotripsy

Şok Dalga Litotripsi Uygulanan Hastalarda İnhaler Aromaterapinin Ağrı ve Anksiyete Üzerine Etkisi

Barış SAYLAM[1]
0000-0003-3256-8752
Ozan EFESOY[1]
0000-0002-5650-1686
Erdem AKBAY[2]
0000-0001-7669-414X
Erim ERDEM[2]
0000-0003-1754-4365

[1]Department of Urology, Mersin City Training and Research Hospital, Mersin, Turkey

[2]Department of Urology, Mersin University School of Medicine, Mersin, Turkey

Corresponding Author
Sorumlu Yazar
Barış SAYLAM
drsaylam@gmail.com

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ABSTRACT

Aim: The aim of this study was to determine the effects of inhaler aromatherapy on pain and anxiety during the shock wave lithotripsy (SWL) protocol.
Material and Methods: This placebo-controlled prospective double-blind study included 120 patients who were scheduled to undergo SWL. Patients were randomly assigned a control placebo group 1 (n=40), the lavender group 2 (n=40), and the frankincense group 3 (n=40). The nebulizer (a rate of 2%) was prepared and operated on in the SWL room before the procedure. Data were collected using the visual analog scale (VAS) and the state-trait anxiety inventory (STAI-I).
Results: Of the 120 patients included in the study, 67 (55.8%) were male and 53 (44.2%) were female. The mean age of the patients was 37.38±11.35 years. The mean stone size was 11.07±3.09 mm. There were no statistically significant difference between the groups in terms of VAS scores of the patients after the procedure (p=0.557). While there was no statistically significant change in the STAI-I score in group 1 and group 2 after the procedure compared to the pre-procedure, a statistically significant decrease was detected in group 3 (p=0.030). The percentages of patients with changing STAI-I scores were compared, it was observed that the decrease in STAI-I score in group 2 and group 3 at a higher rate compared to group 1 (p=0.018).
Conclusion: Frankincense reduced anxiety more significantly than lavender. Both had no significant effect on pain. Inhaler aromatherapy can be used in the SWL process to reduce anxiety and improve treatment compliance.
Keywords: Aromatherapy; SWL; lavender; frankincense; anxiety; pain.

ÖZ

Amaç: Bu çalışmanın amacı şok dalga litotripsi (shock wave lithotripsy, SWL) protokolü sırasında inhalasyon aromaterapisinin ağrı ve anksiyete üzerindeki etkilerinin belirlenmesidir.
Gereç ve Yöntemler: Bu plasebo kontrollü prospektif çift kör çalışmaya SWL yapılması planlanan 120 hasta dahil edildi. Hastalar rastgele kontrol plasebo grubu 1 (n=40), lavanta grubu 2 (n=40) ve günlük yağı grubu 3 (n=40) olarak ayrıldı. Nebülizör (%2'lik bir oranda) işlemden önce SWL odasında hazırlandı ve çalıştırıldı. Veriler görsel analog skala (visual analog scale, VAS) ve durumluk-sürekli kaygı envanteri (state-trait anxiety inventory, STAI-I) kullanılarak toplandı.
Bulgular: Çalışmaya alınan 120 hastanın 67'si (%55,8) erkek, 53'ü (%44,2) kadındı. Hastaların yaş ortalaması 37,38±11,35 yıl idi. Ortalama taş boyutu 11,07±3,09 mm idi. İşlem sonrası hastaların VAS skorları bakımından gruplar arasında istatistiksel olarak anlamlı bir farklılık yoktu (p=0,557). Grup 1 ve grup 2'de işlem öncesine göre karşılaştırıldığında işlem sonrası STAI-I skorlarında istatistiksel olarak anlamlı bir değişiklik yokken, grup 3'te istatistiksel olarak anlamlı bir düşüş saptandı (p=0,030). STAI-I skoru değişen hastaların yüzdeleri karşılaştırıldığında, STAI-I azalma oranının grup 2 ve grup 3'te grup 1'e göre karşılaştırıldığında daha yüksek oranda olduğu gözlendi (p=0,018).
Sonuç: Günlük yağı kaygıyı lavantadan daha önemli ölçüde azalttı. Her ikisinin de ağrı üzerinde anlamlı bir etkisi olmadı. Anksiyeteyi azaltmak ve tedaviye uyumu artırmak için SWL sürecinde inhaler aromaterapi kullanılabilir.
Anahtar kelimeler: Aromaterapi; SWL; lavanta; günlük yağı; kaygı; ağrı.

(ClinicalTrials.gov Identifier: NCT04848350, 09.04.2021, retrospectively registered)

INTRODUCTION

Shock wave lithotripsy (SWL) is widely and effectively used in the treatment of urinary tract stone disease (1). In the first applied lithotriptors, the energy density and maximum energy application at the skin level revealed the need for anesthesia in terms of pain. Later, with the development of new devices, the physics parameters were changed and shock was applied with lower energy levels, using large ellipsoids, the area where shock waves enter the skin is reduced and anesthesia is restricted (2). During the SWL procedure, patients can identify pain in the form of a subjective sensation that is difficult to identify. Pain occurs for two reasons. The first is the trauma caused by shock waves moving towards the kidney as they pass through the skin and muscles, and the second is the deep organ pain due to the trauma caused by shock waves in the kidney (2). Also, various patient-related and environmental factors affecting pain should be considered. The sedation of patients greatly facilitates SWL tolerance (3). However, it has been noted in various studies that patients with anxiety experience more pain during SWL (4). Aromatherapy is a tapas acupressure technique (TAT) method in which essential oils created from fragrant parts of plants are absorbed from the body and show their effects. The therapeutic, disease-relieving or preventive properties of essential oils are utilized (5). Essential oils can be applied to the body by topical, internal, oral, and inhalation methods (6). The fastest and easiest way of entry of essential oils into the body is inhalation. When applied through breathing, evaporating molecules reach the olfactory bulb through the nose and the limbic system in the brain. Aromatherapy provides control of pain and anxiety by affecting the amygdala and hippocampus, which regulate fear and aggressive behaviors in the limbic system (5). Aromatherapy by inhaler is used in hemodialysis, dental procedures, intrauterine contraceptive placement, pain and anxiety control in patients with arthritis (7-10). The literature shows that lavender (Lavandula officinalis) and frankincense (Boswellia) essential oils are used in pain and anxiety management in procedural pain and anxiety control (11,12).

The aim of the study was to investigate the effects of inhaler aromatherapy on pain and anxiety during the SWL protocol.

MATERIAL AND METHODS

Study design

The study was carried out in the SWL unit of Mersin City Training and Research Hospital. The study was designed as a placebo-controlled prospective double-blind and approved by the Clinical Researches Ethics Committee of Mersin University (13.05.2020, 376). All patients who participated in the study were informed about the study and signed written consent forms 1 day before the SWL procedure. A total of 120 patients undergoing SWL unit were included in the study. Exclusion criteria were identified as; patients with pain before the procedure (VAS value is non-zero), and respiratory disease (lung cancer, asthma, bronchitis, chronic obstructive pulmonary disease, etc.), patients using painkillers in the last 3 hours, patients who were disturbed by the smell of used essential oils and patients with a known allergy to used essential oils with a history of contact dermatitis against cosmetic odor. Also, patients who used anxiolytic agents and narcotics were excluded. Patients with these characteristics were not included in the study. SWL was performed with an electro-hydraulic lithotripter (Multimed Classic, Elmed Lithotripsy Systems, Ankara, Turkey) with fluoroscopic guidance. The intensity of the shock wave started from 8 kV and gradually increased to less than 18 kV. The number of shock waves was 2000 per session at a rate of 90 shocks per minute. The patients received the first session of their treatment.

Randomization and Interventions

The patients were grouped by using simple randomization method as placebo control group 1 (n=40), lavender aromatherapy group 2 (n=40) and frankincense aromatherapy group 3 (n=40). They were filled out discomfort intolerance scale-Revised (DIS-R) forms before the procedure. Aromatherapy agents were added to the nebulizer at a rate of 2% (120 ml of water). Only a saline solution was applied to the placebo group. The nebulizer was prepared and operated on in the SWL room before the procedure. The patients were taken to the room 5 minutes before the procedure and the nebulizer was operated on during the procedure. The level of anxiety was evaluated by the state-trait anxiety inventory (STAI) forms. The STAI index has 2 subheadings, STAI-1 (state index) and STAI-2 (trait index), with a total of 40 questions. The STAI form was used to state the transitory emotional state and anxiety level of the participants before and after the procedure. The interrogation procedures were carried out by an assistant doctor who did not know the study. After the patient was rested after the procedure, the patients have again filled out the STAI form and VAS pain scores (Figure 1). After that, the data of all the patients involved in the study were compared.

Outcome Measurements

The scale that evaluates the perceived physical discomfort intolerance, beliefs about physical discomfort, and responses to feelings of physical discomfort has been revised DIS-R (13). The DIS-R is a 5-item measure on which participants indicate, on a 7-point Likert-type scale

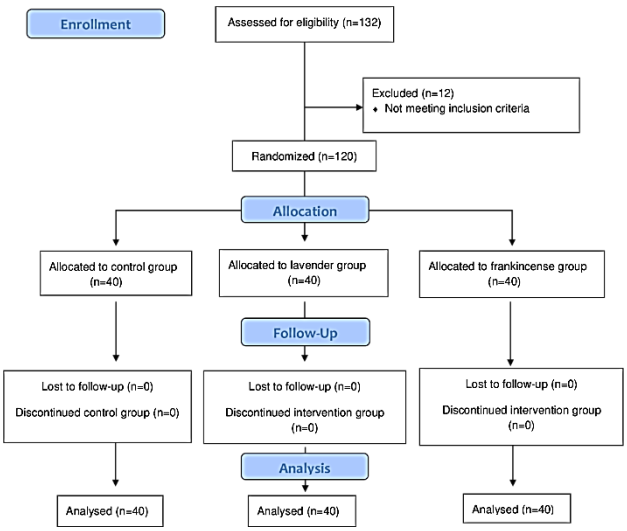

Figure 1. Flowchart of the study protocol

(0: not at all like me to 6: extremely like me), the degree of agreement towards statements related to their tolerance of discomfort. The VAS pain scale was used to measure patients' pain levels. A patient is asked to indicate his/her perceived pain intensity (most commonly) along a 100 mm horizontal line, and this rating is then measured from the left edge, VAS score (14). The level of anxiety was assessed by the STAI-I. The STAI form is a psychological inventory based on a 4-point Likert scale and consists of 40 questions on a self-report basis. The STAI measures two types of anxiety; state anxiety, or anxiety about an event, and trait anxiety, or anxiety level as a personal characteristic. Higher scores are positively correlated with higher levels of anxiety (15).

Statistical Analysis

For statistical analyses SPSS (Statistical Package for the Social Sciences Inc, Chicago, IL, USA) version 21.0 package program was used, and p values less than 0.05 were qualified as statistically significant. Shapiro-Wilk as a normality test has performed for all parameters, and skewness and kurtosis were also evaluated. Descriptive statistics for continuous variables were expressed, and also tabulated as mean±standard deviation or median, interquartile range, minimum-maximum, as appropriate. Categorical variables were presented as frequencies, and percentages. One-Way ANOVA or Kruskal-Wallis test was used to compare groups. Pearson's chi-square test was used to analyze categorical variables and the percentage change of the scores in groups.

RESULTS

Of the 120 patients included in the study, 67 (55.8%) were male and 53 (44.2%) were female. The mean age of the patients was 37.38±11.35 years. Sixty-five (54.2%) of the stones was renal while 55 (45.8%) were proximal ureteral. In total, 52 (43.3%) of the stones were right sided, while 68 (56.7%) were left, and the mean stone size was 11.07±3.09 mm. No statistically significant difference was found between the groups in terms of socio-demographic data of the patients, DIS-R scores and the properties of the stones undergoing SWL (Table 1).

While there was no statistically significant change in the STAI-I score in group 1 and group 2 after the procedure compared to the pre-procedure, a statistically significant decrease was detected in group 3 (p=0.030). However, when the percentages of patients with changing STAI-I scores were compared, it was observed that the decrease in STAI-I in group 2 and group 3 at a higher rate compared to group 1 (p=0.018, Table 2). While there were 15 (37.5%) patients in group 1 with decreased STAI scores after the procedure, there were 21 (52.5%) patients in group 2 and 22 (55.0%) patients in group 3. Accordingly, there were 15 (37.5%) patients in group 1 whose STAI-I scores did not change from before to after the procedure, while there were only 3 (7.5%) patients in group 2 and 8 (20.0%) patients in group 3. In some patients, the STAI-I score was elevated, similarly in the groups; 10 (25.0%), 16 (40.0%), and 10 (25.0%), respectively.

When the VAS scores of the patients were compared after the procedure, no statistically significant difference was found between the groups (p=0.557, Table 2). Similarly, when the VAS values predicted before the procedure and the VAS values experienced after the procedure were compared, it was found that in all groups, the majority of patients experienced less and as much pain as they predicted, and there was no statistically significant difference between the groups (p=0.708). None of the patients had allergic reactions due to aromatherapy.

Table 1. Comparison of sociodemographic characteristics and kidney stone locations and sizes of the patients

	Group 1 (n=40)	Group 2 (n=40)	Group 3 (n=40)	p value
Gender, n (%)				
Male	18 (45.0)	24 (60.0)	25 (62.5)	0.234
Female	22 (55.0)	16 (40.0)	15 (37.5)	
Age (year), mean±SD	36.93±10.56	36.58±11.35	38.65±12.57	0.685
BMI (kg/m²), mean±SD	26.09±2.93	26.17±3.98	28.23±4.35	0.020
Educational level, n (%)				
Illiterate	4 (10.0)	3 (7.5)	2 (5.0)	0.857
Primary school	24 (60.0)	23 (57.5)	22 (55.0)	
High school	12 (30.0)	14 (35.0)	16 (40.0)	
Stone size (mm), mean±SD	11.23±3.47	11.43±2.55	10.55±3.19	0.419
Stone position, n (%)				
Renal	19 (47.5)	24 (60.0)	22 (55.0)	0.528
Proximal ureter	21 (52.5)	16 (40.0)	18 (45.0)	
Stone side, n(%)				
Right	12 (30.0)	18 (45.0)	22 (55.0)	0.076
Left	28 (70.0)	22 (55.0)	18 (45.0)	
DIS-R, mean±SD	22.10±5.44	19.63±5.81	20.73±7.44	0.216

BMI: body mass index, DIS-R: discomfort intolerance scale-revised, SD: standard deviation

Table 2. VAS scores and STAI-I scores after intervention in the groups

	Group 1 (n=40)	Group 2 (n=40)	Group 3 (n=40)	p
VAS, median (IQR) [min-max]	3 (4) [2-10]	4 (4) [1-9]	3 (2) [1-10]	0.557
Decrease in STAI-I, n (%)	15 (37.5)	21 (52.5)	22 (55.0)	**0.018**

VAS: visual analog scale, STAI: state-trait anxiety inventory, IQR: interquartile range

DISCUSSION

Pain is an unpleasant feeling felt in the face of potential harm (16). Therewithal anxiety is an uncomfortable state of mind or feeling of helplessness about a threatening situation or forecast (17). Aromatherapy is thought to have a therapeutic effect in conditions such as anxiety, depression, and chronic pain induced by stress by affecting the hippocampal formation and regions of the limbic system such as the hypothalamus and piriform cortex (18). Therefore, the present study aimed to compare the effect of aromatherapy using lavender and frankincense essential oils on the severity of anxiety and severity of pain during SWL. Less pain and anxiety can positively affect the success of SWL as it will increase the SWL duration and the amount of energy used (19). There are many studies in the literature examining the effect of lavender oil on anxiety and pain. However, fewer studies are available on the effect of frankincense oil on anxiety and pain. Frankincense has an anti-inflammatory effect by inhibiting 5 lipoxygenases and cyclooxygenase in the complement system and used has been used against many inflammatory diseases. It also has anxiety and pain relief effect (20). Studies reported that frankincense has antidepressant effects in addition to its protective effects in Alzheimer's patients (21).

In this study, we compared the effects of these two aromatic agents on anxiety and pain during the SWL procedure by an inhaler. We used the placebo group as the control group and randomized the patients according to their DIS-R scores. Abbasijahromi et al. (22) compared the effect of using lavender and Damask rose essential oils on the level of anxiety and severity of pain following C-section and they could not find a statistically significant difference between anxiety levels. Ayan et al. (18) investigated the effect of aromatherapy in patients with renal colic and found the effect of reducing pain. In our study, inhaler aromatherapy had no pain-reducing effect in both the lavender group and the frankincense group. In another study, inhaler lavender aromatherapy significantly decreased pain in female patients with renal colic. The pain did not significantly decrease in male patients, this condition may be due to differences in the gonadal hormones (23). But in our study, there is no difference between male and female patients. In the literature, the effect of lavender oil on pain and anxiety has always been emphasized. It has been shown that the use of lavender oil by inhalation significantly decreased pain after cesarean section and during needle insertion in hemodialysis patients (24,25). Today, aromatherapy is used as therapeutic and ancillary therapy in many centers. Aromatherapy has become a legal part of holistic nursing (26).

CONCLUSION

In this study, it was found that frankincense reduced anxiety more significantly than lavender. Both had no significant effect on pain. Frankincense can be used via inhaler in certain procedures such as lavender to reduce the anxiety of patients and increase treatment compliance. More clinical studies are needed in different areas related to this. We used aromatherapy during SWL in urology practice. Urologists may perform aromatherapy for procedures that are usually done in the office setting such as biopsies, cystoscopies, and vasectomies.

Ethics Committee Approval: The study was approved by the Clinical Researches Ethics Committee of Mersin University (13.05.2020, 376).

Conflict of Interest: None declared by the authors.

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Extensive, Idiopathic, Recurrent, Spontaneous Coronary Artery Dissection Healed With Medical Treatment

Medikal Tedavi ile İyileşen Yaygın, İdiyopatik, Tekrarlayan Spontan Koroner Arter Diseksiyonu

Ertan AKBAY[1]
0000-0002-9146-0621
Orhan ÖNALAN[2]
0000-0001-9780-7051
Adem ADAR[2]
0000-0002-2404-6447
Fahri ÇAKAN[2]
0000-0002-5427-3480

[1]Department of Cardiology, Başkent University Hospital, Alanya Medical and Research Center, Antalya, Turkey

[2]Department of Cardiology, Karabük University Faculty of Medicine, Karabük, Turkey

ABSTRACT

Spontaneous coronary artery dissection is a rare cause of sudden cardiac death and acute coronary syndrome, and mainly affects young female. Treatment of this condition depends on the clinical features, hemodynamic and angiographic findings of the patient, but due to the rarity of the disease, guideline support was insufficient until the last period. Depending on the condition of the patient and the coronary blood flow, medical observation, percutaneous coronary intervention or coronary artery bypass graft surgery are used in the treatment. We present a young female patient who underwent emergency coronary artery bypass graft surgery after spontaneous left main coronary artery dissection four years ago and was recently diagnosed with extensive spontaneous coronary artery dissection in her right coronary artery. After the diagnosis, the patient was given medical treatment and recovered completely.
Keywords: Spontaneous coronary artery dissection, acute coronary syndrome; coronary artery disease.

ÖZ

Spontan koroner arter diseksiyonu, ani kardiyak ölüm ve akut koroner sendromun nadir bir nedenidir ve özellikle genç kadınları etkiler. Bu durumun tedavisi hastanın klinik özelliklerine, hemodinamik ve anjiyografik bulgularına bağlıdır, ancak hastalığın nadir olması nedeniyle son döneme kadar kılavuz desteği yetersizdi. Hastanın ve koroner kan akımının durumuna göre tedavide medikal gözlem, perkütan koroner girişim ya da koroner arter bypass greft cerrahisi kullanılmaktadır. Dört yıl önce spontan sol ana koroner arter diseksiyonu sonrası acil koroner arter bypass greft cerrahisi uygulanan ve yakın zamanda sağ koroner arterinde yaygın spontan koroner arter diseksiyonu tanısı alan genç bir kadın hastayı sunuyoruz. Teşhisin ardından hasta medikal tedavi ile takip edildi ve tamamen iyileşti.
Anahtar kelimeler: Spontan koroner arter diseksiyonu; akut koroner sendrom; koroner arter hastalığı.

INTRODUCTION

A spontaneous coronary artery dissection (SCAD) occurs when a tear is formed in the arterial wall, which results in the separation of intimal and medial layers and the formation of a pseudo lumen (1,2). Coronary blood flow in the real lumen deteriorates due to hematoma formation inside the pseudo-lumen which compresses the actual lumen (1). This may lead to myocardial ischemia, acute coronary syndrome, cardiogenic shock and even sudden cardiac death (3,4). The peripartum state, oral contraceptive use, connective tissue disease, and notably fibromuscular dysplasia are some of the etiological factors of SCAD (1). This case pertains to a young female who had idiopathic SCAD in the right artery and fully recovered with the non-invasive treatment.

Corresponding Author
Sorumlu Yazar
Ertan AKBAY
drertanakbay@gmail.com

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CASE REPORT

A thirty-six-year-old female was admitted to the emergency service with excruciating chest pain and numbness in the left arm. Her anamnesis revealed the history of coronary artery bypass graft surgery (CABG) four years ago. The patient had no cardiovascular risk factors; she had three children and seven years have passed since her last birth. Although no ischemic changes were observed in the ECG, the patient stated that her symptoms were similar to what she had experienced prior to her CABG (Figure 1). Her vital signs were stable (blood pressure: 120/170 mmHg, breathing rate: 12/min, heart rate: 60bpm and SpO2: 95%), laboratory tests revealed high levels of CK-MB (26 U/L, N: 0-25 U/L) and normal levels of troponin (0.000 ng/ml, N: 0.000-0.006 ng/ml). Other laboratory parameters were completely normal (creatinine: 0.54 mg/dl, hemoglobin: 12.1 g/dL, TSH: 0.65 uIU/ml). When we detailed the anamnesis, it was learned that the patient presented with chest pain similar to the emergency department four years ago and had a cardiac arrest in the follow-up. Since anterior wall myocardial infarction had been observed in her ECG after defibrillation, she had been transferred to the nearest interventional cardiology center. Coronary angiography had showed a dissection extending from the left main coronary artery (LMCA) to the left anterior descending artery (LAD), following which the patient was given an emergency CABG at that time (5). With her current clinical symptoms, the patient was admitted to coronary intensive care unit and given anti-platelet and anticoagulation treatment. The coronary angiography was performed twelve hours after hospitalization and revealed non-critical obstructions in the LAD and circumflex artery (CX) (Figure 2). The flow in the distal portion of the LAD was competitive between native LAD and left internal mammary artery (LIMA). LIMA was selectively imaged and seen as normal. Extensive dissection line was seen in the right coronary artery (RCA), starting from the right coronary sinus and extending to the posterior descending artery (Figure 3). The previous dissection extending from LMCA to LAD, which had been present in her previous angiography, was seen as recovered totally (Figure 2). Since the clinical condition of the patient was stable, she was given dual antiplatelet and heparin infusion. Thorax CT angiography was performed whether to detect the aortal extension of the dissection in the right coronary sinus. No dissection was observed in the ascending aorta at its distal end. As the condition of the patient remained stable, her medication was changed to low molecular weight heparin and she was monitored at the service unit. The control coronary artery angiography was performed two weeks later and revealed that the right coronary sinus dissection had regressed, the hazy appearance of the RCA right ventricle branch had improved and the distal flow became better (Figure 4).

The patient was discharged after being electrically and hemodynamically stable at mobilization. At the 6th month follow-up, she did not report any complaints and there was no ischemic event in this period.

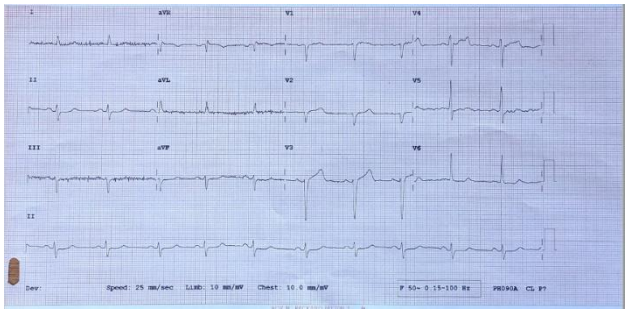

Figure 1. Electrocardiography at hospital admission

DISCUSSION

Although SCAD is rare in the general population, it is more common in female, younger than 50 years of age, pregnancy and peripartum period (6,7). While the treatment was planned with case reports and retrospective study information before, it found a place in the "2020 European Society of Cardiology, Acute Coronary Syndromes in Patients Presenting without Persistent ST-Segment Elevation Guidelines" (8). Our case differs since she had a coronary bypass surgery due to SCAD and this time presented with spontaneous dissection of a different coronary artery and recovered without complications with medical treatment.

SCAD is classified according to the occlusion and severity of the occlusion in the coronary. SCAD types have been identified, and the visual appearance of multiple lumen in the coronary is determinant of type 1 dissection (6). Although coronary CT angiography (CCTA) is useful in diagnosis, its negative nature is not sufficient to exclude the diagnosis of SCAD (9). In addition, intravascular ultrasound (IVUS) and intracoronary imaging (optical coherence tomography, OCT) are used in its diagnosis (10).

Non-invasive treatment, percutaneous coronary intervention (PCI) and CABG are some clinical options for

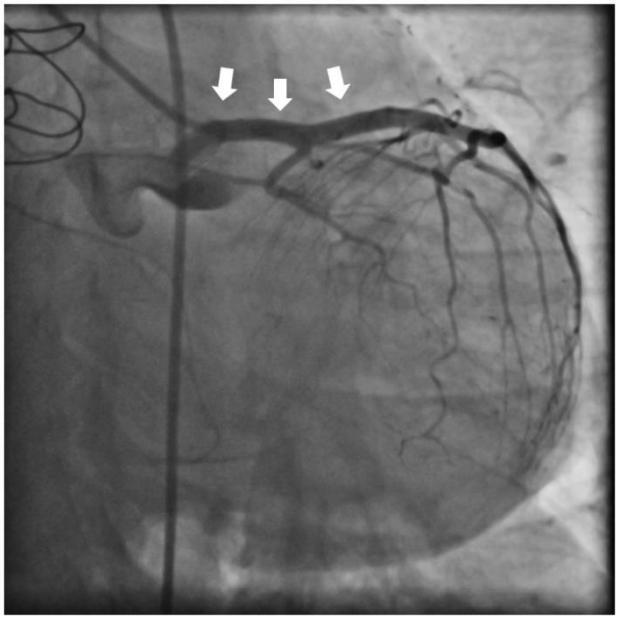

Figure 2. The main coronary and proximal left anterior descending artery area with previous dissection and requiring coronary artery bypass surgery

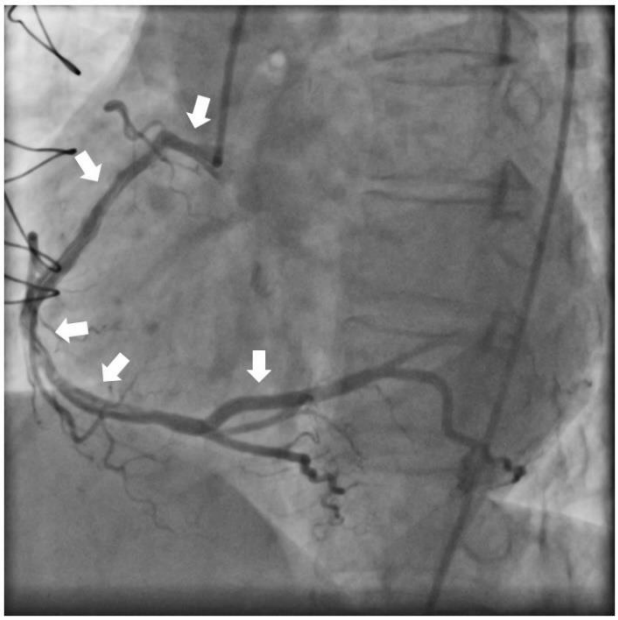

Figure 3. Long and wide dissection in the right coronary artery starting from the coronary sinus

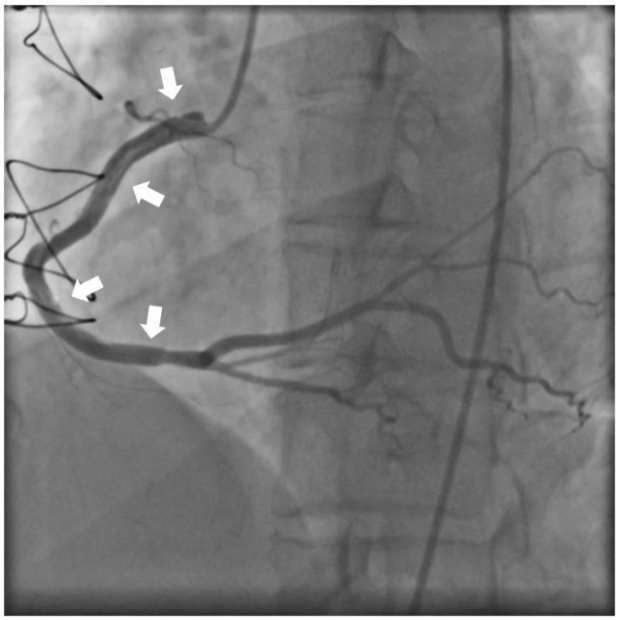

Figure 4. Control coronary angiography performed two weeks later

SCAD (6,8). The hemodynamic status of the patients, whether the lesion is obstructive or not, and distal coronary flow status affect the treatment strategy (6,8). In addition to patient-specific treatment, recent studies suggest that non-invasive treatment is superior to PCI and CABG in terms of mortality and recurrence (11-13). In her previous admission, the patient had applied to the emergency department with similar symptoms and had experienced a cardiac arrest during her follow-up. Emergency CABG had been performed after LMCA dissection was observed in her coronary angiography (5). This time, patient's hemodynamics were stable and dissection in LMCA was observed healed by the time and a new diffuse dissection arose in RCA. Since the vital signs of the patient were stable, there was no chest pain and TIMI 3 flow was present in the distal proportion of RCA, we decided to continue medication with non-invasive treatment. In addition, since the RCA is dissected from the initiation to the distal, we decided not to perform PCI as it requires penetration through the pseudo-lumen, which may increase the hematoma inside the pseudo-lumen and disrupt the flow in the true lumen. If PCI is unavoidable, it should be performed together with IVUS or OCT (14). Increased complication risk of a second CABG led us to primarily consider non-invasive treatment.

When the clinic is unstable, as in the first application of our patient, we frequently apply to interventional treatment strategies (13,15). Cardiogenic shock is observed more frequently in SCAD patients presenting with ST elevation compared to atherosclerotic lesions (15). In addition, the dissected area is observed most frequently in the LMCA and proximal LAD (15). However, survival is better in SCAD patients presenting with ST elevation (13,15).

Coronary angiography performed on the 15th day of hospitalization showed that the dissection regressed. Our case showed that even a diffused dissection could be treated without any complications by non-invasive treatment, consistent with current reports (8,16). In addition, due to the absence of common predisposing factors like vasculitis, systemic inflammatory disease, pregnancy, substance use and stress, we identified this case as idiopathic SCAD. Studies similar to our patient showed that SCAD recurs with a frequency of more than 10% and can often occur in a different coronary artery (17,18). In addition, it suggests that recurrence may be associated with hypertension and coronary tortuosity (18). Although long-term treatment is not clearly known, medical treatment and follow-up seem to be a good alternative in patients with recurrent SCAD (13,18).

In conclusion, even in the presence of extensive dissection, if the clinical characteristics of SCAD patients are stable, the dissection can regress with a non-invasive treatment.

Informed Consent: Written informed consent was obtained from the patient for publication and accompanying images.

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AUTHOR GUIDELINES

SCIENTIFIC RESPONSIBILITY

In terms of scientific publishing standards, articles to be submitted should be prepared in accordance with the criteria of the International Committee of Medical Journal Editors (ICMJE), the World Association of Medical Editors (WAME) and the Committee of Publication Ethics (COPE).

- All articles must be complied with the research and publication ethics. The responsibility of the articles belongs to the authors.
- Articles are required to have not been published in anywhere previously, and/or are not in the evaluation process for publication.
- Articles must be submitted with the Copyright Transfer Form signed by all authors to begin the evaluation process. For authors' order, the signature order in the Copyright Transfer Form is based on.
- The corresponding author is responsible for the final version of the article on behalf of all authors.

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- Compliance with The Principles of Helsinki Declaration (https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/) is required in all studies including "human" factor. In this kind of studies, authors must state that they perform the study in compliance with these principles, they have taken the approval from ethics committee of their institution and the "informed consent" from people participating the study, in the MATERIAL AND METHODS section.
- If "animal" factor was used in the study, authors must state that they have protected the animal rights in line with the principles of Guide for the Care and Use of Laboratory Animals (https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf) and they have taken the approval from ethics committee of their institution, in the MATERIAL AND METHODS section.
- In case reports, informed consent must be taken from patients.
- The information of the ethics committee approval should be indicated together with the name of the committee, approval date and number, in the MATERIAL AND METHODS section.
- If there is a direct-indirect commercial relation or an institution giving financial support in the study, authors must state that they have no commercial relationship with the commercial product, medicine, company etc. used, or if any, what kind of a relationship they have (consultant, other agreements), in the cover letter to the editor.
- The authors are responsible for reporting all personal and financial relationships that may be related with the study. It is necessary to state clearly whether there is any conflict of interest related to the submission and/or evaluation of the article.
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Articles must be uploaded to the system as separate files as described below.

Copyright Transfer Form: The Copyright Transfer Form to be obtained from the system during the submission must be signed by all authors in accordance with the authorship order in the article.

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Research Article

TITLE (English and Turkish), SHORT TITLE, ABSTRACT (English and Turkish), Keywords (English and Turkish), INTRODUCTION, MATERIAL AND METHODS, RESULTS, DISCUSSION, CONCLUSION, REFERENCES
ABSTRACT and ÖZ should be compatible in terms of translation and each should be between 200-250 words.
ABSTRACT should be structured as "Aim, Material and Methods, Results, Conclusion".
ÖZ, should be structured as "Amaç, Gereç ve Yöntemler, Bulgular, Sonuç".

Review (Invited Only)

TITLE (English and Turkish), SHORT TITLE, ABSTRACT (English and Turkish), Keywords (English and Turkish), INTRODUCTION, Subtitles Related to the Subject, CONCLUSION, REFERENCES
ABSTRACT and ÖZ should be compatible in terms of translation and each should be between 150-200 words.

Case Report

TITLE (English and Turkish), SHORT TITLE, ABSTRACT (English and Turkish), Keywords (English and Turkish), INTRODUCTION, CASE REPORT, DISCUSSION, REFERENCES
ABSTRACT and ÖZ should be compatible in terms of translation and each should be between 100-150 words.

Other

The general writing rules are applied for the preparation of the writings (letter to the editor, editorial comment/discussion, etc.) except these three basic types of article. There is no title and abstract sections in these writings. The number of references is limited to 5. The dedicated article should be specified by giving the number and date. The name, institution and address of the author should be included at the end of writing. Answer to the letter is given by the editor, or authors of the dedicated article, by publishing again in the journal.

AUTHOR GUIDELINES

WRITING RULES

- Articles should be prepared as Microsoft Word® document.
- The required margins are 2.5 cm on all sides.
- Page numbers should be placed to bottom right corner of pages.
- All texts must be typed with double-space as left-aligned using 12 point Times New Roman font.

KEYWORDS

- Number of the keywords must be at least 2, words should be separated from each other by a semicolon (;).
- Keywords in Turkish must be given in accordance with Türkiye Bilim Terimleri (TBT) (http://www.bilimterimleri.com), and keywords in English must be given in accordance with Medical Subject Headings (MESH) (http://www.nlm.nih.gov/mesh/MBrowser.html).

STATISTICAL METHODS

- All research articles should be assessed in terms of biostatistics and indicated with appropriate plan, analysis and report. In these articles last subtitle of the MATERIAL and METHODS section should be the "Statistical Analysis".
- In this section, the statistical methods used in the study should be written by indicating the purpose of use, package programs and versions used for statistical analysis should be specified.
- p values should be given in three decimal digits (p=0.038; p=0.810 etc.).
- Further information to control the convenience of articles in terms of biostatistics, can obtained from www.icmje.org.

ABBREVIATIONS

- The term should be written in full words with the abbreviation in parenthesis where first mentioned, and the same abbreviation should be used throughout the entire text.
- Abbreviations used internationally should be used in accordance with the Scientific Writing Rules.

TABLES AND FIGURES

- Should be indicated at the end of the relevant sentence in the text as (Table 1) and/or (Figure 1).
- Tables (with headings) and figures (with captions) must be added after references at the end of the text as each to be on a separate page.
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- If any abbreviation or symbol is used in tables and figures, it should be explained as a footnote below.
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- If figure, picture, table, graphic etc. which have been published before is used, written permission must be taken and it should be stated in the explanation of figures, pictures, tables, graphics. The legal responsibility in this regard belongs the authors.

ACKNOWLEDGEMENT

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- Reference list should be formed according to the reference order used in the text.
- If the number of authors are 6 or less, all authors should be specified, if there are 7 or more "et al." should be added after the first 6 authors are specified.
- The conference papers, personal experiences, unpublished papers, theses and internet addresses should not be used as references.
- DOI is the only acceptable online reference.

Article:

Al-Habian A, Harikumar PE, Stocker CJ, Langlands K, Selway JL. Histochemical and immunohistochemical evaluation of mouse skin histology: comparison of fixation with neutral buffered formalin and alcoholic formalin. J Histotechnol. 2014;37(4):115-24.

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Book:

Buckingham L. Molecular diagnostics: fundamentals, methods and clinical applications. 2nd ed. Philadelphia: F.A. Davis; 2012.

Book Chapter:

Altobelli N. Airway management. In: Kacmarek R, Stoller JK, Heuer AJ, editors. Egan's fundamentals of respiratory care. 10th ed. St. Louis: Saunders Mosby; 2013. p.732-86.

YAZARLARA BİLGİLENDİRME

BİLİMSEL SORUMLULUK

Bilimsel yayıncılık standartları açısından, gönderilecek makaleler, Uluslararası Tıbbi Dergi Editörler Kurulu (ICMJE), Dünya Tıbbi Editörler Birliği (WAME) ve Yayın Etik Kurulu (COPE) kriterlerine uygun olarak hazırlanmalıdır.

- Gönderilecek makalelerde araştırma ve yayın etiğine uyulması zorunludur. Makalelerin sorumluluğu yazarlarına aittir.
- Makalelerin daha önce hiç bir yerde yayınlanmamış ve/veya yayınlanmak üzere değerlendirme sürecinde olmaması gerekir.
- Değerlendirme sürecinin başlaması için makaleler, tüm yazarlar tarafından imzalanmış Telif Hakkı Devir Formu ile birlikte gönderilmelidir. Yazar sıralaması için Telif Hakkı Devir Formu'ndaki imza sırası dikkate alınır.
- Sorumlu yazar, tüm yazarlar adına makalenin son halinin sorumluluğunu taşır.

ETİK SORUMLULUK

- "İnsan" öğesini içeren tüm çalışmalarda Helsinki Deklerasyonu Prensipleri'ne (https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/) uygunluk aranır. Bu tip çalışmalarda yazarların, GEREÇ VE YÖNTEMLER bölümünde çalışmayı bu prensiplere uygun olarak yaptıklarını, kurumlarının etik kurullarından onay ve çalışmaya katılmış insanlardan "bilgilendirilmiş olur" (informed consent) aldıklarını belirtmeleri gerekmektedir.
- Çalışmada "Hayvan" öğesi kullanılmış ise yazarların, GEREÇ VE YÖNTEMLER bölümünde Guide for the Care and Use of Laboratory Animals (https://grants.nih.gov/grants/olaw/guide-for-the-care-and-use-of-laboratory-animals.pdf) prensipleri doğrultusunda çalışmalarında hayvan haklarını koruduklarını ve kurumlarının etik kurullarından onay aldıklarını belirtmeleri gerekmektedir.
- Olgu sunumlarında hastalardan "bilgilendirilmiş olur" (informed consent) alınmalıdır.
- Etik kurul onay bilgisi GEREÇ ve YÖNTEMLER bölümünde kurul adı, onay tarihi ve sayısı ile birlikte belirtilmelidir.
- Eğer çalışmada direkt-indirekt ticari bağlantı veya maddi destek veren kurum mevcut ise yazarlar; kullanılan ticari ürün, ilaç, firma vb. ile ticari hiçbir ilişkisinin olmadığını veya varsa nasıl bir ilişkisinin olduğunu (konsültan, diğer anlaşmalar), editöre sunum sayfasında belirtmelidirler.
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- Makalelerin bilimsel ve etik kurallara uygunluğu yazarların sorumluluğundadır.

BAŞVURU DOSYALARI

Makaleler aşağıda belirtilen şekilde ayrı dosyalar halinde sisteme yüklenmelidir.

Telif Hakkı Devir Formu: Başvuru sırasında sistemden alınacak Telif Hakkı Devir Formu tüm yazarlar tarafından makaledeki yazar sıralamasına uygun şekilde imzalanmış olmalıdır.

Başvuru Mektubu: Makalenin türü, daha önce hiç bir yerde yayınlanmamış ve/veya yayınlanmak üzere değerlendirme sürecinde olmadığı, varsa çalışmayı maddi olarak destekleyen kişi ve kuruluşlar ve bu kuruluşların yazarlarla olan ilişkileri (yoksa olmadığı) belirtilmelidir. Makalenin konusuyla ilgili olarak önerilen, yazarlarla ve kurumlarıyla ilgisi olmayan en az iki hakemin adları, akademik unvanları, kurumları, iletişim bilgileri ve e-posta adresleri yazılmalıdır. Editörlerin hakemleri seçme hakkı saklıdır.

Başlık Sayfası: Makalenin başlığını (İngilizce ve Türkçe), 40 karakteri geçmeyen kısa başlık, tüm yazarların adlarını, akademik unvanlarını, ORCID® numaralarını, kurumlarını, e-posta adreslerini ve ayrıca sorumlu yazarın adını, yazışma adresini, telefon numarasını, e-posta adresini içermelidir. Makale daha önce bilimsel bir toplantıda sunulmuş ise toplantı adı, tarihi ve yeri (yoksa sunulmadığı) belirtilmelidir.

Ana Metin: Makalenin başlığı (İngilizce ve Türkçe), 40 karakteri geçmeyen kısa başlık, Öz (İngilizce ve Türkçe), Anahtar kelimeler (İngilizce ve Türkçe), Ana Metin (gönderilen makalenin türüne uygun olarak bölümlere ayrılmış), Kaynaklar, Tablolar ve Şekil açıklamaları yer almalıdır.

Etik Kurul Onay Belgesi: Tüm araştırma makaleleri için Etik Kurul Onay Belgesi ayrı bir dosya olarak yüklenmelidir.

Not: Makalede şekil, resim veya fotoğraf varsa bunların da her biri ayrı birer dosya olarak yüklenmelidir.

MAKALE TÜRÜNE GÖRE KULLANILMASI GEREKEN BÖLÜMLER

Araştırma Makalesi

BAŞLIK (İngilizce ve Türkçe), KISA BAŞLIK, ÖZ (İngilizce ve Türkçe), Anahtar kelimeler (İngilizce ve Türkçe), GİRİŞ, GEREÇ VE YÖNTEMLER, BULGULAR, TARTIŞMA, SONUÇ, KAYNAKLAR

ÖZ ve ABSTRACT çeviri açısından uyumlu olmalı ve her biri kendi içinde 200-250 kelime arasında olmalıdır.

ABSTRACT, "Aim, Material and Methods, Results, Conclusion" şeklinde yapılandırılmalıdır.

ÖZ, "Amaç, Gereç ve Yöntemler, Bulgular, Sonuç" şeklinde yapılandırılmalıdır.

Derleme (Sadece Davetli)

BAŞLIK (İngilizce ve Türkçe), KISA BAŞLIK, ÖZ (İngilizce ve Türkçe), Anahtar kelimeler (İngilizce ve Türkçe), GİRİŞ, Konu ile İlgili Alt Başlıklar, SONUÇ, KAYNAKLAR

ÖZ ve ABSTRACT çeviri açısından uyumlu olmalı ve her biri kendi içinde 150-200 kelime arasında olmalıdır.

Olgu Sunumu

BAŞLIK (İngilizce ve Türkçe), KISA BAŞLIK, ÖZ (İngilizce ve Türkçe), Anahtar kelimeler (İngilizce ve Türkçe), GİRİŞ, OLGU SUNUMU, TARTIŞMA, KAYNAKLAR

ÖZ ve ABSTRACT çeviri açısından uyumlu olmalı ve her biri kendi içinde 100-150 kelime arasında olmalıdır.

Diğer

Bu üç temel makale türü dışındaki (editöre mektup, editöryel yorum/tartışma vb.) yazıların hazırlanmasında da genel yazım kuralları geçerlidir. Bu tür yazılarda başlık ve öz bölümleri yoktur. Kaynak sayısı 5 ile sınırlıdır. İthaf olunan makale sayı ve tarih verilerek belirtilmelidir. Yazının sonunda yazarın ismi, kurumu ve adresi yer almalıdır. Mektuba cevap, editör veya makalenin yazarları tarafından, yine dergide yayınlanarak verilir.

YAZARLARA BİLGİLENDİRME

YAZIM KURALLARI
- Makaleler Microsoft Word® belgesi olarak hazırlanmalıdır.
- Sayfa kenarlarında 2,5 cm boşluk bırakılmalıdır.
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- Anahtar kelime sayısı en az 2 olmalı, kelimeler birbirlerinden noktalı virgül (;) ile ayrılmalıdır.
- Türkçe anahtar kelimeler Türkiye Bilim Terimleri (TBT)'ne (http://www.bilimterimleri.com), İngilizce anahtar kelimeler Medical Subject Headings (MESH)'e (http://www.nlm.nih.gov/mesh/MBrowser.html) uygun olarak verilmelidir.

İSTATİSTİKSEL YÖNTEMLER
- Tüm araştırma makaleleri biyoistatistik açıdan değerlendirilmeli ve uygun plan, analiz ve raporlama ile belirtilmelidir. Bu makalelerde, GEREÇ VE YÖNTEMLER bölümünün son alt başlığı "İstatistiksel Analiz" olmalıdır.
- Bu bölümde çalışmada kullanılan istatistiksel yöntemler ne amaçla kullanıldığı belirtilerek yazılmalı, istatistiksel analiz için kullanılan paket programlar ve sürümleri belirtilmelidir.
- p değerleri ondalık üç basamaklı (p=0,038; p=0,810 vb.) olarak verilmelidir.
- Makalelerin biyoistatistik açıdan uygunluğunun kontrolü için ek bilgi www.icmje.org adresinden temin edilebilir.

KISALTMALAR
- Terim ilk kullanıldığında parantez içinde kısaltmayla birlikte açık olarak yazılmalı ve tüm metin boyunca aynı kısaltma kullanılmalıdır.
- Uluslararası kullanılan kısaltmalar Bilimsel Yazım Kurallarına uygun şekilde kullanılmalıdır.

TABLOLAR VE ŞEKİLLER
- Metinde ilgili cümlenin sonunda (Tablo 1) ve/veya (Şekil 1) şeklinde belirtilmelidir.
- Tablolar (başlıklarıyla birlikte) ve şekiller (açıklamalarıyla birlikte) kaynaklardan sonra ve her biri ayrı bir sayfada olacak şekilde metnin sonuna eklenmelidir.
- Tablo başlıkları tablo üstünde (Tablo 1. Tablo başlığı), şekil açıklamaları ise şeklin altında (Şekil 1. Şekil açıklaması), ilk harfleri büyük olacak şekilde yazılmalıdır.
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- Şekiller ve fotoğraflar, .png, .jpg vb. formatta ve en az 300 dpi çözünürlükte ayrı dosyalar halinde yüklenmelidir.
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TEŞEKKÜR
- Eğer çıkar çatışması/çakışması, finansal destek, bağış ve diğer bütün editöryel (İngilizce/Türkçe değerlendirme) ve/veya teknik yardım varsa, bu bölümde, KAYNAKLAR bölümünden önce belirtilmelidir.

KAYNAKLAR
- Kaynaklar, kullanım sırasına göre numaralandırılmalı ve metin içinde ilgili cümlenin sonunda parantez içinde numaralarla (1) veya (1,2) veya (3-5) şeklinde verilmelidir.
- Kaynaklar dizini, metin içinde kaynakların kullanıldığı sıraya göre oluşturulmalıdır.
- Yazar sayısı 6 veya daha az ise tüm yazarlar belirtilmeli, 7 veya daha fazla ise ilk 6 yazar belirtildikten sonra "et al." eklenmelidir.
- Kongre bildirileri, kişisel deneyimler, basılmamış yayınlar, tezler ve internet adresleri kaynak olarak gösterilmemelidir.
- DOI tek kabul edilebilir online referanstır.

Makale:

Al-Habian A, Harikumar PE, Stocker CJ, Langlands K, Selway JL. Histochemical and immunohistochemical evaluation of mouse skin histology: comparison of fixation with neutral buffered formalin and alcoholic formalin. J Histotechnol. 2014;37(4):115-24.

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Kitap:

Buckingham L. Molecular diagnostics: fundamentals, methods and clinical applications. 2nd ed. Philadelphia: F.A. Davis; 2012.

Kitap Bölümü:

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