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The European Research Journal 2024;10(1):1-7 Original Article

DOI: https://doi.org/10.18621/eurj.1315058 *Parasitology, Infectious Diseases*

A simple, effective and inexpensive method to isolate the nucleic acid (DNA/RNA) from a single tick for molecular detection of various pathogens

Sudhir Bhatia, Gudrun Baersch

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ABSTRACT

Objectives: Ticks are vectors of a number of pathogens, which cause diseases with fatal consequences, therefore it is essential to detect such pathogens from single tick. Molecular methods like polymerase chain reaction (PCR) are offering such possibilities. At present, cumbersome methods involving liquid nitrogen, cutting ticks with scalpels as well as pooled ticks are being used worldwide. Our goal is to develop a reliable and fast method to obtain nucleic acid (DNA/RNA) from the single tick shipped at room temperature to detect various pathogens.

Methods: We developed a mechanical crushing method with mini column nucleic acid isolation from a single tick shipped at room temperature with postal or currier service in a letter. PCR detection was done for *Borrelia burgdorferi* and tick-borne encephalitis virus as examples.

Results: This method was used successfully for the isolation of nucleic acid from single tick and later used for PCR detection of *B. burgdorferi* and tick-borne encephalitis virus on 17 single tick samples as examples, but for last 18 years, this method was used on more than 250 ticks from Germany. Spectrometric values indicate the presence of sufficient yield of DNA and RNA (up to 900 µg/mL per tick) during the isolation.

Conclusions: This may be the first report about a number of one single tick cases, which were sent at room temperature in letters with postal services for isolation of the nucleic acid with mini column kit and used later on for PCR detection of various pathogens. This inexpensive and simple method may be used in any laboratory worldwide for monitoring the presence of tick-borne pathogens.

Keywords: Tick, tick borne pathogens, nucleic acid isolation, *Borrelia burgdorferi*, polymerase chain reaction

Tick borne pathogens are one of the major threats to human beings and animals because the ticks are vectors of many different pathogens with fatal consequences in many incidences around the world. These pathogens are *Borrelia burgdorferi, Babesia microti, Babesia bovis, Anaplasma genus*, tick borne encephalitis virus etc. Polymerase chain reaction (PCR) is one of the important detection methods to detect various pathogens like SARS CoV-2. Influenza virus, Hepatitis *C virus, Coxiella* burnetii and many other pathogens [1-9]. Moreover there are large number of RNA viruses in ticks, which are needed to be addressed [10].

To conduct the PCR, there is a need of isolated nucleic acid from the source. In the case of ticks, user needs to isolate this from tick itself, but there are many

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methods described in the literature.

Various groups have developed various DNA isolations for conducting molecular analysis. The ticks were preserved and cumbersome nucleic acid isolation method was developed [11]. The ticks were collected in liquid nitrogen and isolated with mini column methods to detect the various RNA viruses [10] In another work, researchers digested the ticks in lysis buffer with Proteinase K overnight at 56 °C, vortexed and centrifuged the tick to get the tick exoskeleton to isolate the nucleic acid from them in order to detect Rickettsia in PCR test [12]. Another group has developed a different method to isolate DNA and RNA from single tick as the tick was washed with PBS and dried. After that it was cut with sterile scalpel and the isolation was performed with chloroform and commercial solution [13]. This method is very cumbersome as there are many ticks, which are very small, hence this method is not suitable. In another method: beads were applied to beat the ticks in a microtube in lysis buffer containing proteinase K [14]. Mortar and Pestle method was being used for a pool of 4-10 ticks, which were washed in ethanol and washed with sterile water. After that they were dried and PBS was added to crush them physically. The DNA from the homogenized extract were isolated with chloroform method [15]. There was a comparison done with different homogenizers to isolate nucleic acid from medical relevant ticks [16]. There are research works, where no mechanical crushing is used to isolate the ticks, but ticks were cut with scalpels in two halves. One part is stored in 80% ethanol and other half was used for isolation and subsequently used molecular detection of Anaplasma and *Ehrlichia gena* [17].

In literature, there are a number of publications about the detection of pathogens from pooled ticks, but there is a need of simple nucleic acid isolation method, which can be applied under field conditions from a single tick, where the tick can be transported under field conditions without any storage chemicals as some research workers are using liquid nitrogen, which is not easily available under fields. Therefore, our laboratory has decided to develop a simple, inexpensive and effective isolation method to detect the various pathogens from single tick. Hence such research about detection of the tick-borne pathogens from single ticks with various molecular methods can be very accurate to have the reliable data about the prevalence of pathogens in a particular area. This type data can be used to develop the preventive and therapeutic measurements in a particularly area.

METHODS

Ticks were sent to Genekam laboratory from different areas at room temperature without any storage chemicals through German postal service in plastic microtubes or glass vials. In many cases, they were fixed with adhesive film on a piece of paper in such a way that the tick was trapped between the folded piece of paper. (Ticks were not pasted on adhesive tape as they were put in the folded paper. If one puts the tick on the adhesive tape, it can cause problem during isolation process) so that they cannot move or go out the containers. On the arrival of these ticks, they were kept at room temperature in restricted area before their processing. Most of the shipments or letters were containing one tick, but sometimes, there were two ticks. In this work, each tick was processed as single tick, not pooled samples of two ticks.

Isolation of nucleic acid from single tick: Mortar and pestle (size and dimensions are mentioned elsewhere) was prepared as followings: they were rinsed in soaped water for 10 minutes, washed with tap water and at the end rinsed with single distilled water twice. After that they were air dried. Before the use, mortar and pestle were sterilized with flame of gas burner while exposing for 1 to 2 minutes that the flame should destroy any remaining DNA from previous isolation, if any and flame should not harm the mortar and pestle. The whole area (particularly bottom) of mortar and head of pestle were exposed to the flame. This is very important step during isolation of nucleic isolation to avoid the contamination from previous samples. It was repeated 3 to 4 times. After that mortar and pestle allowed to cool.

Pincettes were also cleaned with soaped water and washed with tap water, but finally washed with single distilled water. The ends of pincettes were exposed to flame in order to destroy any contamination on them. They were allowed to cool. Alternatively, these were washed thoroughly with soap solution and later with distilled water twice.

Three hundreds µL of lysis buffer from Genekam DNA/RNA kit was also added to mortar. Now single

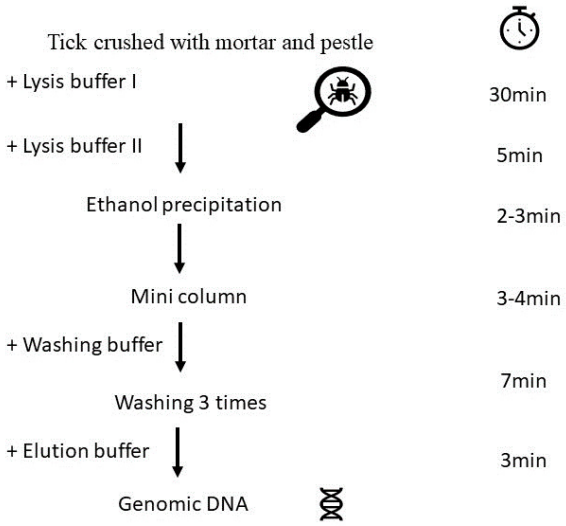

Fig. 1. The sheme of isolation method of almost 50-55 minutes.

tick was observed with a hand lens and with help of pincettes put in lysis buffer (dead ticks) or the single tick was just thrown into the lysis buffer from container (This was used with live tick, donot use pincettes to catch live ticks as they may fall or escape!). The tick was crushed with pestle to get the extract solution. The extract solution was put in microtube. After that 30 µL Proteinase K was added. This was put at 56 °C for 30 minutes or overnight in the heating block. After that DNA/RNA was isolated with standard method from Genekam nucleic acid kit. The protocol is available with manufacturer. The eluted volume was 50 to 100 µl for conducting the PCR testing for different pathogens. The diagram of steps of DNA isolation is shown with the time line in Fig. 1.

Detection of Borrelia. Burgdorferi

Two µL of isolated nucleic acid was used to perform the real time or conventional PCR with Genekam PCR kits. The conventional PCR was a nested PCR. It was performed with thermoycler (Biometra) and results were observed in 2% gel agarose. Positive and negative controls were also included. Real time PCR was also performed with 2 µL of isolated nucleic acid on ABI 7500 (Thermofisher, USA). Positive and negative controls were used.

Detection of Tick-Borne Encephalitis Virus

It was done with conventional PCR kit (Genekam). 2 µl RNA was used here to perform the PCR kit on conventional thermocycler (Biometra) and results were seen on gel agarose. Positive and negative controls were also included. Real time PCR was performed on ABI7500 with 2µl of isolated nucleic acid with real time PCR kit (Genekam, Germany). Negative and positive controls were used.

Protocols for conducting the conventional and real time PCR for both pathogens are available with manufacturer.

Spectrometric measurements of isolated DNA and RNA were measurement with Nanodrop (Thermofischer, USA). 2 µL of elution buffer of isolation kit was used to calibrate the instruments and 2 µL each sample was used to measure the amount of DNA and RNA in each sample. After the measurement, each amount of isolated DNA and RNA was calculated in µg per mL.

RESULTS

We are working with this method since 2005, during these 18 years more than 250 ticks have been used to isolate their nucleic acid and a number of different assays are conducted on them. In this research work, the results of 17 ticks are presented, where isolated nucleic acid was analyzed with real time and conventional PCR for Borrelia burgdorferi and Tick-borne encephalitis virus.

The flame to sterilize the mortar and pestle was used. It is shown as Fig. 2. The size of mortar and pestle is an important factor; therefore, a smallest mortar

Fig. 2. White colored mortar and pestle.

and pestle has the size of 8 cm in diameter with a wall thickness of 0.7 cm and depth of 4 cm. The pestle has a length of 11.5 cm and thickness of 2.5 cm. A white mortar is advantageous because the tick is then easily visible. The ticks (also removed from body as well as live) were shipped to laboratory in a glass vial, small box, carton as well as microtube or taped between a folded paper.

The results showed that nucleic acid was isolated successfully from all ticks with mortar and pestle method using mini column isolation method. Out of 17 ticks, there were only 8 ticks were positive for *B.*

Table 1. Molecular and spectrometric values of isolated nucleic acid of different *Borrelia burgdorferi* positive and negative ticks

Sample	*Borrelia burgdorferi* PCR	DNA (µg/mL)	RNA (µg/mL)
1	positive	17.2	12.7
2	positive	24.6	16.1
3	positive	83.3	61.95
4	positive	41.1	25.15
5	positive	20.2	12,1
6	positive	8.75	4.85
7	positive	41.65	37.65
8	positive	66.45	52.1
9	positive	3.65	1.15
10	positive	10.65	8.6
11	negative	3.3	0.75
12	negative	8.15	5.3
13	negative	2.5	0.8
14	negative	33.8	20.65
15	negative	5.05	5.05
16	negative	7.15	4.5
17	negative	16.15	11.85

burgdorferi in conventional and real time PCR test. There was no signal in negative controls and positive control gave the signal. These are indicators of successful real time and conventional PCR detection (Table 1). Out of 17 ticks, there was only 1 tick positive for tick borne encephalitis virus.

These all results of spectrometric measurements for DNA and RNA are shown in a table 1, where the values show that amount of DNA per tick varies between 3.65 µg/mL to 83.6 µg/mL in ticks with PCR positive results for *B. burgdorferi*, but in *B. burgdorferi* negative ticks DNA yield varies between 3.3 and 33.8 µg/mL.

It is found from results of Table 1 that there is a tend to have less amount DNA in PCR negative ticks. Similarly, RNA yield varies between 1.15 and 61.95 µg/mL for *B. burgdorferi* positive ticks and it varies between 0.75 µg/mL and 20.65 µg/mL in *Borrelia burgdorferi* negative ticks. Isolated RNA output was most of the time less than that of isolated DNA in *B. burgdorferi* positive and negative ticks. These measurements are also indicators of successful isolation of nucleic acid.

DISCUSSION

In this research work, we have shown successful isolation of nucleic acid from a single tick. In literature, there are reports of using different methods for collection of the ticks e.g., liquid nitrogen during the transportation, which is not possible under field conditions [10]. During the isolation of nucleic acid, the pooled ticks are washed with ethanol, dried and cut with scalpel. [13].) These all steps are not feasible and cumbersome under field conditions. Our method is very simple and easy to use. User needs a small mortar and pestle, which can be washed and sterilized with a flame of gas burner. Moreover, these articles can be purchased in any store or online store in any country around the world. The tick can be collected in glass vial or taped between a folded piece of paper. It can be shipped to laboratory with normal post or currier service.

This method may be used with other mini column isolation kit available on the market as we have used Genekam isolation kit in this work as well as other 2 manufacturers from Germany. (data not shown) The spectrometric measurements gave us a surprise as they show that ticks contain most of the times huge amount of DNA and RNA particularly when they are infected with *B. burgdorferi* against PCR negative ticks. We are going to investigate this aspect whether there is some kind of correlation of amount of isolated nucleic acid and the presence of a pathogen in the tick. The presence of nucleic acid in each sample shows that our isolation method was successful for single tick. There are many publications, where the users are pooling the ticks between 3-10 ticks for PCR analysis, but there are no spectrometric values. Our values per tick are indicating that there is huge amount of DNA or RNA presence in samples particularly pathogen positive ticks. It means that users with pooled ticks might have been gotten huge amount nucleic acid with our method.

In another study, the comparison between different DNA isolation methods were done, but it was found that up to 2000 µg/mL DNA can be isolated with chloroform phenol method, it is not mentioned whether they used pooled samples, but it seems that this study used pooled ticks and used a cumbersome method to remove the parts of ticks, in spite of this, our mentioned method here is also in position to provide high amount of isolated nucleic acid from single tick [7].

Comparison between our results and another study done in 2015 shows that our method is going provide higher yield of DNA as this study compared different isolation commercial methods and the yield of isolated was 0.6 to 7 µg/mL, which is many folds lower against the method mentioned in this research work (up to 900 µg/mL per tick). It may indicate our method is going to achieve better yield of isolated DNA, but a comparison between the studies may be needed [18].

Our results show that isolated DNA and RNA were sufficient to conduct PCR analysis for different pathogens like *B. burgdorferi* and tick-borne encephalitis virus. We are conducting other assays on isolated nucleic acid also to be published later.

The method presented here offers to conduct PCR analysis on a single tick, so that one can conduct the prevalence of different pathogens in particular area to develop the preventive measurements. Such methods should be part and parcel of medical studies in the medical institutes to teach the students new possibili-

ties of molecular medicine. This method should be called Genekam Single Tick nucleic acid isolation method as we developed over the past 18 years to make it prefect to be used under field conditions.

CONCLUSION

In this work, it may be the first report about a new simple and inexpensive method to isolate nucleic acid from SINGLE tick in molecular biology laboratory for detection of various pathogens, which cause a number of fatal diseases. The tick is being transported at room temperature with normal post in currier or postal letter. This method may lead to better preventive measurements as well as quicker therapeutic interventions.

Authors' Contribution

Study Conception: SB, GB; Study Design: SB, GB; Supervision: SB, GB; Funding: Institute; Materials: Institute; Data Collection and/or Processing: SB, GB; Statistical Analysis and/or Data Interpretation: SB, GB; Literature Review: SB, GB; Manuscript Preparation: SB, GB and Critical Review: HS.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. Lee KO, Park MY, Kim LH, Seong HS, Park BH, Jeong SJ. Pandemic novel influenza A (H1N1) virus in Korea: the experience from August to September 2009. Korean J Clin Lab Sci. 2009;41(4):145-152.
2. Muller MG, George AR, Walochnik J. Acinetobacter baumannii in localised cutaneous mycobacteriosis in falcons. Vet Med Int. 2010;2010:321797. doi: 10.4061/2010/321797.
3. Marushchak L, Golovko A, Deriabin O. Epizootic situation and development tools for detection of Q-fever in Ukraine: Abstract number 21.049, International Meeting on Emerging Diseases and Surveillance: Vienna, Austria, February 15-18, 2013.
4. Othman AA, Eissa AA, Markous RD, Ahmed BD, Al-Allawi NA. Hepatitis C virus genotypes among multiply transfused hemoglobinopathy patients from Northern Iraq. Asian J Transfus Sci. 2014;8(1):32-34. doi: 10.4103/0973-6247.126687.
5. Colín-Ferreyra Mdel C, Mendieta-Zerón H, Romero-Figueroa Mdel S, Martínez-Madrigal M, Martínez-Pérez S, Domínguez-García MV. [Expression of gamma interferon during HPV and Chlamydia trachomatis infection in cervical samples]. Enferm Infecc Microbiol Clin. 2015;33(2):105-109. doi: 10.1016/j.eimc.2014.05.014. [Article in Spanish]
6. Norouzian H, Ghobadian Diali H, Azadpour M, et al. PCR detection of Coxiella burnetii in milk samples of ruminants, Iran. J Med Bacteriol. 2018;7(1, 2):31-35.
7. Reifenberger GC, Thomas BA, Rhodes DVL. Comparison of DNA extraction and amplification techniques for use with engorged hard-bodied ticks. Microorganisms. 2022;10(6):1254. doi: 10.3390/microorganisms10061254.
8. Bhatia S. Pitfalls found in SARS CoV-2 specific test performance during the comparison between WHO recommended method and a commercial test. Atlantic J Med Sci Res 2023;3(1):22-26. doi: 10.5455/atjmed.2022.12.024
9. Laroche M, Weeks, ENI. Vector-borne bacterial diseases: a neglected field of infectious diseases research. Med Vet Entomol. 2023;37(2):177-178. doi: 10.1111/mve.12646.
10. Harvey E, Rose K, Eden J-S, et al. Extensive diversity of RNA viruses in Australian ticks. J Virol. 2019;93(3):e01358-18. doi: 10.1128/JVI.01358-18.
11. Hubbard MJ, Cann, KJ, Wright DJM. Validation and rapid extraction of nucleic acids from alcohol-preserved ticks. Exp Appl Acarol. 1995;19(8):473-478. doi: 10.1007/BF00048266.
12. Stanley H, Rhodes DVL. Presence of Rickettsia species in ticks collected from companion animals in Northeastern Georgia, United States. Vet Sci. 2021;8(3):37. doi: 10.3390/vetsci8030037.
13. Cafiso A, Chiappa G, Luzzago C, et al. Protocol optimization for simultaneous DNA and RNA co-extraction from single hard tick specimens. MethodsX. 2021;8:101315. doi: 10.1016/j.mex.2021.101315.
14. Crowder CD, Rounds MA, Phillipson CA, et al. Extraction of total nucleic acids from ticks for the detection of bacterial and viral pathogens. J Med Entomol. 2010;47(1):89-94. doi: 10.1603/033.047.0112.
15. Shan J, Jia Y, Hickenbotham P, Teulières L, Clokie MRJ. Combining citizen science and molecular diagnostic methods to investigate the prevalence of Borrelia burgdorferi s.l. and Borrelia miyamotoi in tick pools across Great Britain. Front Microbiol. 2023;14:1126498. doi: 10.3389/fmicb.2023.1126498.
16. Jones AM, Van de Wyngaerde MT, Machtinger ET, Rajotte EG, Thomas C Baker TC. Choice of laboratory tissue homogenizers matters when recovering nucleic acid from medically important ticks. J Med Entomol. 2020;57(4):1221-1227. doi: 10.1093/jme/tjaa006.
17. Day CA, Butler RA, Durick HE, et al. An ecological and epidemiological single-season survey of Anaplasma and Ehrlichia

positive ticks in Victoria Falls National Park, Zimbabwe. Med Vet Entomol. 2023;37(2):195-208. doi: 10.1111/mve.12640.
18. Ammazzalorso AD, Zolnik CP, Daniels TJ, Kolokotronis S. To beat or not to beat a tick: comparison of DNA extraction methods for ticks (Ixodes scapularis). PeerJ. 2015;3:e1147. doi: 10.7717/peerj.1147.

The European Research Journal 2024;10(1):8-16 Original Article

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Genetically determined plasma trefoil factor-3 levels are causally associated with the risk of ulcerative colitis: a Mendelian randomization study

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ABSTRACT

Objectives: Ulcerative colitis (UC) is an inflammatory disease restricted to the colon's mucosal layer. UC is a complex disease with a largely unknown etiology. Mendelian Randomization (MR) is a method that uses variations in genes that have a causal effect of a modifiable exposure to the disease, in genetic epidemiological studies. Trefoil factor 3 (TFF3) is a secreted protein expressed mainly in the colonic mucosa that binds with the mucin 2 protein, forming a protective barrier for the colon mucosa from bacteria and other insults. This study aimed to identify if TFF3 levels in plasma are causally associated with UC.

Methods: We performed a two-sample MR study. For exposure instrumental variables (IVs), genetically determined TFF3 levels in plasma proteome quantitative trait locus data were obtained from the published literature. Outcome data were obtained from the GWAS catalog. The "TwoSampleMR" R package was used for MR. The statistical significance of IV effect sizes on the outcome is mainly evaluated by the inverse variance weighted (IVW) method.

Results: The IVW test showed considerable statistical significance in all analyzed outcomes except for Crohn's disease (CD) samples. Heterogeneity and horizontal pleiotropy tests showed no significant results for MR sensitivity analysis.

Conclusions: We showed that TFF3 levels in plasma were causally associated with the risk of UC. Increased levels of TFF3 are reversely associated with the risk of UC. The absence of any causal relationship between TFF3 and CD from the same study cohort also supports our causal inference.

Keywords: Ulcerative colitis, trefoil factor-3, genome-wide association study, Mendelian randomization analysis

Inflammatory bowel disease (IBD) is a condition characterized by chronic inflammation of the digestive tract. IBD is separated into two subforms of the condition known as Crohn's disease (CD) and ulcerative colitis (UC) [1]. UC is a usually relapsing auto-inflammatory disease, which is restricted to the mucosal layer of the large intestine [2]. UC is a complex disease, and its etiology is largely unknown. However, several genetic, environmental, and autoimmune factors are suspected to be causative for UC [1]

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genetic heritable factors play a role in the etiology of IBD [3, 4]. It has also been shown that genetic heritable factors are stronger in CD than in UC [3, 4]. To date, more than 120 genes/genomic loci have been associated with CD [5]. However, although genetics plays a significant role in the cause of UC, only twenty-nine genes, which are cataloged in large databases, were associated with UC [6]. Genetic epidemiological studies use two approaches to identify risk genes or genetic factors in the etiology of complex diseases such as UC. These are hypothesis-driven candidate genes or hypothesis-free genome-wide association studies (GWAS) [7]. GWAS are powerful techniques for identifying risk genes or genetic factors in the etiology of complex traits or diseases [8, 9]. Published GWAS data statistics were deposited in the GWAS catalog (https://www.ebi.ac.uk/gwas/).

Mendelian Randomization (MR) is a method that uses variations in genes that are a causal effect of exposure to disease in genetic epidemiological studies. The MR method has been increasingly used in genetic epidemiological studies in the last decade [10]. MR is a robust technique for confounding factors (sampling bias, environmental confounders, reverse causation, etc.) that cause incomplete and conflicting results in observational epidemiological studies [11]. MR is analog to randomized controlled trials that are the gold standard for clinical causal inference studies. MR technique is based on the second law of Mendel, which is an independent assortment of parental alleles during meiosis and uses genetic variants as instrumental variables (IVs) for causal inference of risk factors onto outcomes (diseases/traits) [10, 11]. MR studies could be performed in a one-sample (single-study samples) or two-sample (two-independent study samples) manner. Two-sample MR (2S-MR) method has more statistical power when considering single-sample MR in terms of reaching a large sample size and needs only summary statistics, which are properly deposited in large databases, such as the GWAS catalog [10, 12].

Trefoil factors are members of the trefoil family of proteins characterized by having at least one copy of the trefoil domain [13]. In the human genome, they are represented by three genes settled in as a cluster on chromosome 21 known as Trefoil factor 1, Trefoil factor 2, and Trefoil factor 3 (TFF3). TFF3 is a secreted protein containing a 40-amino acid trefoil domain connected with three disulfide bonds and expressed mainly in the colonic mucosa (produced by goblet cells) and in other luminal tissues of the breast, thyroid, lung, etc. [13]. In the colonic mucosa, TFF3 binds with mucin 2 protein (MUC2), which is a large glycoprotein, and they form a protective barrier for the colonic mucosa from bacteria and other microorganisms [14]. The functions of TFF3 in IBD, various cancers, and various autoimmune diseases have been extensively studied and the level of its expression is associated with protective or adverse effects with these disorders [15]. In this study, we performed a univariable 2S-MR to find whether genetically determined TFF3 levels were associated with the risk of UC. To our knowledge, this is the first MR study on TFF3 and UC association.

METHODS

Exposure (TFF3) Data (For Discovery Analysis)

Instrumental Variables (Single-Nucleotide Polymorphisms-SNPs) Selections for Exposure

This study hypothesizes that levels of TFF3 in plasma could be associated with the risk of UC disease. Thus, this is a hypothesis-driven gene-centric 2S-MR study. For exposure IVs, genetically determined TFF3 protein levels in plasma proteome quantitative trait locus (pQTL) data (significant SNPs) were obtained from the study of Suhre *et al*. [16]. This study was performed on European individuals (approx. 1,000 individuals) and in this dataset 61 SNPs were associated with plasma TFF3 levels (significance levels ranged from 1.21×10^{-12} to 7.9×10^{-6}). Two of these SNPs were trans (on the other chromosomes) rather than chr6 in which the TFF3 gene is located) and the other SNPs were cis. To obtain independent exposure SNPs from those SNPs located on chr6, we performed a linkage disequilibrium (LD) pair-wise correlation (r^2) matrix by using LDlink (selected the European (CEU) as a reference population) [17]. We pruned out SNPs that had $r^2 \geq 0.30$ on chr6. Then, 6 out of 61 SNPs were determined as independent exposure IVs (Fig. 1, Table 1). F-statistics for each IV were calculated using $F = \frac{R^2 \times (n-2)}{(1-R^2)}$ the formula, where R^2 = explained variance of IVs on exposure, n = sample size of pQTL GWAS exposure [18]. It is generally accepted that the F-statistic of each SNP should be >10 in MR studies

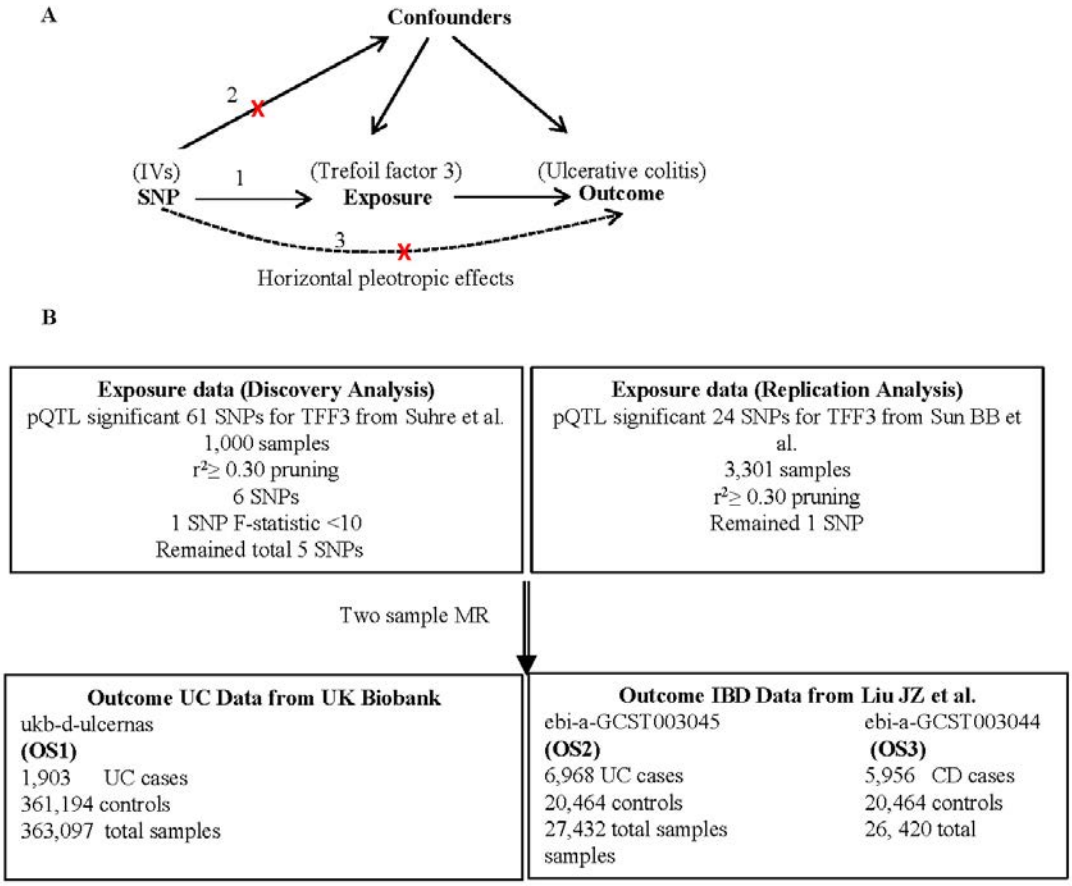

Fig. 1. (A) Mendelian Randomization acyclic graph (causal diagram) 1. SNP is associated with the exposure (relevance), 2. SNP is not associated with confounders (independence), 3. SNP only associated with outcome through the exposure (exclusion restriction). (B) Present study design flowchart.

to avoid weak instrument bias [19]. R^2 was calculated from $2\times EAF\times(1-EAF)\times Beta^2$, where Beta=effect size of IVs, EAF=effect allele frequency of GWAS exposure [18, 20] (Table 1).

Exposure (TFF3) Data (For Replication Analysis)

Independent exposure data for TFF3 plasma levels were obtained from the study of Sun BB *et al*. In this study, 2,994 proteins of plasma in 3,301 individuals from European were analyzed by protein aptamers (SOMAmers) [21]. After pruning only one SNP (rs2524277) remained (Table 1) as an independent SNP and we performed Wald ratio analysis for the effect estimate of MR.

Outcome (UC) Data

For outcome data, we searched the GWAS of UC that the summary statistics deposited in the GWAS catalog, and samples of European descent. The Open GWAS database was especially suitable for this purpose because it has been created for MR studies and contains thousands of summary statistics (https://gwas.mrcieu.ac.uk/). To obtain the most statistical power, we selected two studies that had larger sample sizes and were performed on people of European descent. The first study ID in the Open GWAS database was ukb-d-ulcernas, in which UC GWAS summary-statistic were derived from the United Kingdom (UK) Biobank. These data contained 1,903 UC cases and 361,194 controls (total sample size 363,097). We denoted this study as outcome-study 1 (OS1). The second independent study ID was ebi-a-GCST003045, in which UC GWAS summary statistics were derived from a GWAS meta-analysis study of Liu *et al*. [22] (OS2). OS2 contains 6,968 UC cases and 20,464 population controls of European descent. Additionally, the second study contained 5,956 CD cases (ebi-a-GCST003044) (OS3) and together have

Table 1. SNPs used as instrumental variables in present study

SNP	beta	Se	ea	P value	oa	eaf	R^2	F-statistic
rs2524277	0.7819	0.1086	A	1.22×10^{-12}	G	0.054113	0.0625	66
rs2523586	0.2671	0.0484	G	4.58×10^{-8}	T	0.177316	0.0208	21
rs2523535	0.2205	0.0449	G	1.09×10^{-6}	A	0.249601	0.0182	18
rs3025650	0.4772	0.0987	C	1.55×10^{-6}	T	0.100240	0.0410	42
rs12925077	*-0.3983*	*0.0848*	*A*	*3.08×10^{-6}*	*G*	*0.028155*	*0.0086*	*9*
rs13398473	0.2243	0.0483	T	3.94×10^{-6}	C	0.769369	0.01785	18
rs2524277*	0.4576	0.0506	A	1.58×10^{-19}	G	0.054113	0.02010	70

SNP = single-nucleotide polymorphism, Se = standard error; ea =effect-allele; oa = other allele; eaf = effect-allele frequency, * = Sun *et al.* [21]

been pooled as IBD cases (Fig. 1).

In all original studies in which we used summary statistics of them, it is reported that informed consent and ethical approvals were acquired from the local research ethics committees.

Statistical Analysis

Power was calculated using the total sample size of the outcome data using an online MR-power calculation tool (https://sb452.shinyapps.io/power/) (Table 2). The "TwoSampleMR" (0.5.6), "Mendelian Randomization" (0.5.0), and "'devtools'" (2.4.4) packages were used for MR and statistical analyses. The "ggplot2" package is used for data visualizations. All analyses were conducted on R-platform (version 4.1.0). The statistical significance of IV effect sizes on the outcome is mainly evaluated by the inverse variance weighted (IVW) method. MR-Egger, weighted median, and weighted mode methods were also evaluated as supportive evidence. Heterogeneity and directional (horizontal) pleiotropy of IVs were evaluated using Cochrane's Q statistic and the MR-Egger intercept test, respectively. While reporting our MR study, we have considered the STROBE-MR guidelines (https://www.strobe-mr.org/).

RESULTS

All selected SNPs used in this study are listed in Table 1. These data indicate that rs2524277 has the maximum effect size (beta) and minimum P-value on the TFF3 protein levels in the plasma. The SNP rs12925077 has inverse effect size and the F-statistic of this SNP is 9 (<10), and has a weak-instrument bias; therefore, it has been excluded from subsequent analysis.

IVW test showed considerable statistical significance in all analyzed outcomes except for OS3, which is the CD samples (Table 2). Cochran's Q statistic for testing heterogeneity of IVs by the 2S-MR analysis on OS1 showed no statistically significant heterogeneity (IVW Q=4.00, Q degrees of freedom (Q_df)=4 and Q_pval=0.40; MR Egger Q=3.23, (Q_df)=3 and P= 0.35). Testing for directional horizontal pleiotropy (that is, a violation of the IV assumption 3) by Egger intercept showed no significant horizontal pleiotropy (egger intercept=-0.00018, se=0.00022 and P=0.46). Again, heterogeneity tests of IVW analysis on OS2 and OS3 have not shown any significant heterogeneity (IVW Q=0.03, Q_df=1, Q_pval=0.86 for OS2 andIVW Q= 0.20, Q_df=1, Q_pval=0.65 for OS3). Horizontal pleiotropy could not be evaluated for OS2 and OS3 because only two SNPs remained after the harmonization of SNPs of exposure and outcomes (it should be at least three IVs). The absence of heterogeneity in all study groups also means that the causal relationship is identical between groups (exposure IVs and outcomes). For visual inspection of the results, we created the forest plot and scatter plots from 2S-MR analysis (Fig. 2, Fig. 3, and Fig. 4).

In replication analysis for 2S-MR, we used the plasma proteome data of Sun *et al*. [21]. Wald ratio test showed considerable statistical significance in

Table 2. Results from two sample MR studies performed on three outcomes

Exposure study	Outcome study	Method	nsnp	Beta (se)	OR* (95%CI)	P value	Power
Suhre *et al.* [16] (Discovery)	ukb-d-ulcernas (OS1)	**IVW**	5	-0.0016 (0.0002)	**0.99** (0.98-0.99)	**3.2×10^{-8}**	**0.96**
		Weighted median	5	-0.0014 (0.0004)	0.99 (0.98-1)	**3.7×10^{-4}**	
		MR Egger	5	-0.0011 (0.0006)	0.99 (0.98-1)	0.1850	
		Weighted mode	5	-0.0014 (0.0005)	0.99 (0.98-1)	**0.0327**	
	ebi-a-GCST003045 (OS2)	**IVW**	2	-0.2775 (0.0350)	**0.75** (0.59-0.95)	**2.029×10^{-15}**	**1.00**
		Weighted median	NA#	NA	NA	NA	
		MR Egger	NA	NA	NA	NA	
		Weighted mode	NA	NA	NA	NA	
	ebi-a-GCST003044 (OS3)	**IVW**	2	0.0065 (0.0313)	**1.00** (0.95-1.06)	**0.8333**	**1.00**
		Weighted median	NA	NA	NA	NA	
		MR Egger	NA	NA	NA	NA	
		Weighted mode	NA	NA	NA	NA	
Sun *et al.* [21] (Replication)	ukb-d-ulcernas (OS1)	**Wald ratio**	1	-0.0022 (0.0007)	0.99 (-0.99-1)	**0.0034**	**1.00**
	ebi-a-GCST003045 (OS2)	**Wald ratio**	1	-0.4805 (0.0697)	0.61 (0.53-0.70)	**5.76×10^{-12}**	
	ebi-a-GCST003044 (OS3)	**Wald ratio**	1	0.0258 (0.0623)	1.02 (0.90-1.15)	0.6789	

SNP = single-nucleotide polymorphism, Nsnp = number of SNP, CI = confidence interval, se = standard error, OR = Odds ratio, NA = Not available

*OR was calculated by the formula $OR=e^{\beta}$, where "e" is the base of the natural logarithm, β is the mean effect size of the IVs.

#Because after analysis by the "TwoSampleMR" for OS2 and OS3 there were two SNPs left, outcomes of the weighted median, MR-Egger and weighted mode could not be calculated.

OS2 outcome (OR=0.61; %95 CI 0.53-0.70) except for OS3, which is the CD samples (Table 2). Again, the Wald ratio test showed a statistical significance result in OS1 (OR=0.99; %95 CI-0.99-1). Taken together, we showed that the genetically determined protein levels of the plasma TFF3 are causally associated with the risk of UC in both discovery and replication studies. Increased levels of the plasma TFF3 are inversely correlated with the risk of UC.

DISCUSSION

In this study, we performed 2S-MR to understand whether genetically determined levels of TFF3 in the plasma were associated with the risk of UC. To our knowledge, this is the first MR study on TFF3 and UC association. Also, we could not find any hypothesis-driven observational genetic association study on TFF3 and UC in the published literature. In this study,

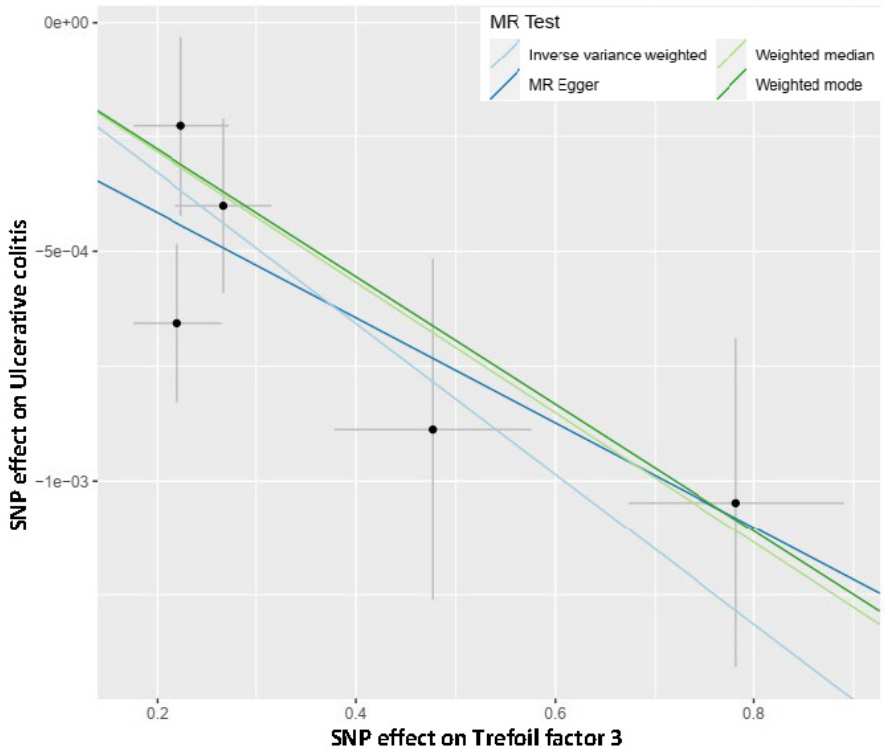

Fig. 2. The scatter plot of causal relationships between Trefoil factor 3 (TFF3) SNPs and ulcerative colitis (UC) in OS1. Each dot represents a SNP, vertical and horizontal black lines around points show 95% confidence intervals (CI) for each polymorphism's associations. The SNPs that increasing TFF3 levels are inversely correlated with the risk of UC. Four different color lines show MR association tests.

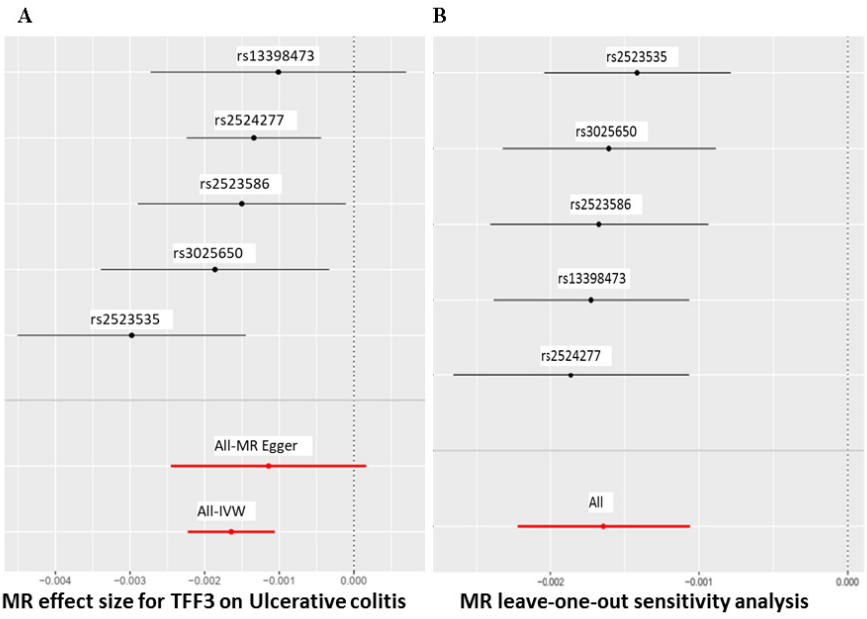

Fig. 3. Forest plots of the OS1 MR results. (A) Forest plot showing the effect estimates of each SNP on UC. (B) Forest plot showing the leave-one-out sensitivity analysis that is the estimated causal effect is shown for each excluded SNP and the overall estimate using all the SNPs. All association is shown in red. Each black dot represents a SNP and horizontal lines around points show 95% CI.

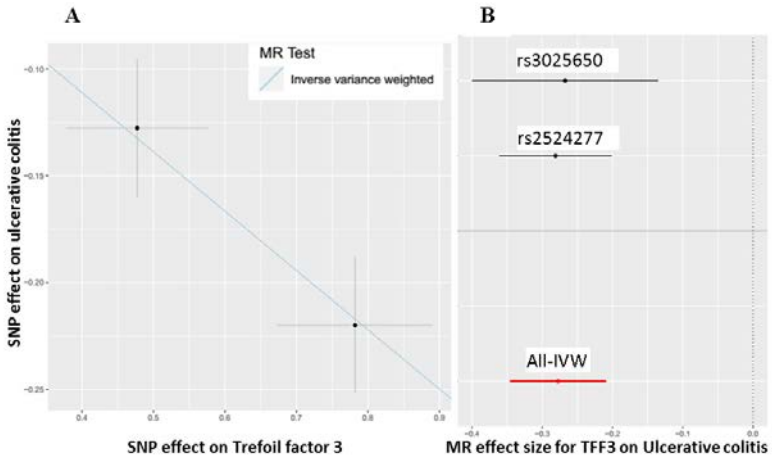

Fig. 4. Scatter and forest plots of the OS2 MR results. (A) The scatter plot of causal relationships between Trefoil factor 3 SNPs and ulcerative colitis. Each dot represents a SNP, vertical and horizontal black lines around points show 95% CI for each polymorphism's associations. (B) Forest plot showing the effect estimates of each SNP on UC. All association is shown in red. Each black dot represents a SNP and horizontal lines around points show 95% CI.

we showed that the effect of increasing TFF3 levels in plasma was associated with the decreasing risk of UC in all study groups of UC (Fig. 2, Fig. 4A). However, the OR of this effect seems quite small in OS1 (OR = 0.99, 95% CI=0.988–0.999) (Table 2). In OS2, the effect of increasing TFF3 levels also decreased the risk of UC (Figure 4A). Nevertheless, the OR was quite larger (significant) than OS1 (OR=0.75, 95% CI=0.59–0.95). One SD increase of the TFF3 level in plasma is associated with a 25 and 39% decreasing the risk of UC in OS2 in the discovery and replication studies, respectively (Table 2). Having more patients with UC in OS2 than OS1 (approximately three-fold) may more accurately reflect the effect of TFF3 on the risk of UC in statistical analysis. Interestingly, in OS3 there was not any effect of TFF3 levels on the risk of CD (OR=1.00, 95% CI=0.95–1.06) (Table 2). These findings support the view that the genetic basis of UC and CD are different [23]. In one study in which it was used 2S-MR approach and performed on the inflammatory proteins in plasma, it was found that an increased level of the vascular endothelial growth factor A (VEGF-A) is associated with a decreased risk of UC [24]. VEGF-A levels were not associated with the risk of CD in that study. In another 2S-MR study by which is performed by Parisinos *et al*. [25], the SNP rs2228145 was used as IV and was showed that the soluble interleukin 6 receptor (sIL6R) levels in plasma are associated with decreased risk of CD and UC. To our knowledge, our 2S-MR study is the 3rd study in which plasma protein levels were used as a biomarker of UC risk assessment. Other studies on plasma TFF3 mainly focused on the possible biomarker role of the plasma TFF3 in the UC disease activity/severity and outcome [26, 27]. Experimental studies performed on the intestinal mucosal barrier function of TFF3 show that TFF3 plays a major role in protecting and repairing the mucosal epithelia from various insults [28]. TFF3 and MUC2 are primarily secreted protein forms of the goblet cells in the colon, they create a protective barrier and a natural immune response in terms of the first line of defense [28]. When Tff3 knockout mice (Tff3-/-) were exposed to dextran sulfate sodium (DSS) induced UC, it has been observed that they had impaired mucosal healing, poor epithelial regeneration, and died from extensive colitis [29]. Thus, our MR study is consistent with the mentioned experimental studies in terms of the crucial role of TFF3 in the etiology and/or pathophysiology of UC. Nevertheless, in the first human clinical study of recombinant TFF3, it was not observed any additional benefits of TFF3 treatment when compared with the effect of corticosteroid treatment alone [30]. However, more clinical trials should be performed to see the exact outcomes of the effects of recombinant TFF3.

Limitations

MR studies are based on some assumptions. Apart

from relevance independence, and exclusion restriction (Fig. 1A), the homogeneity assumption (that is the association of the IVs with the risk factor (TFF3) is homogeneous in the population) is based on acceptance and there is no process of objectively validating it. Our 2S-MR results are based on summary statistics derived from people of European ancestry; it should be careful when extending to other populations due to linkage disequilibrium (LD) differences among different ethnicities. The UC diagnostic criteria and disease definition, as well as other unmeasurable factors, may be different between outcome study groups. Our SNPs, which are used as IVs explain a relatively small variance of the genetically determined plasma TFF3 level (approx. 16%). Therefore, the more independent IVs that explain the greater variance of the levels of TFF3 in plasma will explain the risk of UC more robustly.

CONCLUSION

In the present 2S-MR study, we showed that TFF3 levels in plasma are causally associated with the risk of UC. Increased levels of TFF3 are reversely associated with the risk of UC. The absence of any causal relationship between TFF3 and CD from the same study cohort also supports our causal inference.

Authors' Contribution

Study Conception: BT; Study Design: BT; Supervision: BT; Funding: N/A; Materials: N/A; Data Collection and/or Processing: SF, GY; Statistical Analysis and/or Data Interpretation: BT, SF, GY; Literature Review: BT, SF, GY; Manuscript Preparation: BT and Critical Review: BT, SF, GY.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

The authors disclosed that they did not receive any grant during conduction or writing of this study.

REFERENCES

1. Baumgart DC, Carding SR. Inflammatory bowel disease: cause and immunobiology. Lancet. 2007;369(9573):1627-1640. doi: 10.1016/S0140-6736(07)60750-8.
2. Baumgart DC, Sandborn WJ. Inflammatory bowel disease: clinical aspects and established and evolving therapies. Lancet. 2007;369(9573):1641-1657. doi: 10.1016/S0140-6736(07)60751-X.
3. Orholm M, Binder V, Sørensen TI, Rasmussen LP, Kyvik KO. Concordance of inflammatory bowel disease among Danish twins. Results of a nationwide study. Scand J Gastroenterol. 2000;35(10):1075-1081. doi: 10.1080/003655200451207.
4. Tysk C, Lindberg E, Järnerot G, Flodérus-Myrhed B. Ulcerative colitis and Crohn's disease in an unselected population of monozygotic and dizygotic twins. A study of heritability and the influence of smoking. Gut. 1988;29(7):990-996. doi: 10.1136/gut.29.7.990.
5. Garza-Hernandez D, Sepulveda-Villegas M, Garcia-Pelaez J, et al. A systematic review and functional bioinformatics analysis of genes associated with Crohn's disease identify more than 120 related genes. BMC Genomics. 2022;23(1):302. doi: 10.1186/s12864-022-08491-y.
6. Welter D, MacArthur J, Morales J, et al. The NHGRI GWAS Catalog, a curated resource of SNP-trait associations. Nucleic Acids Res. 2014;42:D1001-1006. doi: 10.1093/nar/gkt1229.
7. Kitsios GD, Zintzaras E. Genome-wide association studies: hypothesis-"free" or "engaged"? Transl Res. 2009;154(4):161-164. doi: 10.1016/j.trsl.2009.07.001.
8. Manolio TA. Genomewide association studies and assessment of the risk of disease. N Engl J Med. 2010;363(2):166-176. doi: 10.1056/NEJMra0905980.
9. Pearson TA, Manolio TA. How to interpret a genome-wide association study. JAMA. 2008;299(11):1335-1344. doi: 10.1001/jama.299.11.1335.
10. Davies NM, Holmes MV, Davey Smith G. Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians. BMJ. 2018;362:k601. doi: 10.1136/bmj.k601.
11. Gagliano Taliun SA, Evans DM. Ten simple rules for conducting a mendelian randomization study. PLoS Comput Biol. 2021;17(8):e1009238. doi: 10.1371/journal.pcbi.1009238.
12. Rasooly D, Patel CJ. Conducting a reproducible mendelian randomization analysis using the R analytic statistical environment. Curr Protoc Hum Genet. 2019;101(1):e82. doi: 10.1002/cphg.82.
13. Gött P, Beck S, Machado JC, Carneiro F, Schmitt H, Blin N. Human trefoil peptides: genomic structure in 21q22.3 and coordinated expression. Eur J Hum Genet. 1996;4(6):308-315. doi: 10.1159/000472224.
14. Järvå MA, Lingford JP, John A, Soler NM, Scott NE, Goddard-Borger ED. Trefoil factors share a lectin activity that defines their role in mucus. Nat Commun. 2020;11(1):2265. doi: 10.1038/s41467-020-16223-7.
15. Yang Y, Lin Z, Lin Q, Bei W, Guo J. Pathological and therapeutic roles of bioactive peptide trefoil factor 3 in diverse diseases: recent progress and perspective. Cell Death Dis. 2022;13(1):62. doi: 10.1038/s41419-022-04504-6.
16. Suhre K, Arnold M, Bhagwat AM, et al. Connecting genetic risk to disease end points through the human blood plasma proteome. Nat Commun. 2017;8:14357. doi: 10.1038/ncomms14357.
17. Machiela MJ, Chanock SJ. LDlink: a web-based application

for exploring population-specific haplotype structure and linking correlated alleles of possible functional variants. Bioinformatics. 2015;31(21):3555-3557. doi: 10.1093/bioinformatics/btv402.
18. Pierce BL, Ahsan H, Vanderweele TJ. Power and instrument strength requirements for Mendelian randomization studies using multiple genetic variants. Int J Epidemiol. 2011;40(3):740-752. doi: 10.1093/ije/dyq151.
19. Davies NM, Holmes MV, Davey Smith G. Reading Mendelian randomisation studies: a guide, glossary, and checklist for clinicians. BMJ. 2018;362:k601. doi: 10.1136/bmj.k601.
20. Ma K, Song P, Liu Z, et al. Genetic evidence suggests that depression increases the risk of erectile dysfunction: A Mendelian randomization study. Front Genet. 2022;13:1026227. doi: 10.3389/fgene.2022.1026227.
21. Sun BB, Maranville JC, Peters JE, et al. Genomic atlas of the human plasma proteome. Nature. 2018;558(7708):73-79. doi: 10.1038/s41586-018-0175-2.
22. Liu JZ, van Sommeren S, Huang H, et al. Association analyses identify 38 susceptibility loci for inflammatory bowel disease and highlight shared genetic risk across populations. Nat Genet. 2015;47(9):979-986. doi: 10.1038/ng.3359.
23. Lawrance IC, Fiocchi C, Chakravarti S. Ulcerative colitis and Crohn's disease: distinctive gene expression profiles and novel susceptibility candidate genes. Hum Mol Genet. 2001;10(5):445-456. doi: 10.1093/hmg/10.5.445.
24. Ek WE, Karlsson T, Höglund J, Rask-Andersen M, Johansson Å. Causal effects of inflammatory protein biomarkers on inflammatory diseases. Sci Adv. 2021;7(50):eabl4359. doi: 10.1126/sciadv.abl4359.
25. Parisinos CA, Serghiou S, Katsoulis M, et al. Variation in interleukin 6 receptor gene associates with risk of Crohn's disease and ulcerative colitis. Gastroenterology. 2018;155(2):303-306.e2. doi: 10.1053/j.gastro.2018.05.022.
26. Nakov R. New markers in ulcerative colitis. Clin Chim Acta. 2019;497:141-146. doi: 10.1016/j.cca.2019.07.033.
27. Nakov R, Velikova T, Nakov V, Ianiro G, Gerova V, Tankova L. Serum trefoil factor 3 predicts disease activity in patients with ulcerative colitis. Eur Rev Med Pharmacol Sci. 2019;23(2):788-794. doi: 10.26355/eurrev_201901_16893.
28. Aamann L, Vestergaard EM, Grønbæk H. Trefoil factors in inflammatory bowel disease. World J Gastroenterol. 2014;20(12):3223-3230. doi: 10.3748/wjg.v20.i12.3223.
29. Mashimo H, Wu DC, Podolsky DK, Fishman MC. Impaired defense of intestinal mucosa in mice lacking intestinal trefoil factor. Science. 1996;274(5285):262-265. doi: 10.1126/science.274.5285.262.
30. Mahmood A, Melley L, Fitzgerald AJ, Ghosh S, Playford RJ. Trial of trefoil factor 3 enemas, in combination with oral 5-aminosalicylic acid, for the treatment of mild-to-moderate left-sided ulcerative colitis. Aliment Pharmacol Ther. 2005;21(11):1357-1364. doi: 10.1111/j.1365-2036.2005.02436.x.

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Early results of fluorescence lymphatic mapping for right colon cancer: a case-matched study

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ABSTRACT

Objectives: The complete mesocolic excision (CME) technique has been described to improve the surgical outcomes of colon cancer. Collecting more lymph nodes is one of the goals of CME. In our study, indocyanine green (ICG) injection was applied to the anterior and posterior walls at certain levels of the right colon. The aim of this study is to determine the impact of lymphangiography in right colon cancer surgery.

Methods: The data of patients were analyzed who underwent colectomy surgery between 1.1.2018-1.1.2022 and using our mapping technique. A case-match study was performed at a ratio of 1:2 (Study group [group S; n=10], Control group [group C; n=20]). Case-matched criteria were age +/-10, T stage +/-1, and tumor location.

Results: There were no differences in terms of age, the American Society of Anesthesiologists score, tumor location, tumor T and N stages, and pathological markers affecting prognosis between the groups. Although 10% of intraoperative complications were seen in group C, it was not statistically significant (P=0.540). After lymphangiography, lymph nodes containing ICG were detected in the resection site and these lymph nodes were sent to pathology in separate containers. Considering the number of lymph nodes sent separately, it was determined that significantly more lymph nodes were sent in the group S (P=0.001).

Conclusions: We have shown that the ICG mapping can be applied safely in the surgical treatment of right colon cancer.

Keywords: Colonic neoplasms, Indocyanine green, lymphatic metastasis, fluorescence lymphatic mapping

Colorectal cancer is the third most common cancer worldwide. Nodal involvement is one of the crucial factors affecting survival, as 5-year survival can range from 90% in early disease to 10% in metastatic disease [1]. To improve outcomes, the complete mesocolic excision (CME) technique was identified [2].

Fluorescence imaging (FI) technology makes intraoperative lymphangiography and lymphatic mapping possible. Several studies demonstrate that peritumoral indocyanine green (ICG) injection makes lymphatic flow and lymph nodes visible in the mesocolon [3, 4]. However, pure peritumoral injection of ICG may not be sufficient for demonstrating whole lymphatic drainage of the right colon, and lymphatic flow and mapping may not be reliable since lymph vessels might be blocked by tumoral cells.

The present study aimed to investigate the effect of intraoperative lymphangiography used in right colon cancer surgery on the number of lymph nodes

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collected intraoperatively, the number of lymph nodes left in the patient (especially D3 lymph nodes) after removal of the resected right colon, and the total number of collected lymph nodes. We also aimed to determine the impact of ICG lymphatic mapping on postoperative complications and its effects on short-term oncological outcomes.

METHODS

Patient Selection and Data Collection

After obtaining Institutional Review Board approval (approval number 2022-6/19) patients who underwent elective right hemicolectomy due to right colon cancer between 1.1.2018 and 1.1.2022, since the lymphatic mapping technique with ICG became available in Bursa Uludag University School of Medicine General Surgery Department, were included in the study.

Data of patients older than 18 years old who had tumors in the right colon, including the cecum and hepatic flexure during the preoperative colonoscopy, and whose histopathological diagnosis was adenocarcinoma, were accessed via patient charts. Computed tomography imaging (thorax and abdomen) was performed for metastasis screening in all patients preoperatively. Patients with metastases on tomography images and patients whose imaging findings had a tumor localization in the colon other than the right colon were excluded from the study. Patients who underwent emergency surgery due to obstruction, bleeding, or perforation and patients with distant organ metastases were excluded from the study. Since there is no laparoscopic fluorescence imaging equipment in our center, cases who underwent laparoscopic right hemicolectomy were excluded from the study. Additionally, patients who were found to have a colon tumor outside of the right colon during surgery were excluded from the study.

During the study period, 198 colon cancer surgeries were performed in our center. Sixty-four of these were right colon cancer surgeries. ICG mapping method was applied to 10 patients who fell within the inclusion criteria. Twenty control patients were selected based on matching criteria.

A study group (group S) of 10 colon cancer patients underwent lymphatic mapping with ICG during right hemicolectomy surgery. These patients were case-matched in a 1:2 ratio with 20 right colon cancer patients (group C) who were operated on without using the lymphatic mapping technique. Case-match criteria were age +/-10, T stage +/-1 and same tumor location. Additionally, no significant difference was detected in terms of biological factors of the tumors between the two groups (lymphovascular invasion, perineural invasion, mucinous component, tumor budding, tumor grade, and differentiation).

In group S, 3 (30%) patients had tumors in the cecum, 3 (30%) patients had tumors in the ascending colon, and 4 (40%) patients had tumors in the hepatic flexure. Similarly, 6 (30%) of the patients in the group C had tumors located in the cecum, 6 (30%) in the ascending colon, and 8 (40%) in the hepatic flexure.

In both patient groups, age, gender, American Society of Anesthesiologists (ASA) score, tumor location, T stage, mucinous component, grade, venous/vascular invasion, lymphatic and perineural invasion, tumor budding, N stage, total and metastasized lymph node counts, operation times, intraoperative and postoperative complications, distant metastasis developed in the follow-up, mortality during the follow-up period and survival times were examined. Additionally, total, and metastasized lymph node counts those sent separately after intraoperative fluorescence imaging in group S were examined.

Surgical Technique and ICG Lymphatic Mapping

Indocyanine green (DID Indocyanine Green Inj, Dongindang Pharmaceutical CO., LTD, Gyeonggi-do, Korea) and SPY Elite laser angiographic system (Stryker Corp/Novadaq Technologies, Kalamazoo, MI, USA) imaging system was used in this study.

The patients who were taken to the operating room were placed in a supine position under general anesthesia, and a laparotomy was performed with a median incision. The lateral peritoneal attachments of the right colon were separated by sharp dissections, and the posterior wall of the colon was made visible. Before applying ICG, a concentration of 2.5 mg/mL was obtained and 8 mL solution (20 mg-ICG) was applied subserosal to 8 different points with a 26-gauge needle tip, as 1 mL per injection site. The application points were chosen as 6 points, the anterior and posterior walls of 3 different localizations, namely the cecum, the midpoint of the ascending colon, and the hepatic flexure. Additional 1 ml injections were made at two

different peritumoral points. Thus, eight different injection points were determined as standard (Fig. 1).

Mobilization of the right colon was achieved by proceeding from the avascular embryological plane between the visceral and parietal planes. Next, initial imaging was performed to ensure that ICG was draining into the lymphatics and lymph nodes of the right colon mesentery (Fig. 2).

After this initial imaging central ligation of vascular structures was performed. Then, the right hemicolectomy was completed in a way that the integrity of the right colon mesentery was preserved. For tumors located at the hepatic flexura, the middle colic artery and vein were high ligated. The gastroepiploic omentum was included in the sample. D3 lymph nodes were not removed in the study group. A second fluorescence imaging was performed just before the ileotransversostomy anastomosis was created to identify whether there were any ICG-positive and fluorescent lymph nodes in the right hemicolectomy resection area, especially D3 lymph nodes. All tissues found to retain ICG in the surrounding fatty tissues were excised under immunofluorescence imaging. All excised tissues were sent in a separate pathology container. Afterward, using a surgical stapler, the surgery was terminated by creating a side-to-side anisoperistaltic ileotransversostomy anastomosis.

Postoperative surgery-related complications were scored using the Clavien-Dindo classification.

Statistical Analysis

The normal distribution of the data was tested with the Shapiro-Wilk test. While quantitative variables with normal distribution are given with mean±standard deviation, variables without normal distribution are passed with median (minimum-maximum) values. Mann-Whitney U test was used to compare quantitative variables between two independent groups. Pear-

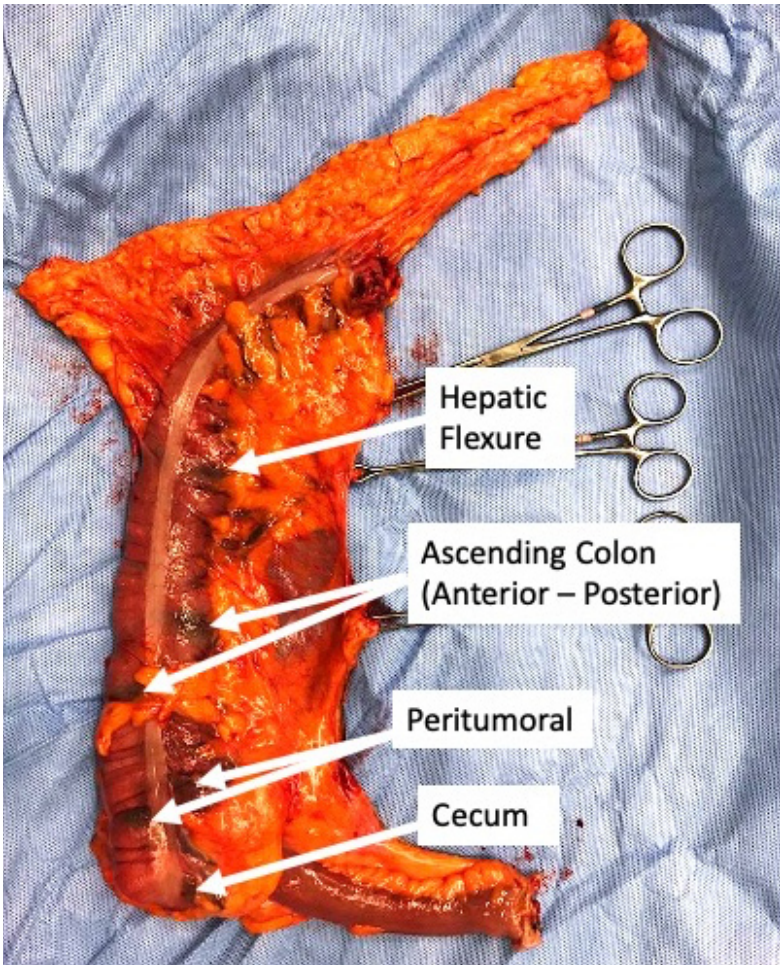

Fig. 1. Subserosal indocyanine green application points seen in right hemicolectomy specimen.

son chi-square, Fisher's exact chi-square, and Fisher-Freeman-Halton tests were used to compare categorical variables between groups, and categorical variables were given with frequency and percentage values. The significance level was accepted as α=0.05. Statistical analyzes were performed using the IBM SPSS Statistics version 23 package program.

RESULTS

The number of female patients was significantly higher in the group S, which was mapped with ICG. There was no significant difference between the two groups regarding age and ASA Class (Table 1).

The median operative time was 60 minutes (60-190 minutes) in the group S, and the median was 75 minutes (60-195 minutes) in the group C. There was no significant difference between the two groups regarding operative time (P=0.948).

There was no significant difference between the two groups regarding tumor stages and pathological factors with prognostic importance which are mucinous component, grade, tumor budding, lymphatic invasion, perineural invasion, and venous/vascular invasion (Table 2).

The median number of lymph nodes in the main pathology specimen were 34 (10-70) in the group S, and the median number metastatic lymph nodes were 0 (0-5). In group C, the median number of total lymph nodes were 38.5 (12-56), and the number of metastatic lymph nodes were 0 (0-4). There was no statistically significant difference between the two groups regarding the total and metastatic lymph node numbers included in the main piece (P=0.983 and P=0.713, respectively).

The median total lymph node number was calculated as 39.5 (14-56) in 12 patients with hepatic flexura tumors (4 patients in group S versus 8 patients in group C). The number of lymph nodes with metastasis detected in the main piece was 0 (0-5). The number of lymph nodes sent separately after resection was 0 (0-4).

After the specimen was taken out in group C, suspicious rest lymph nodes were excised in 2 (10%) patients and sent in a separate pathology container. In the group S, fluorescent lymph nodes were detected in the resection site by the second FI performed in 9 patients. These lymph nodes were sent to pathology in separate containers. While the number of lymph nodes sent separately was 0 (0-5) in the group C, the median was 1 (0-5) in the group S. It was determined that signifi-

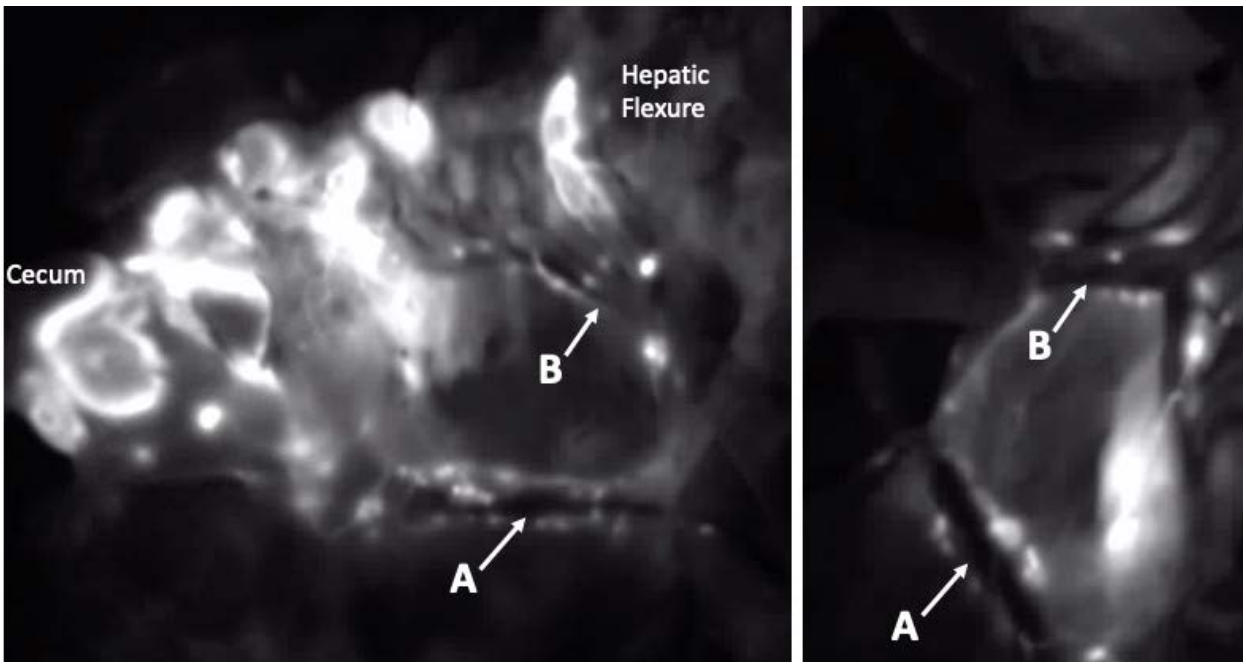

Fig. 2. Lymph nodes around the ileocolic pedicle (A) and right colic vessels (B) visible on the posterior and anterior faces of the mesentery in the first imaging performed after ICG injection.

Table 1. Demographic variables and ASA class

		Group S	Group C	P value
Sex, n (%)	**Male**	4 (40)	16 (80)	**0.045**
	Female	6 (60)	4 (20)	
Age (years), median (min-max)		59 (36-76)	66.5 (28-78)	0.214
ASA class, n (%)	**ASA 1**	3 (30)	6 (30)	> 0.99
	ASA 2	7 (70)	14 (70)	

ASA = American Society of Anesthesiologists, Group S = Study group, Group C = Control group

cantly more lymph nodes were sent separately in the group S (P=0.001). In both groups, no metastatic lymph nodes were detected in the lymph nodes sent separately (Table 3).

In group S, 7 (70%) patients were N0, 3 (30%) patients were N2a; in group C,14 (70%) patients were N0, 3 (15%) patients were N1a, 1 (5%) patient was N1b and 2 (10%) patients were N2a. There was no significant difference between the two groups regarding the N stage (P=0.387).

No intraoperative complications developed in the group S. In group C, there were 2 patients reported intraopereative bleeding from the branches of superior mesenteric vein (right colic vein and the gastrocolic trunc), and bleeding were controlled by surgical intervention. There was no significant difference between the two groups regarding intraoperative complications (P=0.54). Complications experienced in the postoperative period were categorized using the Clavien Dindo Classification as described in the study method section. Accordingly, 9 (90%) patients in group S, who underwent lymphatic mapping with ICG, were in

Table 2. Tumor stages and pathological factors with prognostic importance between two groups

		Group S n (%)	Group C n (%)	P value
Tumor stage	**T1**	1 (10)	0 (0)	0.251
	T2	1 (10)	1 (5)	
	T3	8 (80)	19 (95)	
Mucinous component	**Yes**	3 (10)	7 (35)	> 0.99
	No	7 (70)	13 (65)	
Grade	**1**	1 (33.3)	0 (0)	0.365
	2	1 (33.3)	5 (45.5)	
	3	1 (33.3)	6 (54.5)	
Tumor budding	**Yes**	6 (60)	8 (40)	0.442
	No	4 (40)	12 (60)	
Lymphatic invasion	**Yes**	3 (30)	5 (25)	> 0.99
	No	7 (70)	15 (75)	
Perineural invasion	**Yes**	1 (10)	3 (15)	> 0.99
	No	9 (90)	17 (85)	
Venous/vascular invasion	**Yes**	1 (10)	2 (10)	> 0.99
	No	9 (90)	18 (90)	

Group S = Study group, Group C = Control group

Clavien Dindo class 1, and 1 (10%) patient was in class 2. Ten (50%) patients in group C were in the Clavien Dindo 1 group, 8 (40%) patients were in the Clavien Dindo 2 group, 1 (5%) patient was in the Clavien Dindo 3 group, and 1 (5%) patient was in the Clavien Dindo 4 group (Table 4). There was no significant difference between the two groups in terms of postoperative complications (P=0.171).

There were no patients in both group who developed local recurrence during the follow-up period. There was no significant difference between the two groups regarding distant metastasis at follow-up (P=0.3). The mean postoperative follow-up time was calculated as 21.3±10.72 months. There were no mortalities in two groups. The median survival was 23.5 months (1-36 months) in the group S and 24.5 months (range: 4-37 months) in the group C, and there was no significant difference between the two groups (P=0.846).

DISCUSSION

In the present study, we have shown that ICG and lymphatic mapping techniques can be safely applied in the surgical treatment of right colon cancer and selective D3 lymph node dissection may be possible.

Colorectal cancer is one of the most common malignant tumors [5]. More than one million people worldwide are diagnosed with colorectal cancer each year, and about half of this number die from colorectal cancer [6]. Nodal involvement is one of the crucial factors affecting 5-year survival. Standardized techniques have been developed for maximum lymph node removal during colectomy. The Complete Mesocolic Excision (CME) technique defined by Hohenberger *et al*. [2] was developed by being inspired by the Total Mesorectal Excision (TME) technique, which is a rectal resection technique previously described by Heald [7].

In the standardized right hemicolectomy+CME technique for right colon cancers, ligation of the ileocolic vessel and, if any, right colic vessel(s) at the origin is recommended. For tumors in the transverse colon and both flexures, it is recommended to tie the middle colic artery at its root and to include the lymph nodes in this area in the specimen [2].

Collecting more lymph nodes is one of the goals of the CME technique. Studies have proven that the number of lymph nodes collected in colon cancer surgery is directly proportional to the prognosis [8-10]. These studies have shown the importance of developing new methods to collect more lymph nodes in colon cancer surgery.

Hohenberger *et al*. [2] stated in his original article that the CME technique reduces the local recurrence rate and increases survival. However, the limits of lymphadenectomy in colon cancer surgery are among the most debated issues today. The superiority of the CME technique over conventional right hemicolectomy in terms of survival has been demonstrated in several retrospective studies [11, 12]. These studies have been criticized for examining patients over a long period (>20 years), comparing patients operated by surgical teams with different experiences, different responses to adjuvant chemotherapeutic drug regimens developed by patients, and retrospective design.

No randomized controlled study comparing the CME technique with conventional right hemicolectomy operations exists [13]. Two meta-analyses in this area have shown that CME has no superiority over conventional right hemicolectomy [14, 15].

Many studies criticize the CME technique because of longer operative times, increased intraoperative complications (especially vascular injury), and in-

Table 3. Number of lymph nodes sent with and separately from the main piece

Variables	Group S (n = 10)	Group C (n = 20)	P value
Number of main piece lymph nodes (total)	34 (10-70)	38.5 (12-56)	0.983
Number of main piece lymph nodes (with metastases)	0 (0-5)	0 (0-4)	0.713
Number of lymph nodes sent separately	1 (0-5)	0 (0-5)	**0.001**

Group S = Study group, Group C = Control group

creased rate of autonomic nerve damage [13, 16, 17]. All these discussions encourage research on new techniques that can provide similar local recurrence and survival results by reducing the intraoperative complication rate brought by the CME technique. The central premise of our study is this argument.

Although there was no significant difference in our study, 10% of vascular injuries were observed in group C, and no intraoperative complications were observed in group S, which supports this finding.

Studies showed that intraoperative lymphangiography makes lymph nodes in the mesocolon visible in colon cancer surgery [3, 4]. However, in these studies, it was seen that lymphangiography was performed to show possible spread areas of the tumor by injecting only into the peritumoral area. Since we think that the peritumoral lymphatic pathways may be involved with tumor cells and may prevent the spread of indocyanine green, in addition to the peritumoral area, six more submucosal indocyanine greens were applied as standard to three different anatomical points in the right colon to show the entire lymphatic mapping of the right colon.

In our study, in the group S, there is a phase in which the residual lymph node tissues in the resection area are visualized with ICG and excised separately. The number of additionally excised lymph nodes in the S group was significantly higher than in the C group (P=0.001). This superiority of sampling more lymph nodes is one of the strengths of mapping with ICG.

Our center is one of the reference centers in its region for the surgical treatment of colorectal cancer. However, since intraoperative indocyanine green and lymphatic mapping can be applied to limited patients and this study was conducted in a single center, the number of patients is small.

Limitations

Two major limitations of these study are its retrospective nature and limited number of patients. Not reporting long- term results may be another weak point. However, describing a new surgical technique gives this study its clinical value. This study was planned as a preliminary study to test the applicability of the technique. We believe that if our study is continued with a longer observation period and larger number of cases, it may make a difference in oncological results.

CONCLUSION

In conclusion, we have shown that ICG and lymphatic mapping techniques may be safely applied in the surgical treatment of right colon cancer without prolonging the operation time. With the second imaging performed in our study, we showed that the limits of lymphadenectomy may be extended with the fluorescent lymph nodes that remained in the resection area by avoiding intraoperative complications. Thus, selective D3 lymph node dissection may be possible, avoiding vascular injury, which is a fundamental problem of CME.

Authors' Contribution

Study Conception: ÖI, ATY; Study Design: ÖI; Supervision: ÖI, ATY; Funding: N/A; Materials: MŞ; Data Collection and/or Processing: MŞ, ÖI; Statistical Analysis and/or Data Interpretation: ÖI, DS; Literature Review: MŞ, ÖI; Manuscript Preparation: MŞ, ÖI and Critical Review: ÖI, ATY.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

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REFERENCES

1. Emile SH, Elfeki H, Shalaby M, et al. Sensitivity and specificity of indocyanine green near-infrared fluorescence imaging in detection of metastatic lymph nodes in colorectal cancer: Systematic review and meta-analysis. J Surg Oncol. 2017;116(6):730-740. doi: 10.1002/jso.24701.

2. Hohenberger W, Weber K, Matzel K, Papadopoulos T, Merkel S. Standardized surgery for colonic cancer: complete mesocolic excision and central ligation--technical notes and outcome. Colorectal Dis. 2009;11(4):354-64. doi: 10.1111/j.1463-1318.2008.01735.x.

3. Park SY, Park JS, Kim HJ, Woo IT, Park IK, Choi GS. Indocyanine green fluorescence imaging-guided laparoscopic surgery

could achieve radical D3 dissection in patients with advanced right-sided colon cancer. Dis Colon Rectum. 2020;63(4):441-449. doi: 10.1097/DCR.0000000000001597.
4. Chand M, Keller DS, Joshi HM, Devoto L, Rodriguez-Justo M, Cohen R. Feasibility of fluorescence lymph node imaging in colon cancer: FLICC. Tech Coloproctol. 2018;22(4):271-277. doi: 10.1007/s10151-018-1773-6.
5. Sung H, Ferlay J, Siegel RL, et al. Global cancer statistics 2020: GLOBOCAN estimates of incidence and mortality dorldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71(3):209-249. doi: 10.3322/caac.21660.
6. Siegel RL, Miller KD, Goding Sauer A, et al. Colorectal cancer statistics, 2020. CA Cancer J Clin. 2020;70(3):145-164. doi: 10.3322/caac.21601.
7. Heald RJ. The 'Holy Plane' of rectal surgery. J R Soc Med. 1988;81(9):503-508. doi: 10.1177/014107688808100904.
8. Le Voyer TE, Sigurdson ER, Hanlon AL, et al. Colon cancer survival is associated with increasing number of lymph nodes analyzed: a secondary survey of intergroup trial INT-0089. J Clin Oncol. 2003;21(15):2912-2919. doi: 10.1200/JCO.2003.05.062.
9. Schumacher P, Dineen S, Barnett C Jr, Fleming J, Anthony T. The metastatic lymph node ratio predicts survival in colon cancer. Am J Surg. 2007;194(6):827-831. doi: 10.1016/j.amjsurg.2007.08.030.
10. Mammen JM, James LE, Molloy M, Williams A, Wray CJ, Sussman JJ. The relationship of lymph node dissection and colon cancer survival in the Veterans Affairs Central Cancer Registry. Am J Surg. 2007;194(3):349-354. doi: 10.1016/j.amjsurg.2006.08.092.
11. West NP, Morris EJ, Rotimi O, Cairns A, Finan PJ, Quirke P. Pathology grading of colon cancer surgical resection and its association with survival: a retrospective observational study. Lancet Oncol. 2008;9(9):857-865. doi: 10.1016/S1470-2045(08)70181-5.
12. Bertelsen CA, Neuenschwander AU, Jansen JE, et al. Disease-free survival after complete mesocolic excision compared with conventional colon cancer surgery: a retrospective, population-based study. Lancet Oncol. 2015;16(2):161-168. doi: 10.1016/S1470-2045(14)71168-4.
13. Xu L, Su X, He Z, et al. Short-term outcomes of complete mesocolic excision versus D2 dissection in patients undergoing laparoscopic colectomy for right colon cancer (RELARC): a randomised, controlled, phase 3, superiority trial. Lancet Oncol. 2021;22(3):391-401. doi: 10.1016/S1470-2045(20)30685-9.
14. Killeen S, Mannion M, Devaney A, Winter DC. Complete mesocolic resection and extended lymphadenectomy for colon cancer: a systematic review. Colorectal Dis. 2014;16(8):577-594. doi: 10.1111/codi.12616.
15. Kontovounisios C, Kinross J, Tan E, Brown G, Rasheed S, Tekkis P. Complete mesocolic excision in colorectal cancer: a systematic review. Colorectal Dis. 2015;17(1):7-16. doi: 10.1111/codi.12793.
16. Willaert W, Ceelen W. Extent of surgery in cancer of the colon: is more better? World J Gastroenterol. 2015;21(1):132-138. doi: 10.3748/wjg.v21.i1.132.
17. Kong JC, Prabhakaran S, Choy KT, Larach JT, Heriot A, Warrier SK. Oncological reasons for performing a complete mesocolic excision: a systematic review and meta-analysis. ANZ J Surg. 2021;91(1-2):124-131. doi: 10.1111/ans.16518.

The European Research Journal 2024;10(1):25-36 Original Article

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Dream themes and rejection sensitivity of individuals with and without borderline personality disorder: a comparative study

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ABSTRACT

Objectives: This study aims to determine how the dream themes of borderline personality disorder patients differ from those of without borderline personality disorder diagnosis and the mediating role of rejection sensitivity in this differentiation process.

Methods: The convenience sampling method was used in the study, and the participants were 79 female borderline patients diagnosed with semi-structured interviews with criteria of DSM-5 and 79 female individuals who had not received a psychological diagnosis before and at the study interviews. Sociodemographic Information Form, Dream Themes Scale, Borderline Personality Questionnaire, and Rejection Sensitivity Scale were used in the study. Linear Regression Analysis, Hierarchical Regression Analysis, and Independent Groups T-Test were used during the investigations.

Results: Borderline personality disorder patients scored higher in all sub-dimensions of the dream themes scale and rejection sensitivity scale than the control group. Borderline personality traits predicted rejection sensitivity. Borderline personality traits and rejection sensitivity together predicted dream themes. Borderline personality predicts anxiety dreams, fear dreams, and experience dreams, and rejection sensitivity mediates these predictive relationships.

Conclusions: The dreams of borderline personality disorder patients have negative content covered by the impact of experiences sub-dimension on dream themes, as they contain reflections of negative content in beliefs, thoughts and emotions related to their daily lives. Rejection sensitivity plays a mediator role in borderline patient's dreams. The study results show that it would be beneficial not to ignore the content of dreams in patients with borderline personality disorder, considering the relationship of negative dreams with daily functioning, dissociative symptoms, self-harming behaviors, and suicide attempts. Results also indicate that it might be beneficial to target rejection sensitivity to reduce aggravating dream contents.

Keywords: Borderline personality disorder, dream themes, dreams, negative dream themes, rejection sensitivity

Dreaming is a collection of mental activity that takes place during sleep [1]. Sigmund Freud [2] described the dream as "the royal road to the unconscious" and presented a psychoanalytic framework for dream analysis by penning the books "Interpretation of Dreams 1 and 2" and gave this mys-

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terious phenomenon its place and importance in psychology [2]. He postulated that dreams reflect the subconscious mind's conflicts, desires, and fears [1]. Freud [2] said, "the dream is not only about unconscious conflicts or desires, but it is also very closely related to the personality."

Hobson and Kahn [3] state that obtaining and analyzing dreams with a mental state examination is worth a short interview to obtain the general cognitive functions of patients while they are admitted to the hospital [3]. Kramer and Glucksmann [4] state that dreams reflect the emotional state of patients and states that dreams are a valid and reliable assessment tool in terms of psychodynamic and psychodiagnostic pain, reflecting conflicts, resistances, transferences, and opposing transferences, self-images, and defenses. Clinical states are said to be reflected in a patient's dreams. The themes of dreams tend to reflect the patient's current mood state; bipolar patients mostly have bizarre and grandiose themed dreams when they are in a manic episode. On the other side, depression was characterized by more negative themes, and improvement in depression may be heralded by changes in the patient's dreams [4]. Dream content has also been researched in patients with anxiety disorders; in particular, for PTSD, nightmares are used as a diagnostic symptom, and the content and frequency of nightmares help clinicians infer the severity of the illness [1].

Patients with borderline personality disorder (BPD) have a hypersensitivity to abandonment [5]. This situation results in high rejection sensitivity levels, which can be considered as a precursor or substitute for abandonment [6]. While rejection sensitivity indicates intense sensitivity to signals for rejection, the reactions of individuals with rejection sensitivity in the face of actual and/or perceived rejection experiences are inconsistently high [7]. Rejection sensitivity and sleep disturbance are two factors that may maintain or exacerbate BPD symptoms [8]. Although sleep problems are not included in the diagnostic criteria of BPD, approximately half of the borderline patients reportreport disturbed sleep [9] and have more nightmares and dream anxiety than healthy individuals [10]. Additionally, borderline patients with dreams with negative content show more significant psychopathology than borderline patients without dreams with negative content [9]. Generally, an increased frequency of negative dream content is associated increased anxiety, sleep disturbances, and poor mental health [11, 12].

This study was conducted because there is no comparative study with Turkish BPD patient samples about dreams content and rejection sensitivity's role in it. In this study, it is expected that patients with BPD will encounter more nightmare dreams because of their rejection sensitivity than healthy participants, and it is also thought this might be because of their hyper rejection sensitivity. It is thought that determining the separation of dream themes of patients with borderline personality disorder from individuals who are not sick will be useful in determining the status and severity of the disease in the patient and determining the correct treatment method, both in the academic literature and in clinical treatments. Because it is known that sleep problems in BPD cause difficulties in social, emotional, and cognitive impairment and reduced self-care [13].

Borderline patients can often engage in self-harming and sabotaging behaviors [14]; approximately 70-75% of patients attempt suicide at least once in their lifetime, and approximately 8-10% of suicidal attempts are completed [5]. Besides, it is known that there is a positive relationship between nightmares and non-suicidal self-injury (NSSI) [15] and suicidal behavior [16]. Therefore, it is thought that borderline patients with nightmares have an increased risk of harming themselves and committing suicide. Therefore, this study may be useful for inhibiting the NSSI and suicidal attempts of borderline patients by showing the increase in negative contents in dreams as a sign of future NSSI or suicidal attempts. Sleep disorders deepen the dissociative symptoms of borderline patients and cause dissociative symptoms even in healthy individuals [17]. Because of that, this study might help by stressing the importance of reducing dissociative symptoms in borderline patients.

METHODS

The universe of the study consists of female borderline patients and women without any psychiatric diagnosis in Turkey. Convenience sampling and comparative design was used in the study. Convenience sampling is

a non-random sampling method in which the sample to be selected from the population is determined by the judgment of the researcher.

Population

The sample of the study consists of borderline personality disorder patients who applied to a Psychiatry Clinic between 2021-2022 and individuals without a psychiatric diagnosis.

The ages of participants were between 18-36, and they were residing in Istanbul/Turkey. All participants in the study group (borderline personality disorder patients) were women, 15.2% were high school graduates, 72.2% had undergraduate degrees, 12.7% had graduate degrees, 2.5% perceived their economic level as low, 34.2% perceived their economic level as moderate, 63.3% perceived their economic level as high. The mean age of the participants in the study group (32±8 years).

All participants in the control group (women without borderline personality disorder diagnosis) are women, 3.8% are primary school graduates, 10.1% are high school graduates, 60.8% are undergraduate graduates, 25.3% are postgraduate graduates, 1.3% perceived their economic level as low, 88.6% perceived their economic level as moderate, 10.1% perceived their economic level as high. The mean age of the participants in the control group (30±9 years).

Procedure

The study has been approved by the Istanbul Aydın University Ethics Committee (Date: 09.07.2021, No: 2021/08). Researchers reached out to individuals without any psychiatric disorders from social media platforms by announcing their study. Groups were similar in age. All the participants voluntarily participated in this study between August 2021 and April 2022 by filling out the Dream Themes Scale, Rejection Sensitivity Scale, and sociodemographic data form. The study group consisted of 79 female borderline patients who had borderline personality disorder diagnoses with semi-structured intervies taken by a psychiatrist with criteria of DSM-5, and the control group consisted of 79 female individuals who had not received a psychological diagnosis before and at the studies' interviews. While determining the study group, having a depressive episode or PTSD was scanned as an exclusion criterion, and patients who met the diagnostic criteria for depressive disorder or PTSD comorbid with borderline personality disorder were not included in the study. Besides semi-structured interviews with criteria of DSM-5, the Borderline Personality Questionnaire was given to the control group to measure their borderline personality traits to see the effect of borderline traits, which are not as high as to get a diagnosis on dream themes and rejection sensitivity.

Scales Used

The Sociodemographic Data Form which developed by the researcher, the Borderline Personality Questionnaire, the Dream Themes Scale and the Rejection Sensitivity Scale used for collecting data.

Sociodemographic Data Form

The sociodemographic data form includes gender, age, education level, economic level, and marital status.

Borderline Personality Questionnaire (BPQ)

It has Principal Components Analysis scores ranging between 0.40-0.81 [19]. Turkish BPQ internal consistency coefficient Cronbach αlpha value was determined as 0.89. The scale consists of 80 questions and 9 sub-dimentions [20]. Their Cronbach αlpha value was determined as: impulsivity: 0.50, emotional lability: 0.77, abandonment: 0.40, relationships: 0.68, self-image: 0.72, suicidal/self-injurious behavior: 0.48, feeling of emptiness: 0.73, intense anger: 0.74, and psychosis-like states: 0.62. In this study, the total score of the Borderline Personality Questionnaire was used, and the total Cronbach alpha value of the scale was determined as .929.

Dream Themes Scale (DTS)

The Dream Themes Scale (DTS) is a measurement tool developed by Genç *et al*. [21]. As a result of the reliability analysis, Cronbach's alpha internal consistency coefficient was found to be .94, and the item-total correlations were found to be between .34 and .72 [21]. In this study, the Cronbach alpha values of the sub-dimensions of the Dream Themes Scale were respectively: Negative Themes: .711, Anxiety Themes: .613, Fear Themes: .736, Effect of Experiences: .786, Frustration Themes: .872. The scale consists of 29 items and five sub-dimensions. It evaluates anxiety

themes, fear themes, effects of experiences, frustration themes, and negative themes. It is a valid and reliable scale. The increase in the scores obtained from the sub-dimensions of the DTS and the overall scale indicates the results of the dream themes.

Rejection Sensitivity Scale (RSS)

Downey and Feldman's [22] Rejection Sensitivity Scale (RSS) has 18 hypothetical situations in which the individual is likely to be rejected by others that are meaningful to the individual, and the person is asked to answer the rejection and acceptance expectations about the stated situation in a 6-point Likert type. Item scores can vary from 1 to 36. The Turkish validity and reliability study of the scale was conducted by Özen *et al*. [23], and the Cronbach's alpha value for the total score was found to be 0.85. In this study, the Cronbach alpha value of the rejection sensitivity scale was determined as .858.

Statistical Analysis

In the study, the relational screening model, which is the method in which the event, individual, or object subject to the research is tried to be defined as it exists, was used. Analyzes were made using the SPPS 25 program. The normality test, which is the first stage of the analysis, was applied, and when the skewness and kurtosis values of the variables were examined, it was seen that the relevant values were between -2 and +2. After deciding on the existence of a normal distribution, it was decided to use parametric analysis. Descriptive statistics for the demographic variables in the study were calculated immediately after the normality analysis in order to evaluate variables independent samples T-Test, Simple Linear Regression Analysis Test, and Hierarchical Regression Analysis Test used.

RESULTS

When we compared the scores obtained from the Negative Themes subscale (t=115.79, mean: 17.07±115.79, P<0.05), from the Anxiety Themes subscale (t=132.38), mean: 11.11±132.38, P<0.05), from the Fear Themes subscale (t=109.10, mean: 14.84±109.10, P<0.05), Effect of Experiences subscale (t=126.79, mean: 17.14±126.79, P<0.05), Frustration Themes subscale (t=109.67, mean: 19.53±109.67, P<0.05), and from Rejection Sensitivity Scale (t=88.22, mean: 5.27±88.22, P<0.05) according to the study and control group variables, a significant difference was found between the means. When we evaluate the results, it is seen that the scores of the participants in the study group are higher than

Table 1. Comparison of dream themes scale and rejection sensitivity scale scores between study and control group

		n	Mean±SD	*t*	df.	P value*
Negative themes	Study group	79	23.65±2.27	17.07	115.79	**<0.001**
	Control group	79	14.03±4.47			
Anxiety themes	Study group	79	16.52±2.73	11.11	132.38	**<0.001**
	Control group	79	10.18±4.28			
Fear themes	Study group	79	11.91±1.78	14.84	109.10	**<0.001**
	Control group	79	4.76±3.90			
Influence of experiences	Study group	79	16.62±2.06	17.14	126.79	**<0.001**
	Control group	79	8.84±3.47			
Frustration themes	Study group	79	13.01±1.52	19.53	109.67	**<0.001**
	Control group	79	5.01±3.31			
Rejection sensitivity scale	Study group	79	60.34±4.84	5.27	88.22	**<0.001**
	Control group	79	48.78±18.87			

**Independent samples t-test*

Table 2. Predicting rejection sensitivity of borderline personality

	B	*SH*	*β*	*t*	P value*
(Constant)	32.51	3.48		9.34	**<0.001**
Borderline personality questionnaire	0.76	0.14	0.53	5.48	**<0.001**

**Simple linear regression analysis. R=.53, R^2=.28, F=30.01;* P<0.001

the scores of those in the control group (Table 1).

Borderline personality was found to predict rejection sensitivity (R=.53, R2=.28, P<0.05). It was determined that the independent variable in the established regression model explained 28% of the change in rejection sensitivity. When we evaluate the results, borderline personality has a positive effect on rejection sensitivity (β=.53, P<0.05) (Table 2).

When the findings in Table 3 were evaluated, it was seen that in the first model, borderline personality explained 40% of the variance in anxiety-themed dreams. In the second process, the rejection sensitivity variable was added to the model. Rejection sensitivity explained 3% of the variance in anxiety-themed dreams. It was observed that rejection sensitivity and borderline personality variables explained 43% of the variance in anxiety-themed dreams. With the addition of rejection sensitivity in the second process, the beta value of the borderline personality variable decreased from .64 to .53. This difference in Beta value with the bootstrapping method was found to be significant since the lower and upper limit values of the indirect total effect did not contain zero. According to this result, it was determined that there was partial mediation (Fig. 1).

When the findings in Table 4 were evaluated, it was seen that borderline personality explained 30% of the variance in fear-themed dreams in the first model. In the second process, the rejection sensitivity variable was added to the model. Rejection sensitivity was found to explain 3% of the variance in fear-themed dreams. It was observed that rejection sensitivity and borderline personality variables explained 33% of the variance in the score in fear-themed dreams. With the addition of rejection sensitivity in the second process, the beta value of the borderline personality variable decreased from .55 to .41. This difference in Beta value with the bootstrapping method was found to be significant since the lower and upper limit values of the indirect total effect did not contain zero. According to this result, it was determined that there was partial mediation (Fig. 2).

As a result of the evaluation of the findings in Table 5, it was seen that in the first model, borderline

Table 3. Mediator role of rejection sensitivity in predicting anxiety-themed dreams of borderline personality in the control group

Model		*R*	R^2	*B*	*SH*	*β*	*t*	P value*	Lower bound	Upper bound
	(Constant)	.64	.40	5.71	0.71		8.00	**<0.001**	4.29	7.13
1	**Borderline personality questionnaire**			0.21	0.03	0.64	7.33	**<0.001**	0.15	0.27
	(Constant)	.68	43	4.11	1.02		4.04	**<0.001**	2.09	6.14
	Borderline personality questionnaire			0.17	0.03	0.53	5.23	**<0.001**	0.11	0.24
2	**Rejection sensitivity scale**			0.05	0.02	0.22	2.15	**0.035**	0.00	0.09
	Undirect total effect (mediator)			0.04	0.02				0.00	0.09

**Hierarchical regression analysis*

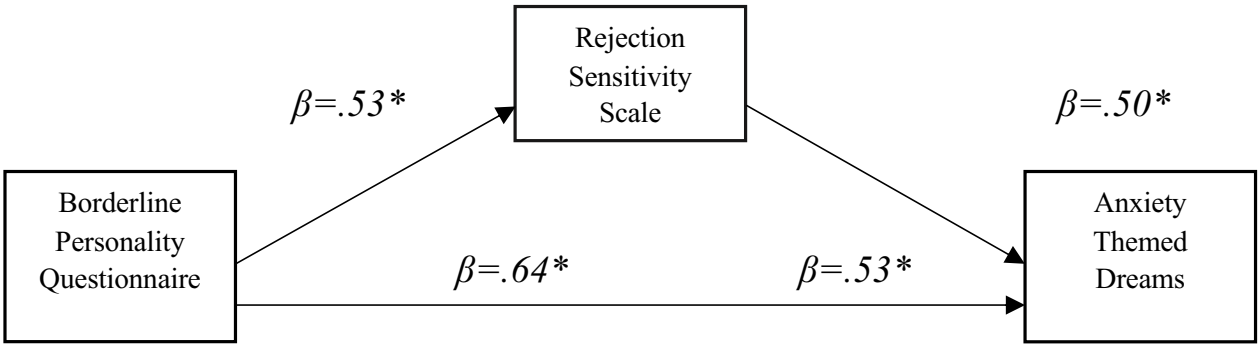

Fig. 1. **Beta values on the mediator role of rejection sensitivity in predicting anxiety-themed dreams of borderline personality in the control group. *P<0.05**

Table 4. **The mediating role of rejection sensitivity in predicting fear-themed dreams of borderline personality in the control group**

Model		*R*	*R^2*	*B*	*SH*	*β*	*t*	P value*	Lower bound	Upper bound
	(Constant)	.55	.30	1.28	0.71		1.81	0.074	-0.13	2.70
1	**Borderline personality questionnaire**			0.16	0.03	0.55	5.74	**<0.001**	0.11	0.22
	(Constant)	.59	33	-0.44	1.01		-0.43	0.665	-2.44	1.57
	Borderline personality questionnaire			0.12	0.03	0.41	3.77	**<0.001**	0.06	0.19
2	**Rejection sensitivity scale**			0.05	0.02	0.26	2.35	**0.021**	0.01	0.10
	Undirect total effect (mediator)			0.04	0.02				0.02	0.09

**Hierarchical regression analysis*

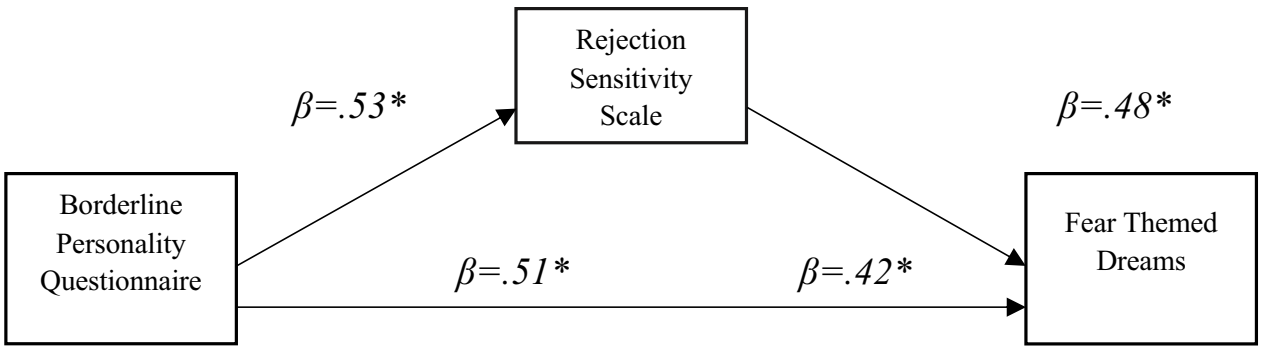

Fig. 2. **Beta values on the mediator role of rejection sensitivity in predicting fear-themed dreams of borderline personality in the control group. *P<0.05**

Table 5. Results on the mediator role of rejection sensitivity in predicting borderline personality effects of experiences-themed dreams in the control group

Model		*R*	*R^2*	*B*	*SH*	*β*	*t*	P value*	Lower bound	Upper bound
	(Constant)	.55	.30	1.28	0.71		1.81	0.074	-0.13	2.70
1	**Borderline personality questionnaire**			0.16	0.03	0.55	5.74	**<0.001**	0.11	0.22
	(Constant)	.59	33	-0.44	1.01		-0.43	0.665	-2.44	1.57
	Borderline personality questionnaire			0.12	0.03	0.41	3.77	**<0.001**	0.06	0.19
2	**Rejection sensitivity scale**			0.05	0.02	0.26	2.35	**0.021**	0.01	0.10
	Undirect total effect (mediator)			0.04	0.02				0.02	0.09

**Hierarchical Regression Analysis*

personality explained 30% of the variance in the Dreams themed Effect of Experiences. In the second process, the rejection sensitivity variable was added to the model. Rejection sensitivity was found to explain 3% of the variance in Dreams with the theme of Effect of Experiences. It was observed that rejection sensitivity and borderline personality variables explained 33% of the variance in the score of Dreams with the theme of the Effect of Experiences. With the addition of rejection sensitivity in the second process, the beta value of the borderline personality variable decreased from .55 to .41. This difference in Beta value with the bootstrapping method was found to be significant since the lower and upper limit values of the indirect total effect did not contain zero. According to this result, it was determined that there was partial mediation (Fig. 3).

As a result of the evaluation of the findings in Table 6, it was seen that borderline personality explained 54% of the variance in frustration-themed dreams in the first model. In the second process, the rejection sensitivity variable was added to the model. Rejection sensitivity explained 4% of the variance in frustration-themed dreams. It was observed that rejection sensitivity and borderline personality variables explained 58% of the variance in the scores of frustration-themed dreams. With the addition of rejection sensitivity in the second process, the beta value of the borderline personality variable decreased from .74 to .62. This difference in Beta value with the bootstrapping method was found to be significant since the lower and upper limit values of the indirect total effect did not contain zero. According to this result, it was determined that there was partial mediation (Fig. 4).

It was determined that the rejection sensitivity scale predicted the dreams with the theme of the Effect of Experiences (R=.26, R2=.07, P<0.05). It was determined that the independent variable in the estab-

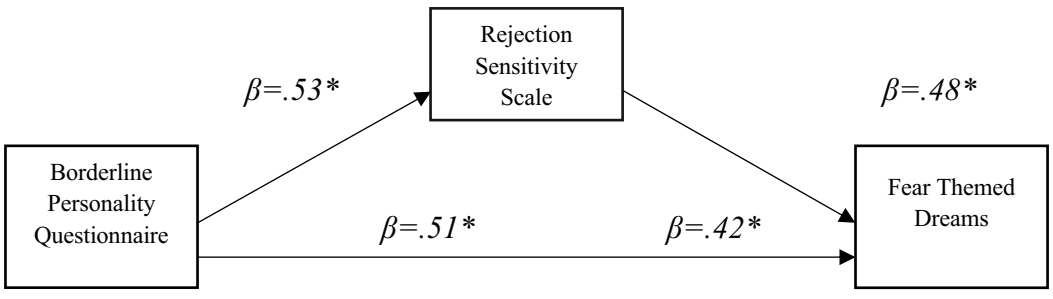

Fig. 3. Beta values on the mediator role of rejection sensitivity in the prediction of borderline personality effects of experiences-themed dreams in the control group. *P < 0.05

Table 6. Results on the mediator role of rejection sensitivity in predicting frustration-themed dreams of borderline personality in the control group

Model		*R*	R^2	*B*	*SH*	*β*	*t*	P value*	Lower bound	Upper bound
	(Constant)	.74	.54	1.02	0.48		2.12	**0.037**	0.06	1.99
1	**Borderline personality questionnaire**			0.19	0.02	0.74	9.67	**<0.001**	0.15	0.23
	(Constant)	.77	.58	-0.29	0.68		-0.42	0.674	-1.64	1.07
	Borderline personality questionnaire			0.16	0.02	0.62	7.11	**<0.001**	0.11	0.20
2	**Rejection sensitivity scale**			0.04	0.02	0.23	2.65	**0.010**	0.01	0.07
	Undirect total effect (mediator)			0.03	0.02				0.00	0.07

**Hierarchical regression analysis*

lished regression model explained 07% of the change in the Dreams-themed Effect of Experiences. When we evaluate the results, the rejection sensitivity scale has a positive effect on Dreams with the theme of the Effect of Experiences (β=.26, P<0.05) (Table 7).

DISCUSSION

According to Freud [2], the materials of dreams can be chosen from any segment of the dreamer's life. The only condition here is the existence of a line of thought that connects the life of the day before the dream to earlier ones. Although Freud [2] defined dreams as the "royal road to the unconscious" of neurotic patients, [24] states that dreams are a very important element in the understanding of borderline patients. Borderline personality disorder patients report much more negative themes in their dreams than other individuals during their psychodynamic treatment [25]. Stone [24] states that the evaluation of dreams of borderline patients is critical in two respects. The first of these is that the dreams of borderline patients can often contain frightening themes, and the second is that the interpretation of their dreams is meaningful because they often use defense mechanisms such as denial and negation, and their awareness of their actual motivations is low. In this study, it was determined that borderline patients have more dreams with frightening themes, anxiety themes, and negative themes than the control group. A quarter of adult nightmare sufferers are borderline patients [26], and patients with borderline personality disorder have high (%49) comorbidity with night mare disorder [9]. Individuals with vulnerable psychological boundaries, like borderline patients, experience longer, more intensely emotional, stressful, vivid, and

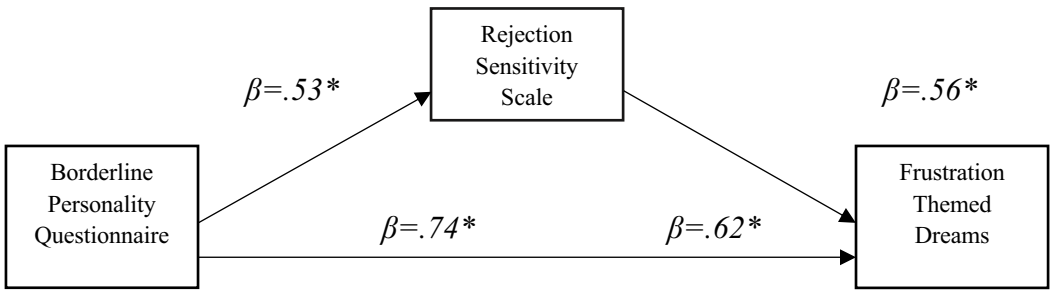

Fig. 4. Beta values on the mediator role of rejection sensitivity in predicting frustration-themed dreams of borderline personality in the control group. *P<0.05

Table 7. Findings regarding the prediction of rejection sensitivity of the study group on the effect of experiences themed dreams

	B	*SH*	*β*	*t*	P value*
(Constant)	6.25	2.45		2.55	**0.013***
Rejection sensitivity scale	0.09	0.04	0.26	2.32	**0.023***

**Simple linear regression analysis; Stepwise method. R=.26, R^2=0.07, F=5.37;* P=0.023

frightening dreams that resemble nightmares than individuals with thick boundaries [27].

In this study, it was determined that the effect of the daily experiences of the borderline patient group on dream themes was higher than the control group. Freud [2] says that the dream is not only about unconscious conflicts or desires; it is very closely related to the personality, age, gender, living standards, daily customs, and what he has been through [2]. Yu [28] stresses that the dreaming procedure is sensitive to affect valence and emotional concerns experienced during wakefulness. Borderline personality disorder patients have a high level of rejection sensitivity, and this sensitivity leads to emotional instability [29]. Aumann *et al*. [30] found that individuals with emotional instability have more dreams as a kind of continuation of daily life, and also, more than the present, their dreams are rooted in the past or the future. They also showed there is a relationship between dream bizarreness and emotional instability.

In this study, it was determined that the negative dream themes level of the borderline patient group was higher than the control group. Simor *et al*. [31] found that BDP patients report their dreams as more distressing, and when 68.1% of BPD patients experience night terror-like symptoms, the rate comes down to 22% in the control group. Some theories explain negative dream themes in borderline patients. At first, the negative dream content of borderline patients can be explained with the ironic process theory [32]. İronic process theory postulates that deliberate attempts to think about different thoughts other than the negative ones that are target for suppression go together with a monitoring procedure. This process looks for instances where the negative thought was unsuccessfully suppressed, which ironically can trigger thoughts of it, particularly during periods of high cognitive load, such as stressful situations. This process looks for instances where the negative thought was unsuccessfully suppressed, which ironically can trigger thoughts of it, particularly during periods of high cognitive load, such as stressful situations.

The second theory for explaining negative dream contents in borderline patients is the classical continuity hypothesis. Borderline patients suffer more from adverse life events than other personality disorders patients. This theory hypothesis dreams are a reflection of the dreamer's waking life, thus individuals with BPD may have more bad dreams than those of other people, even those with other personality disorders [33, 34]. Classical continuity theory has been confirmed by the study of Schredl *et al*. [35]. Kramer and Nuhic's [36] study of inpatients with mental disorders showed that psychopathological symptoms are reflected in the patients' dreams, and their finding supports the continuity hypothesis of dreaming. Therefore, it is thought that the continuity hypothesis makes the difference between anxiety themes, fear themes, negative themes, frustration themes, and the effects of experiences unclear in the case of patients with BPD and explains why all these themes are reported more by BPD patients in our research results.
The third theory stresses emotional dysregulation's effect on negative dream themes like nightmares [37]. Emotional instability, which is a result of emotion regulation difficulty, has a close relationship with nightmare frequency and/or distress [34, 35] and negative dream content [38]. Additionally, Selby *et al*. [39] highlight it in their Emotional Cascade Model (ECM). The ECM makes an effort to identify the cause of the disturbing nightmares BPD sufferers encounter. Emotion regulation difficulty is one of the core factors of

BPD. According to ECM, nightmares or any unpleasant dreams in addition to the terror they cause, may result in weaknesses in the ability to appropriately regulate one's emotions, which makes it harder to deal with stress the next day. During the waking state, borderline patients experience emotional cascades [31], and this adverse consequence leads to rumination, which is the repetitive thinking of mostly unpleasant thoughts.

Because of their difficulty in emotion regulation, ruminations increase negative affect, which, in turn, intensifies ruminations and starts a circulation. These processes result in increased negative emotions and increased cognitive activity during sleep that favors the appearance of nightmares and maladaptive behaviors during the waking state that are intended to regulate negative emotions. So borderline patient lives the day and night continuity; negative emotional experiences during day life increases the escalation of negative emotions, increased negative emotions increase ruminations, increased ruminations create a very aversive emotional state, aversive emotional state triggers dysfunctional coping skills and makes the emotions of patients even more dysregulated. Catastrophic emotions elevate cognitive activity during sleep, and they create negative dream themes or nightmares. Nightmares increase negative affect and vulnerability to stressors and negative emotional experiences during wakefulness. So, it seems that there is a bipolar relationship between unfavorable dream themes and borderline personality disorder severity. Frequent bad dreams in persons with BPD may influence the occurrence of negative life events [39]. Additionally, the emotional cascade model says that behavioral symptoms of BPD, such as substance use, binge eating, or self-harm, represent the person's attempt to interrupt the cascade [40]. Although, results show that dreams have an important role in BPD symptoms and their severity.

Furthermore, BPD has high comorbidity with axis I and axis II disorders. Especially it shows high comorbidity with depressive disorders [41]. It is known that the dreams of depressive patients mostly contain negative themes such as rejection, defeat, and loss [42], and the severity of depression during the daytime is correlated with the intensity of negative dream emotions [43]. But even when the level of depressive disorder is controlled for, BPD continues to be associated with negative dream content [44]. Post-traumatic stress disorder (PTSD) is the second most reported co-occurring axis I disorder in BPD. Individuals who have a PTSD diagnosis have dreams that include more negative themes than individuals with no PTSD diagnosis [45]. BPD patients often experience various traumas not in their adulthood but in their childhood which makes them even more vulnerable, and it increases nightmare frequency [38], thus explaining heightened nightmare frequency in BPD patients with comorbid PTSD, especially [46]. In this study, although both PTSD and depressive disorders were excluded in the borderline patient group, the higher negative dream content in the borderline patient group indicates that rejection sensitivity may be more determinant than comorbid diagnoses.

The study findings show that it may be beneficial to devote more space to dreams in clinical practice and scientific research practice related to borderline patients. Our recommendation in this direction is to investigate the relationship between complex post-traumatic stress disorder and dreams in patients with BPD. Complex posttraumatic stress disorder (cPTSD) is a new diagnosis in ICD-11 that considers the potentially more extensive posttraumatic consequences, including emotional dysregulation, particularly after prolonged and repeated trauma [47]. The diagnostic criteria consist of the three PTSD criteria and the additional symptoms of emotional dysregulation, interpersonal difficulties, and negative self-concept which are the core factors of BPD [5].

Limitations

The limitation of the study is that only female participants were included in the current study. It is well-known that borderline personality disorder affects women more frequently (up to 76%) than men, and men with borderline personality disorder may exhibit severe antisocial personality traits or drug use symptoms. [18]. So, in order to provide a higher level of access to the literature and it was selected to work primarily with female borderline patients for convenience of comparison.

CONCLUSION

As a result of this study, in which the dream themes

of BPD patients and undiagnosed individuals were compared, it was determined that all dream theme dimensions were seen at a higher level in BPD patients. It has been determined that the dreams of BPD patients generally have negative content (the concept of negative is used to indicate the content, not the negative themes sub-dimension), and it shows that the negative content in the daily life of borderline patients and the effect of intense experiences sub-dimension also cause negative content in these patients. The results of the present study reveal the relationship between BPD and negative dream content and the important role of rejection sensitivity in this relationship. The study's results emphasize the importance of addressing dreams in patients with BPD, considering the relationship of negative dreams with daily functioning, dissociative symptoms, self-harming behaviors, and suicide attempts. Results also indicate that it might be beneficial to target rejection sensitivity to reduce aggravating dream contents.

Ethical Considerations

The study has been approved by the Istanbul Aydın University Ethics Committee (Date: 09.07.2021, No: 2021/08). An informed consent form was sent to the participants and their consent was obtained. The entire study was performed in accordance with the Declaration of Helsinki.

Authors' Contribution

Study Conception: HF; Study Design: HF; Supervision: HF; Funding: HF; Materials: HF; Data Collection and/or Processing: HF; Statistical Analysis and/or Data Interpretation: HF; Literature Review: HF; Manuscript Preparation: HF and Critical Review: HF.

Conflict of interest

The author disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

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REFERENCES

1. Stamm D, Hardin DA. Dreaming and psychiatric disorders. In: Kushida CA. (ed.), The Encyclopedia of Sleep. Vol. 1, Waltham, MA: Academic Press. 2013: pp. 144-15.
2. Freud S, 2019. Rüyaların Yorumu (Muradoğlu D-Çeviri) (5th ed.). Ankara: Say Yayınları; 2019.
3. Hobson A, Kahn D. Dream content: Individual and generic aspects. Conscious Cogn. 2007;16(4):850-858. doi: 10.1016/j.concog.2007.07.010.
4. Kramer M, Glucksman ML. (eds.), Dream Research: Contributions to Clinical Practice. 1st ed., New York, Routledge; 2015.
5. Faraji H. [Evaluation of Determining Borderline Personality Disorder in Adolescence]. OPUS Uluslararası Toplum Araştırmaları Dergisi 2021;18(43):7139-66. doi: 10.26466/opus.898571 [Article in Turkish].
6. Kartal F, Uğur K, Mete B, Demirkol ME, Tamam L. The relationship between the oxytocin level and rejection sensitivity, childhood traumas, and attachment styles in borderline personality disorder. Psychiatry Investig. 2022;19(3):239-246. doi: 10.30773/pi.2021.0358.
7. Downey G, Khouri H, Feldman SI. Early interpersonal trauma and later adjustment: The mediational role of rejection sensitivity. In: Cicchetti D, Toth SL. (eds.), Developmental perspectives on Trauma: Theory, Research, and Intervention, New York: University of Rochester Press; 1997: pp. 85-114.
8. Barros K. Borderline personality disorder features, perceived social support, sleep disturbance, and rejection sensitivity. Arizona, Arizona State University ProQuest Dissertations Publishing; 2016.
9. Semiz UB, Basoglu C, Ebrinc S, Cetin M. Nightmare disorder, dream anxiety, and subjective sleep quality in patients with borderline personality disorder. Psychiatry Clin Neurosci. 2008;62(1):48-55. doi: 10.1111/j.1440-1819.2007.01789.x.
10. Jenkins CA, Thompson KN, Nicholas CL, Chanen AM. Sleep in Young People With Features of Borderline Personality Disorder: A Scoping Review. J Pers Disord. 2022;36(1):19-39. doi: 10.1521/pedi_2021_35_525.
11. Lemyre A, Bastien C, Vallières A. Nightmares in mental disorders: a review. Dreaming 2019;29(2):144-166. doi: 10.1037/drm0000103
12. Alghamdi AA, Alafif NO, BaHammam AS, et al. Nightmares' pattern and predictors among a Saudi University students during COVID-19 Pandemic. Int J Environ Res Public Health. 2022;19(3):1776. doi: 10.3390/ijerph19031776.
13. Selby EA. Chronic sleep disturbances and borderline personality disorder symptoms. J Consult Clin Psychol. 2013;81(5):941-947. doi: 10.1037/a0033201.
14. Faraji H, Güler K. Borderline personality traits and self handicapping. Int J Curr Res 2021;13(6):17683-17689. doi: 10.24941/ijcr.41401.06.2021
15. Bandel SL, Brausch AM. Poor sleep associates with recent nonsuicidal self-injury engagement in adolescents. Behav Sleep Med. 2020;18(1):81-90. doi: 10.1080/15402002.2018.1545652.
16. Liu X, Liu ZZ, Chen RH, et al. Nightmares are associated with future suicide attempt and non-suicidal self-injury in adolescents. J Clin Psychiatry. 2019;80(4):18m12181. doi:

10.4088/JCP.18m12181.
17. Giesbrecht T, Smeets T, Leppink J, Jelicic M, Merckelbach H. Acute dissociation after 1 night of sleep loss. J Abnorm Psychol. 2007;116(3):599-606. doi: 10.1037/0021-843X.116.3.599.
18. Shenoy SK, Praharaj SK. Borderline personality disorder and its association with bipolar spectrum and binge eating disorder in college students from South India. Asian J Psychiatr. 2019;44:20-24. doi: 10.1016/j.ajp.2019.07.017.
19. Poreh AM, Rawlings D, Claridge G, Freeman JL, Faulkner C, Shelton C. The BPQ: a scale for the assessment of borderline personality based on DSM-IV criteria. J Pers Disord. 2006;20(3):247-260. doi: 10.1521/pedi.2006.20.3.247.
20. Ceylan V, Borderline Kişilik Ölçeği (Türkçe Bpq): Geçerlik, Güvenirliği, Faktör Yapısı, Hasan Kalyoncu Üniversitesi Sosyal Bilimler Enstitüsü (Yayımlanmamış Yüksek Lisans Tezi); 2017.
21. Downey G, Feldman SI. Implications of rejection sensitivity for intimate relationships. J Pers Soc Psychol. 1996;70(6):1327-1343. doi: 10.1037//0022-3514.70.6.1327.
22. Özen A, Sümer N, Demir M. Predicting friendship quality with rejection sensitivity and attachment security. J Soc Pers Relat 2011;28(2):163-181. doi: 10.1177/0265407510380607
23. Genç A, Çelikel FÇ, Başol G. [Development, validity and reliability of the dream themes scale]. The Journal of Academic Social Science Studies 2013;6:295-308. [Article in Turkish]
24. Stone MH. Dreams of fragmentation and of the death of the dreamer: a manifestation of vulnerability to psychosis. Psychopharmacol Bull. 1979;15(1):12-14.
25. Taubner S, Benecke C. [How do patients with borderline personality disorder dream?]. PTT: Persönlichkeitsstörungen Theorie und Therapie 2009;13:151-167. [Article in German]
26. Hartmann E, Russ D, van der Kolk B, Falke R, Oldfield M. A preliminary study of the personality of the nightmare sufferer: relationship to schizophrenia and creativity? Am J Psychiatry. 1981;138(6):794-797. doi: 10.1176/ajp.138.6.794.
27. Hartmann E, Kunzendorf RG. Boundaries and dreams. Imagin Cogn Pers 2006;26(1):101-115. doi: 10.2190/HK76-038K-407M-86
28. Yu CKC. Dream motif scale. Dreaming 2012;22(1):18-52. doi: 10.1037/a0026171
29. Strimpfel JM, Macfie J, Elledge LC, Watson AJ. Childhood disruptions in caregiving, adult parental attachment, and borderline features in emerging adulthood: rejection sensitivity as a mechanism of ınfluence. J Cogn Psychother. 2022;36(2):129-145. doi: 10.1891/JCPSY-D-20-00047.
30. Aumann C, Lahl O, Pietrowsky R. Relationship between dream structure, boundary structure and the big five personality dimensions. Dreaming 2012;22(2):124-135. doi: 10.1037/a0028977
31. Simor P, Csóka S, Bódizs R. Nightmares and bad dreams in patients with borderline personality disorder: fantasy as a coping skill? Eur J Psychiatr 2010;24(1):28-37. doi: 10.4321/S0213-61632010000100004
32. Wegner DM. Ironic processes of mental control. Psychol Rev. 1994;101(1):34-52. doi: 10.1037/0033-295x.101.1.34.
33.Pagano ME, Skodol AE, Stout RL, et al. Stressful life events as predictors of functioning: findings from the collaborative longitudinal personality disorders study. Acta Psychiatr Scand. 2004;110(6):421-429. doi: 10.1111/j.1600-0447.2004.00398.x.
34. Schredl M. Continuity between waking and dreaming: a proposal for mathematical model. Sleep Hypn 2003;5:26-39.
35. Schredl M, Paul F, Reinhard I, Ebner-Priemer UW, Schmahl C, Bohus M. Sleep and dreaming in patients with borderline personality disorder: a polysomnographic study. Psychiatry Res. 2012;200(2-3):430-436. doi: 10.1016/j.psychres.2012.04.036.
36. Kramer M, Nuhic Z. Are view of dreaming by psychiatric patients: an update. In: Pandi S, Ruoti R, Kramer M, eds., Sleep and Psychosomatic Medicine. New York: Taylor and Francis; 2007: pp. 137-155.
37. Levin R, Nielsen TA. Disturbed dreaming, posttraumatic stress disorder, and affect distress: a review and neurocognitive model. Psychol Bull. 2007;133(3):482-528. doi: 10.1037/0033-2909.133.3.482.
38. Pesant N, Zadra A. Dream content and psychological well-being: a longitudinal study of the continuity hypothesis. J Clin Psychol. 2006;62(1):111-211. doi: 10.1002/jclp.20212.
39. Selby EA, Ribeiro JD, Jr. Joiner TE. What dreams may come: emotional cascades and nightmares in borderline personality disorder. Dreaming 2013;23(2):126-144. doi: 10.1037/a0032208
40. Baer RA, Peters JR, Eisenlohr-Moul TA, Geiger PJ, Sauer SE. Emotion-related cognitive processes in borderline personality disorder: a review of the empirical literature. Clin Psychol Rev. 2012;32(5):359-369. doi: 10.1016/j.cpr.2012.03.002.
41. El Ghamry R, Omar AN, Zaki N, et al. Profile of egyptian patients with borderline personality disorder with and without comorbidity. Egypt J Psychiatr 2021;42:115-122.
42. Türkçapar MH, Sargın AE. [Cognitive Behavioral Psychotherapies: History and Development]. Davranışçı Psikoterapi ve Araştırmalar Dergisi 2012;1:7-14. [Article in Turkish]
43. Solomonova E, Picard-Deland C, Rapoport I, et al. Stuck in a lockdown: dreams, bad dreams, nightmares, and their relationship to stress, depression and anxiety during the COVID-19 pandemic. PLoS One. 2021;16(11):e0259040. doi: 10.1371/journal.pone.0259040.
44. Harty L, Duckworth R, Thompson A, Stuewig J, Tangney JP. Are inmates' subjective sleep problems associated with borderline personality, psychopathy, and antisocial personality independent of depression and substance dependence? J Forens Psychiatry Psychol. 2010;21(1):23-39. doi: 10.1080/14789940903194095.
45. Pigeon W, Carr M, Mellman T. Dream content associated with the development of PTSD. Int J Dream Res 2021;14(1):136-140. doi: 10.11588/ijodr.2021.1.75883
46. Ford JD, Courtois CA. Complex PTSD and borderline personality disorder. Borderline Personal Disord Emot Dysregul. 2021;8(1):16. doi: 10.1186/s40479-021-00155-9.
47. Jowett S, Karatzias T, Shevlin M, Albert I. Differentiating symptom profiles of ICD-11 PTSD, complex PTSD, and borderline personality disorder: A latent class analysis in a multiply traumatized sample. Personal Disord. 2020;11(1):36-45. doi: 10.1037/per0000346.

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Comparison of platelet-to-lymphocyte and neutrophil-to-lymphocyte ratios with epithelial ovarian cancer stages

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ABSTRACT

Objectives: Epithelial ovarian cancer (EOC) is the most common histologic type among ovarian cancers. It is usually diagnosed at an advanced stage and the prognosis worsens. The aim of our study was to investigate the predictive value of serum platelet-to-lymphocyte ratio (PLR) and neutrophil-to-lymphocyte ratio (NLR), which are systemic inflammatory response markers in EOC stages.

Methods: In this study, 140 patients diagnosed with primary EOC in İzmir Katip Çelebi University Atatürk Training and Research Hospital Gynecology and Obstetrics Clinic between 01.01.2012-01.07.2019 were included. The cases were staged using the FIGO 2014 Ovarian Cancer Staging system. Whether the PLR and NLR values were different between the stages were analyzed with appropriate statistical analysis methods.

Results: A total of 140 patients, 54 were in the early stage (Stage I: 47; Stage II: 7) and 86 were in the advanced stage (Stage III: 73; Stage IV: 13). The PLR and NLR values differed between the four stages (P=0.003 and P=0.032, respectively). The PLR value was different between the early and advanced stages (P=0.033), the AUC value was 0.607, the optimum cut-off was 220, the sensitivity was 47%, and the specificity was 81% in the early and advanced stage discrimination. Accordingly, the Odds ratio of PLR for advanced EOC was 3.82 (95% CI: 1.70-8.57, P=0.0011).

Conclusions: The NLR and PLR values were found to have a prognostic value in the discrimination of EOC stages. It has been determined that PLR value may play a predictive role in advanced EOC before surgery.

Keywords: Epithelial ovarian cancer, stage, prognosis, neutrophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio

Ovarian cancer consists of different histological subtypes with different risk factors, cells of origin, clinical features, and treatments. Of these histological subtypes, epithelial ovarian cancer (EOC) accounts for approximately 90% and is classified as serous, endometrioid, clear cell, and mucinous carcinomas. Germ cell tumors and sex cord-stromal tumors, which constitute approximately 10% of ovarian cancers, are defined as non-epithelial ovarian cancers [1].

The prognosis of ovarian cancer can be poor, despite advances in surgery and chemotherapy. Although ovarian cancer ranks 8th among female cancers, it re-

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mains the leading cause of death from gynecological cancer and the fifth leading cause of cancer-related death in women. The incidence of new ovarian cancer cases ranges from 9 to 15 per 100,000 women per year, and the death rate averages 5.4 to 11.6 deaths per 100,000 women [2]. According to the Global Cancer Observatory data, 313,959 women were diagnosed with ovarian cancer in 2020. 1.6% of new cancer cases in the world and 1.7% in Turkiye are ovarian cancer [3]. Since the early stages of the disease are usually asymptomatic, approximately 75% of cases are diagnosed in the advanced stages (III and IV). The 5-year survival rate in EOC is directly related to the stage of the disease at the time of initial diagnosis. While the 5-year average survival rate for all EOCs diagnosed at stage 4 is around 20% for all races, this rate rises to 89% in those diagnosed at stage I [4].

Recently, there has been great interest in the role of cancer-associated inflammation in the burden and prognosis of the disease. Inflammation is known to be associated with different stages of tumor development, including initiation, elevation, malignant transformation, invasion, and metastasis. The association between poor prognosis and elevation of white blood cells, platelets, or their ratios can be explained by an inflammatory process elicited by cancer cells [5]. Therefore, systemic inflammatory response (SIR) markers such as hypoalbuminemia, hyperfibrinogenemia, C-reactive protein (CRP), cancer antigen-125 (CA-125), absolute white blood cell count (WBC), neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) were investigated as prognostic factors in cancer patients [6-8].

Prognostic factors in ovarian cancer include age at diagnosis, The International Federation of Gynecology and Obstetrics (FIGO) tumor stage, histological type, tumor grade, and presence of residual disease after the first surgery [1]. All of these known variables, except age, are only suitable for post-operative evaluation. For this reason, there is no defined screening program for ovarian cancers and there is no biomarker currently used.

The aim of our study is to investigate the prognostic relationship between preoperative NLR and PLR rates and EOC stages, which are easy, inexpensive, and can be used in practice.

METHODS

Clinical Data

Patients who were operated for suspected ovarian cancer and/or adnexal mass in Izmir Katip Celebi University Atatürk Training and Research Hospital, Department of Gynecology and Obstetrics between 01.01.2012 and 01.07.2019, were scanned through the medical records and 223 cases were identified. Among these cases, 83 cases who were diagnosed as benign or borderline ovarian tumors, non-epithelial malignant ovarian tumors, recurrent ovarian malignancies and epithelial ovarian carcinomas in pathological microscopic examination but who had preoperative blood transfusion were excluded from the study. 140 patients diagnosed with primary EOC in the final pathology were included in the study.

Preoperatively studied complete blood count results of 140 EOC cases included in the study were obtained from the medical records. Preoperative neutrophil-to-lymphocyte ratio (NLR) and platelet-to-lymphocyte ratio (PLR) were calculated at all stages. The cases were staged using FIGO 2014 Ovarian Cancer Staging System [9].

This study was approved by Izmir Katip Çelebi University Non-invasive Clinical Research Ethics Committee (Date: 26.09.2019, Decision no. 434).

Statistical Analysis

The data were evaluated in the statistical package program IBM SPSS Statistics 25.0 (IBM Corp., Armonk, New York, USA). The dependent variables of the study were the NLR and PLR; the independent variables were determined as the groups obtained by the FIGO 2014 Ovarian Cancer Staging System. Descriptive statistics were given as median ± standard deviation (SD), percent (%). The normal distribution of the data of numerical variables was evaluated with the Shapiro-Wilk normality test. Comparisons of two groups including continuous variables were evaluated with the Mann-Whitney U test according to the results of the normality test. Comparisons of more than two groups were evaluated by Kruskal-Wallis analysis according to the normality test result. In case of difference in Kruskal-Wallis analysis, Dunn-Bonferroni multiple comparison test was used as a post-hoc test.

Table 1. Comparison results of platelet-to-lymphocyte ratio by epithelial ovarian cancer stages

	n	Median±SD	P value (Kruskal-Wallis)		Median±SD	P value (Mann-Whitney U)
Stage I	47	156.67±75.93	0.003	Early stages (I+II)	160.68±83.54	0.033
Stage II	7	177.71±118.42				
Stage III	73	190.04±151.9		Advanced stages (III+IV)	207.15±156.28	
Stage IV	13	303.16±155.13				

SD = standard deviation

ROC analysis was used to calculate the AUC value. The optimum cut-off was calculated with the Youden index. A value of P<0.05 was considered statistically significant.

RESULTS

According to FIGO 2014 Ovarian Cancer Staging System of 140 patients diagnosed with histopathologically EOC, 54 were in the early stage (Stage I: 47; Stage II: 7) and 86 were in the advanced stage (Stage III: 73; Stage IV: 13). Descriptive statistical data of the PLR and NLR values of the stages are shown in Tables 1 and 2.

In the normality analysis, it was observed that the data were not normally distributed (P>0.05). As a result of analysis of variance, a statistically significant difference was found between the PLR and NLR values of the four stages (P=0.003 and P=0.032, respectively) (Tables 1 and 2).

When the PLR values of the four stages were compared, there was a statistically significant difference between stage I and stage IV (P=0.001) and between stage III and stage IV (P=0.03). No difference was found between the other stages (P>0.05). When the NLR values of the four stages were compared, a significant difference was found only between stage III and stage IV (P=0.031). There was no statistically significant difference between the other stages (P>0.05).

When the patients were grouped as early stage (stage I+stage II) and advanced stage (stage III+stage IV), while the PLR value was different between the two groups (Fig. 1), there was no difference in NLR value (P=0.033 and P=0.831, respectively) (Tables 1 and 2).

In correlation analysis, it was observed that PLR values were correlated with stages at a low level (r = 0.263, P=0.002). NLR values were not correlated with stages (r = 0.108, P=0.205).

The prognostic efficacy of PLR and NLR values in the discrimination of stages were evaluated. The AUC value was 0.607, the optimum cut-off was 220, the sensitivity was 47%, and the specificity was 81%

Table 2. Comparison results of neutrophil-to-lymphocyte ratio by epithelial ovarian cancer stages

	n	Median±SD	P value (Kruskal-Wallis)		Median±SD	P value (Mann-Whitney U)
Stage I	47	2.66±2.14	**0.032**	Early stages (I+II)	2.69±2.32	0.831
Stage II	7	3.26±3.37				
Stage III	73	2.76±2.95		Advanced stages (III+IV)	3.03±2.84	
Stage IV	13	4.61±1.90				

SD = standard deviation.

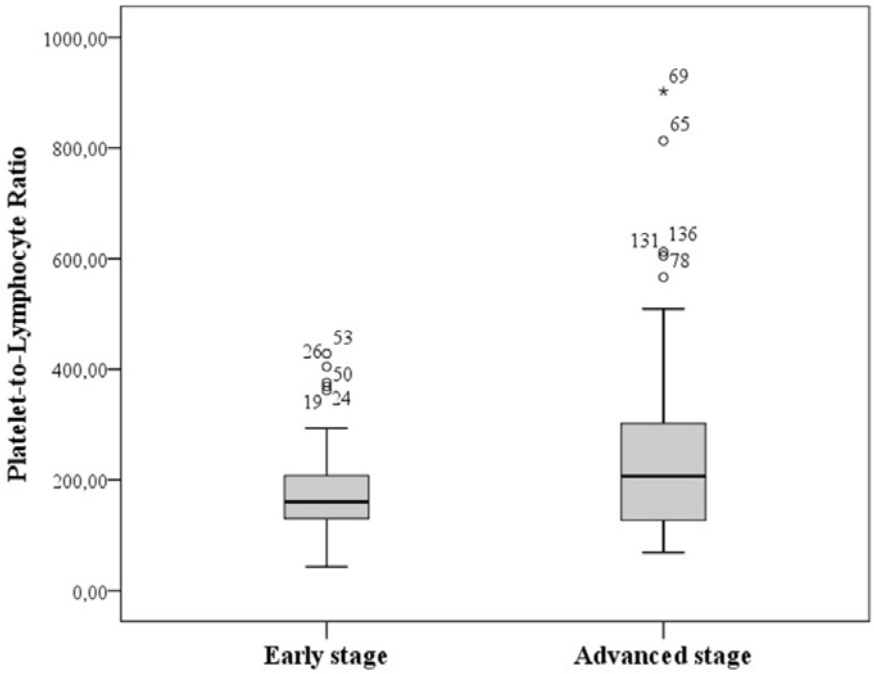

Fig. 1. **Box plot of platelet-to-lymphocyte ratio in early and advanced epithelial ovarian cancer.**

in the discrimination between early stage (stage I+II) and advanced (stage III+IV) for PLR values. When the cut-off was 220 for the PLR value, the Odds ratio for advanced EOC was calculated as 3.82 (95% CI: 1.70-8.57, P=0.0011) (Table 3). The ROC curve for the discrimination of PLR value between early and advanced epithelial ovarian cancer is shown in Fig. 2.

The AUC of PLR was 0.845 (P<0.001) in the discrimination of stage IV from stage I and 0.737 (P=0.007) in the discrimination of stage IV from stage III. The AUC of NLR was 0.730 (P=0.009) in the discrimination of stage IV from stage III. There was no statistically significant difference in PLR and NLR values in the discrimination of the other stages (Table 4). The optimum cut-off value of PLR was calculated as 210 in the discrimination of stage IV from stage I. In this case, the sensitivity of the PLR value was 85% and the specificity was 81%. The optimum cut-off value of the PLR was calculated as 207 in the discrimination of stage IV from stage III. Accordingly, the

Table 3. **Prognostic value of PLR in the discrimination of early and advanced stages of epithelial ovarian cancer**

Stages		PLR<220 (n)	PLR≥220 (n)	AUC (early vs advanced stages)	Odds ratio
Early	FIGO I	40	7	0.607 **(P=0.033)** Sensitivity 47% Specificity 81%	3.82 **(P=0.0011)**
	FIGO II	4	3		
Advanced	FIGO III	42	31		
	FIGO IV	4	9		

AUC= area under the curve, PLR= platelet-to-lymphocyte ratio, FIGO= The International Federation of Gynecology and Obstetrics

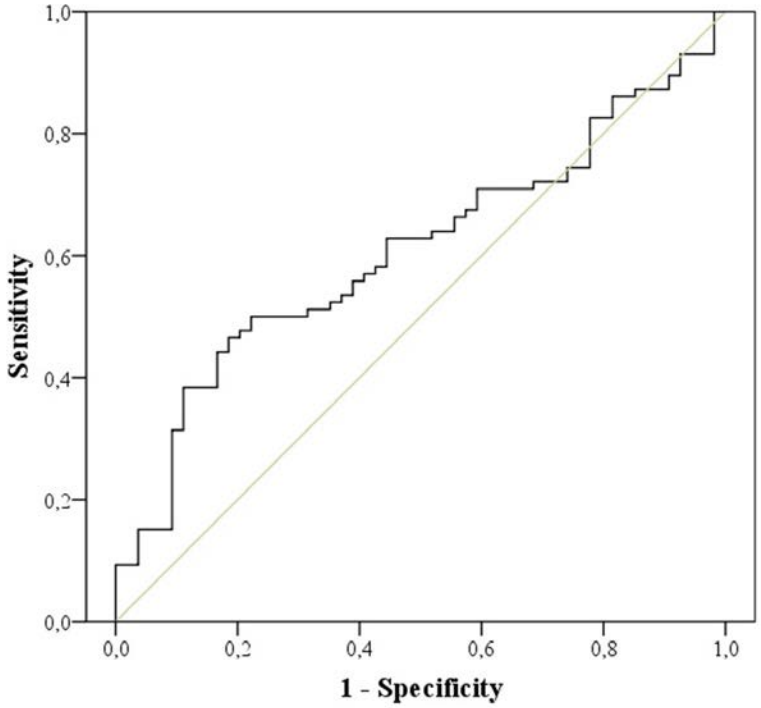

Fig. 2. ROC curve of platelet-to-lymphocyte ratio in the discriminating early and advanced epithelial ovarian cancer.

sensitivity of the PLR value was 85% and the specificity was 56%. The optimum cut-off value of the NLR was calculated as 3.06 in the discrimination stage IV from stage III. In this case, the sensitivity of the NLR value was determined as 85% and the specificity as 59% (Table 4).

DISCUSSION

The role of cancer-associated inflammation theory in oncogenesis and tumor growth has been a major area of study in recent years. Inflammatory cell counts and the ratios derived from these cell counts, such as PLR and NLR, have been investigated for diagnostic, prognostic and treatment follow-up in many cancer types. The biggest advantage of these inflammatory cell numbers and ratios is that they can be easily obtained in complete blood count data and do not require any additional health expenditure. Various studies have shown that these inflammatory parameters, together with CA-125, can contribute to the management of the disease in ovarian cancers [10].

EOC accounts for approximately 90% of ovarian

Table 4. Efficacy of PLR and NLR in discriminating epithelial ovarian cancer stages

FIGO Stages	PLR				NLR			
	Cut-off	AUC	Sen	Spe	Cut-off	AUC	Sen	Spe
I vs IV	210	0.845 **(P<0.001)**	85%	81%	NS	NS	NS	NS
III vs IV	207	0.737 **(P=0.007)**	85%	56%	3.06	0.730 **(P=0.009)**	85%	59%

AUC= area under the curve, PLR= platelet-to-rymphocyte ratio, NLR= neutrophil-to-lymphocyte ratio, Sen=Sensitivity, Spe= Specificity, FIGO= The International Federation of Gynecology and Obstetrics, NS= not significant.

Note: There was no statistically significant difference in PLR and NLR values in the discrimination of the other stages (P>0.05).

cancers in all age and ethnic groups. The fact that patients with EOC are asymptomatic for a long time and therefore are often diagnosed at an advanced stage has intensified studies to determine the diagnostic and prognostic values of inflammatory cell ratios derived from complete blood count parameters such as NLR and PLR [4, 11].

In our study, the effectiveness of NLR and PLR ratios in the evaluation of the stages of patients with EOC was investigated. It was determined that the PLR value increased especially in stage IV compared to other stages. We thought that the lack of a significant difference between stage II and other stages in terms of PLR value may be due to the small number of patients in this group. NLR value was different only between stage III and stage IV. In addition, it was determined that the PLR value was correlated with the stages (stages I-IV), while the NLR value was not. Moreover, while PLR value differs between early stage and advanced stage EOC, there is no difference in NLR value. In summary, it was observed that the PLR value increased as the stage increased. We think that the PLR value may be important in terms of giving an idea about the stage of the disease at the initial diagnosis stage.

In studies in the literature on the subject, it is reported that PLR and NLR values increase as the stage increases, similar to our findings. In a study by Kökçü *et al*. [12], which included 100 patients with epithelial ovarian cancer, it was shown that NLR and PLR levels were increased in advanced stage compared to early stage. In the study, the prognostic value of PLR was found to be better than blood parameters such as platelet and NLR ratio [12]. In a study by Zhang *et al*. [13] in which 190 patients with ovarian cancer were included, it was revealed that while the mean PLR values were 182.6 in stage I, it increased to 234.50 in stage IV, and this increase was statistically significant ($p = 0.032$). In the recently published study by Huang *et al*. [14], it was shown that the value of PLR and NLR did not differ between histological grade, age of the patient or type of ovarian cancer, but increased in advanced ovarian cancer compared to early-stage ovarian cancer. Thus, it has been reported that high PLR and NLR values at the time of diagnosis can be interpreted in favor of probable advanced stage ovarian cancer. Although there are studies in the literature that PLR values increase as the stage increases, which is largely consistent with our findings, there are findings in some studies that the PLR value does not change between stages. In the study of Wang *et al*. [15], it was reported that while there was no increase in PLR values, NLR values increased in advanced EOC compared to the early stage.

In the literature, studies have been conducted to differentiate ovarian cancer from the healthy group, generally for the effectiveness of PLR and NLR in the diagnosis of epithelial ovarian cancer. Considering the results of these studies, the AUC value of PLR in differentiating ovarian cancer from the healthy group varies between 0.621 and 0.684, while the AUC value of NLR varies between 0.604 and 0.737 [16-18]. We were able to find only one study evaluating the efficacy of PLR and NLR in staging ovarian cancers. In this study, using the Multivariate Logistic Regression Analysis method, it was reported that PLR is an independent risk factor associated with the distinction between early and advanced stages in EOC. However, such a result could not be reached for the NLR ratio. In the same study, when the cut-off was determined as 200 for PLR, the odds ratio was calculated as 1.0105 [16]. In our study, the prognostic value of PLR and NLR ratios was investigated in the discrimination of early and advanced stages by ROC analysis. Accordingly, the AUC value of PLR values in the discrimination between early and advanced stages in EOC were found to be 0.607, sensitivity 47%, and specificity 81% (cut-off: 220). In our study, the odds ratio for PLR was found to be 3.82. In the light of these findings, we think that PLR values can be considered in estimating the stage of epithelial ovarian cancer at the initial diagnosis stage.

There are also studies to reveal the prognostic value of PLR and NLR ratios for survival prediction. In a meta-analysis evaluating the results of a total of 3467 patients and 13 studies, it was reported that an increase in NLR had a poor prognostic effect (hazard ratios 1.70 and 1.77, respectively) on overall survival (OS) and progression-free survival (PFS). In the same study, it was stated that the increase in PLR had a slightly higher risk of poor prognosis for OS and PFS (hazard ratio 2.05 and 1.85, respectively) [19]. In our study, an analysis for survival prediction was not performed. However, the stage of EOC at diagnosis is one of the most important factors on survival. In this respect, according to the results of our study, the fact that

the PLR value has a prognostic value in the prediction of early and advanced stages is also compatible with studies on survival prediction.

Limitations

Our study has some limitations. The first is that the number of patients in some stages is low. Especially there were few patients in the stage II. This may have affected the power of statistical analyzes regarding the stage II. Not including a healthy group in our study can be considered as a limitation in terms of investigating the diagnostic value of the ratio of PLR and NLR. However, in this study, only a result was revealed for the use of these values for prognostic purposes in the differentiation of stages at the initial diagnosis stage.

CONCLUSION

According to the data of our study, NLR and PLR values were found to have a statistically prognostic value in the discrimination of EOC stages. It has been determined that NLR value is not effective in distinguishing early and advanced EOC, and PLR value may play a predictive role for advanced EOC before surgery. However, there is a need for more comprehensive studies in this area.

Authors' Contribution

Study Conception: ÇA, HİT; Study Design: ÇA, HİT; Supervision: ÇA, YD; Funding: HİT; Materials: HİT; Data Collection and/or Processing: HİT; Statistical Analysis and/or Data Interpretation: HİT, YD; Literature Review: HİT, YD; Manuscript Preparation: HİT, YD and Critical Review: YD.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. Matulonis UA, Sood AK, Fallowfield L, Howitt BE, Sehouli J, Karlan BY. Ovarian cancer. Nat Rev Dis Primers. 2016;2:16061. doi: 10.1038/nrdp.2016.61.
2. Mathis J, Jellouli MA, Sabiani L, Fest J, Blache G, Mathevet P. Ovarian cancer screening in the general population. Horm Mol Biol Clin Investig. 2019;41(3). doi: 10.1515/hmbci-2019-0038.
3. Sung H, Ferlay J, Siegel RL, et al. Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2021;71(3):209-249. doi: 10.3322/caac.21660.
4. Torre LA, Trabert B, DeSantis CE, et al. Ovarian cancer statistics, 2018. CA Cancer J Clin. 2018;68(4):284-296. doi: 10.3322/caac.21456.
5. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. Cell. 2010;140(6):883-899. doi: 10.1016/j.cell.2010.01.025.
6. Liu Y, Chen S, Zheng C, Ding M, et al. The prognostic value of the preoperative c-reactive protein/albumin ratio in ovarian cancer. BMC Cancer. 2017;17(1):285. doi: 10.1186/s12885-017-3220-x.
7. Chen L, Wang X, Shu J, Xu S, Wu Q, Yu Y. Diagnostic value of serum D-dimer, CA125, and neutrophil-to-lymphocyte ratio in differentiating ovarian cancer and endometriosis. Int J Gynaecol Obstet. 2019;147(2):212-218. doi: 10.1002/ijgo.12949.
8. Li L, Tian J, Zhang L, et al. Utility of Preoperative Inflammatory Markers to Distinguish Epithelial Ovarian Cancer from Benign Ovarian Masses. J Cancer. 2021;12(9):2687-2693. doi: 10.7150/jca.51642.
9. Prat J; FIGO Committee on Gynecologic Oncology. Staging classification for cancer of the ovary, fallopian tube, and peritoneum. Int J Gynaecol Obstet. 2014;124(1):1-5. doi: 10.1016/j.ijgo.2013.10.001.
10. Prodromidou A, Andreakos P, Kazakos C, Vlachos DE, Perrea D, Pergialiotis V. The diagnostic efficacy of platelet-to-lymphocyte ratio and neutrophil-to-lymphocyte ratio in ovarian cancer. Inflamm Res. 2017;66(6):467-475. doi: 10.1007/s00011-017-1026-6.
11. Ashrafganjoei T, Mohamadianamiri M, Farzaneh F, Hosseini MS, Arab M. Investigating preoperative hematologic markers for prediction of ovarian cancer surgical outcome. Asian Pac J Cancer Prev. 2016;17(3):1445-1448. doi: 10.7314/apjcp.2016.17.3.1445.
12. Kokcu A, Kurtoglu E, Celik H, Tosun M, Malatyalıoglu E, Ozdemir AZ. May the platelet to lymphocyte ratio be a prognostic factor for epithelial ovarian cancer? Asian Pac J Cancer Prev. 2014;15(22):9781-9784. doi: 10.7314/apjcp.2014.15.22.9781.
13. Zhang WW, Liu KJ, Hu GL, Liang WJ. Preoperative platelet/lymphocyte ratio is a superior prognostic factor compared to other systemic inflammatory response markers in ovarian cancer patients. Tumour Biol. 2015;36(11):8831-8837. doi: 10.1007/s13277-015-3533-9.
14. Huang K, Xu S, Wang J, Ge L, Xu J, Jia X. Combined use of CA125, neutrophil/lymphocyte ratio and platelet/lymphocyte ratio for the diagnosis of borderline and malignant epithelial ovarian tumors. J Ovarian Res. 2023;16(1):37. doi: 10.1186/s13048-023-01106-4.
15. Wang Y, Liu P, Xu Y, et al. Preoperative neutrophil-to-lymphocyte ratio predicts response to first-line platinum-based chemotherapy and prognosis in serous ovarian cancer. Cancer Chemother

Pharmacol. 2015;75(2):255-262. doi: 10.1007/s00280-014-2622-6.
16. Polat M, Senol T, Ozkaya E, et al. Neutrophil to lymphocyte and platelet to lymphocyte ratios increase in ovarian tumors in the presence of frank stromal invasion. Clin Transl Oncol. 2016;18(5):457-463. doi: 10.1007/s12094-015-1387-7.
17. Yildirim MA, Seckin KD, Togrul C, et al. Roles of neutrophil/lymphocyte and platelet/lymphocyte ratios in the early diagnosis of malignant ovarian masses. Asian Pac J Cancer Prev. 2014;15(16):6881-6885. doi: 10.7314/apjcp.2014.15.16.6881.
18. Bakacak M, Serin S, Ercan Ö, et al. Utility of preoperative neutrophil-to-lymphocyte and platelet-to-lymphocyte ratios to distinguish malignant from benign ovarian masses. J Turk Ger Gynecol Assoc. 2016;17(1):21-25. doi: 10.5152/jtgga.2015.0152.
19. Zhao Z, Zhao X, Lu J, Xue J, Liu P, Mao H. Prognostic roles of neutrophil to lymphocyte ratio and platelet to lymphocyte ratio in ovarian cancer: a meta-analysis of retrospective studies. Arch Gynecol Obstet. 2018;297(4):849-857. doi: 10.1007/s00404-018-4678-8.

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Tuberculous peritonitis: an analysis of case series of 49 consecutive patients

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ABSTRACT

Objectives: The incidence of tuberculous peritonitis has been reported between 0.1% and 0.7% among all tuberculosis types. This study, it was aimed to evaluate the cases with tuberculous peritonitis, which has an important place in the differential diagnosis of patients with ascites, clinically, biochemically, microbiologically, and histopathologically.

Methods: Forty-nine patients with a definite clinical, radiological, and histopathological diagnosis of tuberculous peritonitis, which formed the basis of our study, were analyzed retrospectively.

Results: The mean age of patients with tuberculous peritonitis was found to be 39.45±19.02 years. Purified-protein derivative (PPD) was positive in 23 (72%) of 32 patients with tuberculous peritonitis whose PPD results were recorded, in 9 (28%) PPD results were evaluated as anergic or negative. QuantiFERON-TB Gold In-Tube (QFT-GIT) test was sent in 10 of the patients, the result was positive in 9 (90%) patients and negative in 1 (10%) patient. There were 32 patients in whom tuberculosis polymerase chain reaction (PCR) was studied from ascitic fluid, 7 (22%) of the patients were PCR positive and 25 (78%) negative. Mycobacterium tuberculosis culture positivity was found in 18 (69%) of 26 patients who were biopsied. In total, 29 (59%) of the patients had M. tuberculosis culture positivity.

Conclusions: Tuberculous peritonitis constitutes a public health problem in endemic regions of the world and tuberculous peritonitis should be considered in patients presenting with ascites. Despite all diagnostic difficulties, necessary tests, especially peritoneal biopsy, should be performed for early diagnosis, and it should not be forgotten that early initiation of treatment is very important in terms of morbidity and mortality of the disease.

Keywords: Tuberculous peritonitis, diagnostic difficulties, ascites

The differential diagnosis of ascites accumulating between peritoneal leaves remains a problem in many cases, despite the advanced testing possibilities we have. Tuberculous peritonitis (TBP) is an extrapulmonary manifestation of tuberculosis (TB) that involves Mycobacterium seeding of the peritoneum. It is estimated that the prevalence of TBP has decreased and now comprises 4%-10% of all extrapulmonary cases of TB worldwide. The disease occurs equally in both sexes, with most cases between the ages of 21 and 45. Poor hygienic conditions, overpopulation, and consumption of unpasteurized milk pose a risk for the development of TBP. TBP often occurs with the reactivation of dormant TB foci in the

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peritoneum caused by hematogenous spread from the primary lung focus. TBP can also occur by the hematogenous spread in the presence of active pulmonary TB or miliary TB. More rarely, TB mycobacteria enter the peritoneal cavity through contiguous TB or TB salpingitis. The diagnosis of TBP is made by clinical, immunological, microbiological, and, most importantly, histopathological methods. While the frequency of TB is gradually decreasing, there has been an increase in the number of individuals infected with TB as a result of the immunosuppressive Human Immunodeficiency Virus (HIV) infection, the increase in malignancies, and the effect of socioeconomic conditions [1-4].

This study, it was aimed to evaluate the cases with TBP, which has an important place in the differential diagnosis of patients with ascites, clinically, biochemically, microbiologically, and histopathologically. In addition, in our study, we aimed to show that serum CA-125 level may be a predictive factor in the differential diagnosis of ovarian carcinoma and TBP.

METHODS

The patients were grouped according to their clinical, serological, radiological, and histopathological analyzes and were grouped under four main headings according to their incidence (Table 1). While approximately half of the cases were decompensated liver cirrhosis, most of the remaining cases were peritonitis carcinomatosis. 49 patients with a definite clinical, microbiological, and histopathological diagnosis of TBP, which formed the basis of our study, were analyzed retrospectively, age, gender, comorbid diseases, peritoneal fluid analysis (Lactate dehydrogenase (LDH), albumin, glucose, Adenosine deaminase (ADA), Polymerase Chain Reaction (PCR)), histopathological diagnosis, Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), serum CA-125 level and serum albumin values of the patients were recorded, purified protein derivative (PPD), QuantiFERON-TB Gold In-Tube (QFT-GIT) test, posteroanterior (PA) chest radiographs, computed tomography (CT) reports were evaluated.

For the study, approval of the ethics committee for clinical research of Erzurum Ataturk University Faculty of Medicine Ethics Committee was obtained 2019/1-51.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS v20) program was used to analyze the data. Categorical variables were presented as numbers and percentages, and numerical variables were presented as mean and standard deviation. The suitability of the numerical variables to the normal distribution was checked using the Kolmogorov-Smirnov Test, z values calculated for skewness and kurtosis, and graphing methods. Mann Whitney U was used for comparisons of non-normally distributed numerical variables between two groups, Kruskal Wallis was used for comparisons between more than two groups, Mann Whitney U with Bonferroni correction was used for posthoc analyses, and χ^2, χ^2 Trend tests were used for comparison of categorical variables. The statistical significance level was accepted as $P<0.05$ in all analyses.

RESULTS

The mean age of the patients in our study was 55.6 ± 16.7 years. Of the 618 patients with ascites evaluated, 290 (46.9%) were male and 328 (53.1%) were female. Of the patients, 305 (49.4%) had liver cirrhosis, 126 (20.4%) malignancy, 49 (7.9%) TBP, 32 (5.2%) non-cirrhotic portal hypertension, 32 heart failure (5.2%), 28 (4.5%) mixed (existence of liver cirrhosis and other diagnoses that may cause ascites), 14 (2.3%) nephrotic syndrome, 13 (There were 2.1% pancreatic ascites, 11 (1.8%) undiagnosed, 8 (1.3%) patients with ascites due to other causes (Table 1).

The mean age of patients with TBP was found to be 39.45±19.02 years. Eight (16.3%) of TBP patients

Table 1. Etiological Distribution of the Patients

Diagnosis	n	%
Liver Cirrhosis	305	49.4
Peritonitis carcinomatosa	126	20.4
Tuberculosis Peritonitis	49	7.9
Other	138	22.3

Table 2. Age, gender, and comorbidity distribution of patients with tuberculous peritonitis

Age ranges	n	%
< 20	8	16,3
21-40	20	40,8
41-60	13	26,5
> 60	8	16,3
Gender		
Female	41	84
Male	8	16
Comorbid diseases		
Diabetes mellitus	7	14,3
ESRD*	5	10,2
Steroid use	4	8,2
Cirrhosis	4	8,2
Renal Tx **	1	2
Previous tuberculous	3	6,1
Coexistence of Lung TB	1	2

ESRD = End Stage Renal Failure, **Renal Tx = Renal Transplantation

were male and 41 (83.7%) were female. Patients were grouped according to age ranges.8 (16.3%) patients were <20 years old, 20 (40.8%) patients were 21-40 years old, 13 (26.5%) patients were 41-60 years old, and 8 (16.3%) patients were >60 years old. There were comorbid diseases in 22 (45%) patients with TBP and pulmonary TB in 1 (2%) (Table 2).

PPD was positive in 23 (72%) of 32 patients with TBP whose PPD results were recorded, in 9 (28%) PPD results were evaluated as anergic or negative. QFT-GIT test was sent in 10 of the patients, the result was positive in 9 (90%) patients and negative in 1 (10%) patient. There were 32 patients in whom TB PCR was studied from ascitic fluid, 7 (22%) of the patients were PCR positive and 25 (78%) negative. Mycobacterium tuberculosis culture positivity was found in 18 (69%) of 26 patients who were biopsied. In total, 29 (59%) of the patients had M. tuberculosis culture positivity.

There were 15 patients diagnosed with TBP whose ADA level was studied in ascitic fluid, the ADA cutoff value was taken as 30 U/L, and the ADA level was found to be high in 15 (100%) of the patients. The mean ADA level of the patients was found to be 121.80±42.72 U/L. CRP levels were high in 47 (95.6%) patients with TBP, and CRP values were normal in 2 (4.1%) patients. ESR results from patients with TBP were studied in all patients. ESR elevation was present in 40 (81.6%) patients. ESR was normal in 9 (18.4%). The distribution of microbiological and biochemical data of patients with TBP is shown in Table 3.

The patients were diagnosed with TBP microbiologically, clinically, or histopathologically. While the microbiological diagnosis was made in 11(22,5%) of 49 patients with TB peritonitis, 12 (24,5%) were diagnosed clinically and 26 (53%) histopathologically. In 11 (%22) patients diagnosed microbiologically, M. tuberculosis was isolated in the acidic fluid. Eight (16.3%) of the patients who were diagnosed histopathologically had paracentesis findings supporting TBP and a high ADA level. With high ascites ADA level, 1 (2%) patient had a QTF-GIT test, 3 (6.1%) patients had PCR, and 7 (14.3%) had PPD positivity. Ascites ADA level was not studied in 18 (37%) patients who were diagnosed histopathologically. In these patients, PCR was positive in 2 (4.1%) patients, the QTF-GIT test was positive in 3 (6.1%) patients, and PPD was positive in 10 (20.4%) patients, together with high clinical suspicion and paracentesis findings supporting TBP and 2 (4.1%) patients had a history of previous TB. While paracentesis findings were supporting TBP in 4 (8.2%) patients, a minimally invasive peritoneal biopsy was performed on high clinical suspicion. Since most of our patients with TBP are women, we compared serum CA-125 levels, which may be useful in the differential diagnosis of ovarian malignancies, which is a common cause of ascites in women. Patients with TBP and patients with ovarian carcinoma were divided into 2 groups. There were 49 patients with TBP and 50 patients with ovarian carcinoma. The mean age of patients with TBP was found to be 39.45±19.02 years. The mean age of patients with ovarian carcinoma was 56.68±12.40 years. The mean serum CA-125 level of patients with TBP was found to be 437.78±383.66 U/mL. The mean CA-125 level of patients with ovarian carcinoma was found to be 1814.06±1767.52. When the mean CA-125 levels of the patients were compared, a significant difference was found between the groups (P<0.001). The mean

Table 3. Distribution of microbiological and biochemical data of patients with tuberculous peritonitis

Test type	Tested patients (n)	Positive patients n (%)
Pürified protein derivative	32	23 (72)
Quantiferon	10	9 (90)
Polymerase chain reaction	32	7 (22)
Adenosine deaminas	15	15 (100)
C-reactivated protein	49	47 (95.6)
Erythrocyte sedimentation rate	49	40 (81.6)

CA-125 level of patients with ovarian carcinoma was higher than patients with TBP.

Quadruple anti-TB was given to patients diagnosed with tuberculous peritonitis, and two months later, dual anti-TB treatment was continued and completed for a total of 6 months (The first two months are isoniazid (INH), rifampicin, pyrazinamide, and ethambutol, and after the second month INH and rifampicin for 4 months). It was determined that all patients tolerated the treatment well and no significant adverse events were observed except for mild transaminase elevation observed in 2 (4%) patients. As a result of the clinical and radiological evaluations made after the treatment, ascites disappeared except for 2 (4%) patients, and it was seen that these 2 (4%) patients had Multi-Drug Resistant (MDR-TB).

DISCUSSION

Diseases that can cause ascites to take place in a very wide range. In these diseases whose treatments are completely different, morbidity and mortality of the disease can be reduced by early diagnosis. Although ascites can be seen in many diseases, the most common cause is liver cirrhosis. TBP, which is a rare cause of ascites, is seen at a rate of 10%, especially in underdeveloped countries. Ascites related to TBP were detected in our study at a rate of 7,9%, which is important because it shows that TBP is a more important etiological cause in our country than in Western countries [2-4].

Increasing population migration, the use of more potent immunosuppressant therapies, an increase in the number of immunocompromised HIV-infected individuals, and an increase in the incidence of malignancies have contributed to the reemergence of TB in areas where it was previously largely controlled. TBP often complicates patients with underlying end-stage renal or hepatic disease. However, diagnosing TB disease remains challenging due to the insidious nature of the disease, the many different clinical manifestations, and the limitations of current diagnostic tests. When unexplained ascites are encountered, especially in high-risk patients, the clinician should suspect TBP. TBP should be considered in the differential diagnosis of all patients presenting with unexplained lymphocytic acid and a serum-ascites albumin gradient (SAAG) <11 g/L [4, 5]. Bacteriological performance is imperfect since the culture of ascites was positive in only 58,1% and peritoneal biopsy in 73.3% of cases. Biopsy for bacterial culture and histology is essential for diagnosis [6].

With TB culture being the diagnostic gold standard for TB, ascites fluid ARB and culture positivity rates may be low due to the low density of bacilli, which is one of the difficulties encountered in diagnosis [7, 8]. A positive ascitic fluid ADA can be highly suggestive. Numerous studies have been performed looking at the optimal cutoff for sensitivity and specificity, with most studies suggesting an ADA level of > 30 IU/L as yielding sensitivities of close to 100% and specificity generally greater than 95% for TBP [4]. In our study, PCR positivity, ADA level, and QFT-GIT test positivity were determined by the literature, and these tests can be used when necessary [9].

Currently, TBP is usually diagnosed histopathologically, as it can show malignancy-like features and

bacteriological confirmation is both difficult and time-consuming. While awaiting bacteriological confirmation, the histopathological examination may speed up the diagnostic process and facilitate the early initiation of treatment. The consensus in many studies is that peritoneal biopsy is the best procedure for the diagnosis of TBP [4, 10]. In the case of suspected TBP, the laparoscopic peritoneal biopsy is the preferred diagnostic tool and should be performed without delay [11]. With the help of imaging methods, an increasing number of peritoneal biopsies have been taken recently. This method provides a safer and less expensive alternative to diagnostic laparoscopy. Recently, it has been reported that 95% of the cases are diagnosed with a peritoneal biopsy taken in this way [12].

The incidence of TBP has increased in the presence of cirrhosis, chronic renal failure patients undergoing continuous peritoneal dialysis (CAPD), and immunosuppressive patients such as the presence of HIV infection and steroid use. Almost half of the patients in our study also have comorbid diseases [13].

One of the differential diagnoses in female patients presenting with ascites may be peritoneal infiltration of ovarian cancer. Therefore, the distinction between ovarian cancer and TBP is important. As is seen in our study, serum CA-125 levels are found to be higher in epithelial ovarian malignancies than in TBP. CA-125 levels are not expected as high as in ovarian carcinoma in patients with TBP [14, 15].

Limitations

Since our study is retrospective, it can be said that the shortcomings of our study are that some TBP cases were not recorded and that some diagnostic parameters were recorded in the cases we detected.

CONCLUSION

In conclusion, TBP constitutes a public health problem in endemic regions of the world and TBP should be considered in patients presenting with ascites. Despite all diagnostic difficulties, necessary tests, especially peritoneal biopsy, should be performed for early diagnosis, and it should not be forgotten that early initiation of treatment is very important in terms of morbidity and mortality of the disease.

Authors' Contribution

Study Conception: ES, OY, BA; Study Design: ES, OY, BA, EBY; Supervision: ES, OY, BA, EBY; Funding: ES, OY, BA, EBY; Materials: ES, BA; Data Collection and/or Processing: ES, OY; Statistical Analysis and/or Data Interpretation: ES, EBY; Literature Review: OY, BA; Manuscript Preparation: ES, BA and Critical Review: BA, EBY.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. Demir K, Okten A, Kaymakoglu S, et al. Tuberculous peritonitis--Reports of 26 cases, detailing diagnostic and therapeutic problems. Eur J Gastroenterol Hepatol. 2001;13(5):581-585. doi: 10.1097/00042737-200105000-00019
2. Kushima H, Sakamoto R, Kinoshita Y, et al. Tuberculous peritonitis. BMJ Case Rep. 2021;14(10):e245311. doi: 10.1136/bcr-2021-245311
3. Al-Knawy BA. Etiology of ascites and the diagnostic value of serum-ascites albumin gradient in non-alcohol liver disease. Ann Saudi Med 1997;17(1):26-28. doi: 10.5144/0256-4947.1997.26
4. Sanai FM, Bzeizi KI. Systematic review: tuberculous peritonitis–presenting features, diagnostic strategies, and treatment. Aliment Pharmacol Ther. 2005;22(8):685-700. doi: 10.1111/j.1365-2036.2005.02645.x
5. Pullukçu H, Taşbakan MS, Sipahi OR, et al. Pooled analysis of extra-pulmonary tuberculosis in Turkey: a review of 2,168 patients. In: 18th European Congress of Clinical Microbiology and Infectious Diseases; Barcelona, Spain; 2008. p.1563.
6, Chow KM, Chow VC-Y, Szeto CC. Indication for peritoneal biopsy in tuberculous peritonitis. Am J Surg. 2003;185(6):567-573. doi: 10.1016/s0002-9610(03)00079-5
7. Guirat A, Koubaa M, Mzali R, et al. Peritoneal tuberculosis. Clin Res Hepatol Gastroenterol. 2011;35(1):60-69. doi: 10.1016/j.gcb.2010.07.023
8. Chau TN, Leung VK, Wong S, et al. Diagnostic challenges of tuberculosis peritonitis in patients with and without end-stage renal failure. Clin Infect Dis. 2007;45(12):e141-146. doi: 10.1086/523727
9. Fan L, Chen Z, Hao XH, et al. Interferon-gamma release assays for the diagnosis of extrapulmonary tuberculosis: a systematic review and meta-analysis. FEMS Immunol Med Microbiol. 2012;65(3):456-466. doi: 10.1111/j.1574-695X.2012.00972.x
10. Azghay M, Bouchaud O, Mechaï F, et al. Utility of QuantiF-

ERON-TB Gold In-Tube assay in adult, pulmonary and extrapulmonary, active tuberculosis diagnosis. Int J Infect Dis. 2016;44:25-30. doi: 10.1016/j.ijid.2016.01.004
11. Koning M, van der Pol LM, Schippers EF. [Peritoneal tuberculosis]. Ned Tijdschr Geneeskd 2017;161:D1372. [Article in Dutch]
12. Vardareli E, Kebapçi M, Saricam T, et al. Tuberculosis peritonitis of the wet acidic type: clinical features, and diagnostic value of image-guided peritoneal biopsy. Dig Liv Dis. 2004; 36(3):199-204. doi: 10.1016/j.dld.2003.10.016
13. Khatri GR, Frieden TR. Controlling tuberculosis in India. N Engl J Med. 2002;347(18):1420-1425. doi: 10.1056/NEJMsa020098
14. Woolas RP, Xu FJ, Jacobs IJ, et al. Elevation of multiple serum markers in patients with stage I ovarian cancer. J Natl Cancer Inst. 1993;85(21):1748-1751. doi: 10.1093/jnci/85.21.1748
15. Kosseifi S, Hoskere G, Roy TM, et al. Peritoneal tuberculosis: modern peril for an ancient disease. South Med J. 2009;102(1):57-59. doi: 10.1097/SMJ.0b013e39007c8

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Acute kidney injury in neonatal intensive care unit and the significance of nRIFLE criteria on diagnosis and prognosis

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ABSTRACT

Objectives: The objective of this study is to identify factors that affect the severity of acute kidney injury (AKI) using neonatal RIFLE (Risk, Injury, Failure, Loss of function, End-stage kidney disease) criteria; to identify the impact of these criteria and the factors on mortality rates and to determine the one-year clinical outcome.

Methods: Five hundred and thirty-two inpatients who were admitted to Gazi University, Faculty of Medicine, Neonatal Intensive Care Unit (NICU) between 2006 and 2016 have been examined retrospectively.

Results: Acute kidney injury developed in the first month of life in 85 (16%) patients. Thirty-nine (7.35%) of the cases were term and 46 (8.65%) were preterm. Among these patients, 33 (38.8%) were in the risk group, 18 (21%) in the injury group, and 34 (40%) in the failure group. Metabolic acidosis and edema were the most commonly seen findings as acute kidney injury scores increased. According to the neonatal RIFLE (nRIFLE) criteria, the severity of AKI was significantly correlated ($P<0.05$) with metabolic acidosis (71%) and edema (50.5%). There was a positive correlation between urinary output and pH, bicarbonate, glomerular filtration rate, and sodium values in patients with AKI, while a negative correlation between urinary output and BUN, creatinine, potassium, phosphorus, and uric acid was found. Regarding the nRIFLE criteria, the frequency of hyponatremia and hyperpotassemia was increased as the AKI severity score was increasing ($P<0.05$). The mortality rate was 54% in the newborn period and factors that significantly affect mortality were the need for mechanical ventilation, sepsis, nephrotoxicity, and acidosis ($P<0.05$).

Conclusions: The nRIFLE criteria based on urinary output is a guide for clinicians to diagnose AKI. There is a need to work on new markers in future studies.

Keywords: Acute kidney injury, risk factors, nRIFLE, newborn

Acute kidney injury (AKI) is a sudden renal dysfunction resulting from changes in extracellular fluid volume, fluid electrolyte and acid-base balance, and insufficiency in nitrogen excretion. It is a complex disease with many causes, pathophysiological pathways, and clinical importance [1].

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It peaks in neonatal intensive care unit (NICU) patients and especially affects premature. The incidence of renal failure in newborns ranges from 6% to 24% in the NICU. Clinical manifestations of acute kidney injury range from minimal renal failure to requiring renal replacement therapy [2]. Exposure to perinatal events such as nephrotoxic drugs, sepsis, and asphyxia in the neonatal period carries a great risk for the development of acute kidney injury. Current studies indicate that there is a relationship between morbidity and mortality and acute kidney injury in these patients. Close monitoring of patients at risk and early recognition of changes in kidney function are the keys to improving the process [3].

Due to the use of diverse definitions for AKI and the lack of a standard definition in clinical trials in newborns, the incidence and mortality rates of AKI vary widely. The serum creatinine value is a restricted laboratory parameter in the definition of AKI. Therefore, neonatal RIFLE (Risk, Injury, Failure, Loss of function, End-stage kidney disease) criteria were developed based on urine output (UO). It has been notified that a better classification will be made at this point and diuresis can be used as an alternative method for defining high-risk NICU patients [4, 5].

Our study aimed to determine the risk factors affecting the severity of AKI and to establish the effect of these risk factors on newborn mortality rates by using the neonatal RIFLE (nRIFLE) criteria in newborns. Another aim of the study was to monitor the renal functions of newborns who lived at least one year during follow-up.

METHODS

The outcomes of 532 patients who were admitted to NICU between 2006 and 2016 have been examined retrospectively and 85 newborns diagnosed with AKI were enrolled into the study. The diagnosis of neonatal AKI was based on decreased urine output, taking into account gestational and postnatal age. Urine output was systematically measured by diaper weight or neonates were catheterized. The Ethical Committee for Medical Research of Gazi University approved this study under protocol number 19026.

Classification of AKI severity was done according to the nRIFLE criteria used by Bezerra and his colleagues in 2013. “Risk” was defined as UO<1.5 ml/kg/h in 24 hours, ‘’injury’’ was defined as UO<1 ml/kg/h in 24 hours, and ‘’failure’’ was defined as UO <0.7 ml/kg/h in 24 hours or anuria for 12 hours. “Loss” defined as renal failure>4 weeks and “end-stage renal disease” defined as renal failure>3 months were not used in our study [4, 5].

Patients included in this study were assessed in terms of risk factors that affect AKI severity and mortality.

Blood Urea Nitrogen (BUN), serum creatinine, uric acid, sodium (Na), potassium (K), phosphorus (P), urinalysis, blood gas and blood culture tests were recorded at the time of admission. Electrolyte changes and the presence of acidosis were noted. pH<7.35 with serum bicarbonate<21 mmol/L in blood gas was evaluated as acidosis.

Normal ranges for blood Na, K and Ca levels were accepted as 135-145 mEq/L; 3.5-5.5 mEq/L and 8.5-10.5 mg/dL, respectively.

Microorganisms were grown in the blood and urine cultures of septic patients, antibiotics were administered and anomalies in the abdominal ultrasonography of the patients were recorded. Vancomycin, amikacin, amphotericin B, meropenem, and gentamicin were agreed as nephrotoxic antibiotics (Vancomycin, amikacin, amphotericin B, meropenem and gentamicin were considered nephrotoxic antibiotics.).

Oliguria, hyperuricemia, hyperpotassemia, hypervolemia, and metabolic acidosis were defined as indications for peritoneal dialysis treatment.

The patients older than 28 postnatal days when AKI was diagnosed, patients with <24 hours NICU stay and the 22 newborns without adequately obtainable data were excluded from the study.

Statistical Analysis

IBM Statistical Package for the Social Sciences (SPSS) 21.0 was used for statistical analysis. To examine the impact of the independent variables in the sample group, nonparametric analysis methods were used for the groups composed of less than 30 people whereas parametric analysis methods were used for the group of more than 30 people.

Descriptive analysis methods were used for the sociodemographic data of the sample group. Chi-Square, Kruskal-Wallis analysis, and Mann Whitney U were used to determine whether there is any difference in

the independent variables among the three groups that constitute the sample group. The Spearman correlation method was used to examine the relationship between the independent variables.

RESULTS

Of the 85 newborns evaluated according to the nRIFLE criteria, 33 were in the risk group, 18 in the injury group, and 34 in the failure group, respectively.

Demographic information of the patients was examined. According to the severity of AKI, it was identified that there was no significant difference among groups in terms of gender, gestational age, birth weight, and period of hospitalization. The mean age at diagnosis of AKI was 5.96±6.14 days. The mean and standard derivation (SD) of birth weight of newborns diagnosed as AKI were 2162±1069 g and gestational ages were 33.95±5.52 weeks.

The incidence of metabolic acidosis was 71% and the frequency of edema was 50.5% in newborns diagnosed with AKI. The incidence of acidosis and edema was higher in the failure group (P<0.05) (Table 1).

The relationship between the duration of mechanical ventilation and urine output was assessed and no

Table 1. Comparison of risk factors in the groups

Risk Factors	Risk UO<1.5 mL/ kg/h for 24 h		Injury UO<1.0 mL/ kg/h for 24 h		Failure UO<0.7 mL/ kg/h for 24 h or anuric for 12 h		Total		P value
	n	%	n	%	n	%	n	%	
Acidosis	19	31.7	13	21.7	28	46.7	60	100	**0.049**
Edema	4	9.3	10	23.3	29	67.4	43	100	**<0.001**
Mechanic ventilation	24	34.8	15	21.7	30	43.5	69	100	0.259
Nephrotoxic agent use	29	37.2	16	20.5	33	42.3	78	100	0.347
Surgical intervention	10	27	9	24.3	18	48.6	37	100	0.144
Septicemia	29	37.2	15	19.2	34	43.6	78	100	0.067
Respiratory system problems	19	35.8	13	24.5	21	39.6	53	100	0.691
Congenital heart disease	28	41.8	14	20.9	25	37.3	67	100	0.522
Asphyxia/ hypoxia	7	33.3	3	14.3	11	52.4	21	100	0.385
Congenital urinary anomalies	10	40	2	8	13	52	25	100	0.123
Gastrointestinal tract problems	14	43.8	7	21.9	11	34.4	32	100	0.691
Inotrope requirement	19	31.7	15	25	26	43.3	60	100	0.097
Urinary tract infection	2	28.6	0	0	5	71.4	7	100	0.157
Phototherapy	17	48.6	6	17.1	12	34.3	35	100	0.301
Intracranial hemorrhage	11	57.9	3	15.8	5	26.3	19	100	0.152
Mother's health problems	18	38.3	9	19.1	20	42.6	47	100	0.826
Mother's medicine use	12	41.4	5	17.2	12	41.4	29	100	0.880

UO = urine output

Table 2. Laboratory Findings Based on the Classification of Acute Kidney Injury Severity

Laboratory Findings	Risk UO<1.5 mL/kg/h for 24 h			Injury UO<1.0 mL/kg/h for 24 h			Failure UO<0.7 mL/kg/h for 24 h or anuric for 12 h			Total			P value
	n	mean	SD	n	mean	SD	n	mean	SD	n	mean	SD	
pH	32	7.32	0.1	18	7.29	0.14	33	7.24	0.13	83	7.28	0.13	**0.033**
HCO3 (mmol/L)	32	19.7	3.8	18	18.74	4.68	33	17.5	5.13	83	18.62	4.61	0.085
PCO2 (mm Hg)	32	38.78	10.54	18	38.88	10.71	33	41.51	12.37	83	39.89	11.28	0.602
GFR (mL/min)	7	15.35	6.70	7	15.13	8.31	22	9.72	4.63	36	11.86	6.32	**0.047**
BUN (mg/dL)	33	21.02	13.82	18	33.56	27.78	34	32.72	22.47	85	28.36	21.49	**0.037**
Creatinine (mg/dL)	33	0.89	0.47	18	1.13	0.75	34	2.01	1.28	85	1.39	1.05	**<0.001**
Uric acid (mg/dL)	33	4.92	3.65	18	6.68	4.21	34	8.65	3.98	85	6.79	4.2	**<0.001**
Sodium (mmol/L)	33	140.45	6.66	18	135.78	7.94	34	134.18	8.17	85	136.95	8	**0.003**
Potassium (mEq/L)	33	4.28	0.85	18	5.13	1.67	34	5.27	1.51	85	4.86	1.4	**0.006**
Calcium (mg/dl)	33	8.89	0.82	18	8.36	1.36	34	8.34	1.14	85	8.56	1.1	0.063
Phosphorus (mg/dL)	33	4.63	1.69	16	5.48	1.89	34	5.63	1.94	85	5.2	1.87	**0.038**
Albumin (g/dL)	32	2.74	.0.54	18	2.71	0.76	31	2.66	0.65	81	2.7	0.63	0.794

BUN = blood urea nitrogen, GFR = glomerular filtration rate, SD = standard deviation, UO = urine output

significant relationship was found (r=0.196, P=0.08). There was no notable difference between groups according to nRIFLE criteria in terms of mechanical ventilation time (P=0.164). The failure group had significantly lower pH, glomerular filtration rate (GFR), and serum sodium values than the risk group. Serum BUN, creatinine, uric acid, potassium, and phosphorus values were significantly higher in the failure group (Table 2). The relationship between laboratory results and the urine output of the study group was shown in Table 3. A significant positive correlation between urinary output and pH (r=0.308, P<0.01), HCO3 (r=0.227, p<0.05), GFR (r=0.372, P<0.05), serum sodium (r=0.357, P<0.01) when the table was observed. A significant negative correlation between urinary output and BUN (r=-0.253, P<0.05), creatinine (r=-0.493, P<0.01), uric acid (r=-0.44, P<0.01), serum potassium (r=-0.33, P<0.01), serum phosphorus (r=-0.248, P<0.05). No important relationship was found between urinary output and the other values (P>0.05) (Table 3).

A significant difference in the albumin values of the edematous and non-edematous patients was found. Eventually, it was observed that there was a difference in albumin values between groups (P<0.05). Patients with edema (mean albumin = 2.56±0.63 g) had significantly lower albumin values than those without edema (mean albumin = 2.85±0.60 g).

According to AKI severity, the distribution of hyponatremia, hypernatremia, and hyperpotassemia was found statistically significant (P<0.05). The majority of patients with hyponatremia and hyperpotassemia were found in the failure group.

Forty-six infants with AKI died in the neonatal period. According to the nRIFLE, no relationship was found between the AKI severity and the increase in

Table 3. Correlation between laboratory values and urine outflow of the all infants

Laboratory Values	Urine Outflow	
	R	P value
pH	0.308	**0.006**
HCO3 (mmol/L)	0.227	**0.044**
PCO2 (mm Hg)	-0.116	0.310
GFR (mL/min)	0.372	**0.036**
BUN (mg/dL)	-0.253	**0.023**
Creatinin (mg/dL)	-0.493	**0.000**
Uric acid (mg/dL)	-0.440	**0.000**
Sodium (mmol/L)	0.357	**0.001**
Potassium (mmol/L)	-0.330	**0.003**
Calcium (mg/dL)	0.180	0.107
Phosphorus (mg/dL)	-0.248	**0.027**
Albumin (g/dL)	0.081	0.483

BUN = blood urea nitrogen, GFR = glomerular filtration rate

mortality rate (P=0.437).

A statistically significant relationship between mechanical ventilation (P<0.001), acidosis (P<0.01), sepsis (P<0.05), and nephrotoxicity (P<0.05) with mortality rate was found (Table 4). The phi values of variables with a significant difference were calculated. Mechanical ventilation (Ø=0.46) had a greater effect on mortality rate than metabolic acidosis (Ø=0.32), sepsis (Ø=0.32), or nephrotoxicity (Ø=0.24).

Most of the patients were followed by local healthcare centers, so the regular follow-up of all the patients in our study in terms of kidney function could not be done. Only 15 of the 36 patients who survived were examined regularly in the pediatric nephrology department. Renal function loss was detected by imaging methods in five patients. An increase in renal parenchymal echogenicity was observed by ultrasonography in three patients. Three patients were followed up with a diagnosis of hydronephrosis. Antihypertensive treatment was started in seven patients during the follow-up. Renal artery stenosis was diagnosed by Doppler ultrasonography in two of seven patients. The mean follow-up period was 20.13 ± 18.68 months and no patient developed chronic kidney failure in this period. However, it is known that our patients have been at risk for late sequelae, especially the patients with renal scintigraphic changes and renal dysfunction should be followed closely.

DISCUSSION

Acute kidney injury is a complicated clinical condition with a sudden decrease in kidney function and a serious problem in terms of morbidity and mortality in newborns. Serum creatinine level and urine output are frequently used in the diagnosis of AKI in newborns. However, there is no consensus on these criteria in the literature and there are different AKI definitions. In 2013, Bezerra and colleagues created the nRIFLE criteria reason that newborns have their physiopathology. In line with the nRIFLE criteria, if oliguria is accepted below 1.5 mL/kg / h, it is stated that risk factors and complications for AKI might increase. It was reported that a better classification would be made on this scale and diuresis could be used as an appropriate alterna-

Table 4. Factors Affecting Mortality Rate in Neonates with Acute Kidney Injury

Parameters	Patients Decreased		Patients Living		Total		P
	N	%	N	%	N	%	
Mechanical ventilation	45	65.2	24	34.8	69	100	**0.000**
Metabolic acidosis	39	65	21	35	60	100	**0.004**
Septicemia	45	57.7	33	42.3	78	100	**0.044**
Dialysis	14	66.7	7	33.3	21	100	0.184
Asphyxia/Hypoxia	15	71.4	6	28.6	21	100	0.067
Surgical intervention	24	64.9	13	35.1	37	100	0.081
Nephrotoxicity	45	57.7	33	42.3	78	100	**0.044**

tive method for defining high-risk NICU patients [4, 5]. The most important reason for this is that the serum creatinine value used in the AKI definition is limiting. The serum creatinine level of the infants reflects the creatinine level of the mother in the early postnatal period. In addition, the decrease in serum creatinine level may last days or weeks depending on gestational age. Especially, premature infants may have a higher serum creatinine level than their mothers. Serum creatinine ultimately does not change until renal function decreases by 25-50% and begins to increase after 24-48 hours from the onset of injury [3, 6, 7].

Complexity in the evaluation of renal physiology and serum creatinine level makes AKI staging difficult. Due to the disadvantages of using serum creatinine levels, recent studies have focused on new markers [8]. nRIFLE criteria were used for the diagnosis of AKI in our study because our study was retrospective, the use of new markers in the diagnosis of AKI was not possible, and achieving serum creatinine values was difficult.

In our study, there was no significant relationship between the distribution of demographic data of newborns with AKI and AKI severity scores. It is stated in the literature that acute renal injury in the neonatal period is usually associated with low gestational weeks and low birth weight in preterm infants [9]. But similar to our study, Cataldi *et al*. [10] found no correlation between low birth weight, low age of gestation, and acute renal injury. This is explained by the difficulty of making comparisons because of the differences in the population and the criteria used to calculate renal function.

Need for mechanical ventilation, the occurrence of sepsis, hypervolemia, metabolic acidosis, and asphyxia was identified as independent risk factors for the development of AKI in the literature [2, 9, 11, 12]. In our study, only metabolic acidosis and hypervolemia were found as risk factors among these factors which significantly increase AKI severity ($P<0.05$). Like our study, Askenazi *et al*. [13] had also shown that hypervolemia was a risk factor for AKI and increased the mortality rate.

Mathur *et al*. [14] stated that sepsis may cause renal failure through shock, hemorrhage, and heart failure. In our research, it was found that sepsis had an important effect on the increase in the severity of AKI, although it was not statistically significant ($P=0.067$). This finding may result from our patient population, the majority of them were severely ill.

Cataldi *et al*. [10] showed that 50% of neonates were exposed to at least one nephrotoxic medication. It was frequently mentioned that non-steroidal anti-inflammatory drugs (NSAIDs) are used for patent ductus arteriosus (PDA) occlusion, and diuretics for fluid electrolyte treatment [10]. There was no substantial relationship between AKI severity and nephrotoxic drug use in our research. This condition can be explained by the adjustment of nephrotoxic drug doses according to the kidney function to prevent any toxic effect on kidney function.

In the study of Bolat *et al*. [15], maternal illness and medication use in pregnancy were described as risk factors affecting AKI. Taking antibiotics during pregnancy is known to affect the renal function of the newborn and antenatal steroids cause low birth weight and worsen organogenesis. In our study, it was observed that the maternal illness rate of neonates with AKI was 55% and the rate of medication use in pregnancy was 34%. As opposed to this result, no significant relationship was found between maternal illness ($P=0.826$) and maternal drug use ($P=0.88$) with increasing AKI severity in our study. Our small sample size may affect this result.

In the literature, the mortality of patients with AKI has ranged from 25% to 78% [4, 5]. In this study, the mortality rate in the group of newborns with AKI was high (54%). Our high mortality rate can be explained by the fact that our clinic is a tertiary center and complicated patients have been referred to us frequently.

In our study, it was observed that metabolic acidosis, sepsis, mechanical ventilation, and nephrotoxicity were significantly associated with the mortality of newborns. We also found that the need for mechanical ventilation was more effective on mortality compared to having metabolic acidosis, sepsis, and nephrotoxicity. In line with our results, Koralkar *et al*. found that the need for mechanical ventilation in AKI increased the mortality rate nine times [16].

In the study of Mathur *et al*. [14], it was found that sepsis was associated with an increased risk of AKI, so most of the patients with sepsis were lost. The mortality rate in infants with sepsis in our study was 57.7%.

There have been some studies in the literature on the long-term prognosis of AKI in newborns. These

studies describe the possibility of long-term injury and emphasize that hypertension, chronic kidney disease (CKD), and renal concentration defects may develop. They also emphasized the importance of long-term follow-up in newborn babies.

In the study of Moghal *et al*. [17], it was emphasized that serum creatinine, blood pressure, urinalysis, and albumin/creatinine ratio should be evaluated, and possible abnormalities should be followed up in the long term. Abitbol *et al*. [18] studied 20 very low birth weight infants and found proteinuria, increased serum creatinine level, and a tendency to obesity as risk factors for AKI.

In our study, 15 patients were followed up for a mean of 20.13±18.68 months. It was found that the renal functions of 5 of 15 patients were decreased in the imaging methods and close follow-up should be suggested.

Limitations

First, it was retrospective, and we couldn't find all the information we needed. Second, it was a single-center study, so the size of the patient population was small. Third, the diagnostic criteria of AKI couldn't be compared with the others.

CONCLUSION

Neonatologists and pediatric nephrologists must work together to reduce the morbidity and mortality of AKI in neonates and to obtain better results in these patients. The rifle criteria can be a guideline for the diagnosis of AKI for clinicians, but they need to be enhanced. Moreover, novel biomarkers to predict AKI severity should be defined. Further studies to identify the long-term outcomes of AKI and the risk of CKD are needed.

Authors' Contribution

Study Conception: ÇC, NB, CT, YA, SABE; Study Design: ÇC, NB, CT, YA, SABE; Supervision: ÇC, NB, CT, YA, SABE; Funding: N/A; Materials: N/A; Data Collection and/or Processing: ÇC, NB, CT, YA, SABE; Statistical Analysis and/or Data Interpretation: ÇC, NB, CT, YA, SABE; Literature Review: ÇC, NB; Manuscript Preparation: ÇC, NB and Critical Review: ÇC, NB, CT, YA, SABE.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. Ottonello G, Dessì A, Neroni P, Trudu ME, Manus D, Fanos V. Acute kidney injury in neonatal age. J Pediatr Neonat Individual Med. 2014;3(2):e030246. doi: 10.7363/030246
2. Andreoli SP. Renal failure in the neonate. In: Oh W, Guignard JP, Baumgart S, eds. Nephrology and Fluid/Electrolyte Physiology. 1st ed. Saunders Elsevier Philadephia; 2008:pp.285-305.
3. Jetton JG, Askenazi DJ. Acute kidney injury in the neonate. Clin Perinatol. 2014;41(3):487-502. doi: 10.1016/j.clp.2014.05.001.
4. Bezerra CT, Vaz Cunha LC, Libório AB. Defining reduced urine output in neonatal ICU: importance for mortality and acute kidney injury classification. Nephrol Dial Transplant. 2013;28(4):901-909. doi: 10.1093/ndt/gfs604.
5. Ricci Z, Ronco C. Neonatal RIFLE. Nephrol Dial Transplant. 2013;28(9):2211-2214. doi: 10.1093/ndt/gft074.
6. Selewski DT, Charlton JR, Jetton JG, et al. Neonatal acute kidney injury. Pediatrics. 2015;136(2):e463-473. doi: 10.1542/peds.2014-3819.
7. Askenazi D, Smith LB, Furth S, Warady BA. Acute kidney injury and chronic kidney disease. In: Gleason CA, Devaskar SU eds. Avery's Disease of the Newborn. 9th ed. Saunders Elsevier Philadelphia; 2012:pp.1281-1300.
8. Askenazi DJ, Montesanti A, Hunley H, Koralkar R, Pawar P, Shuaib F, et al. Urine biomarkers predict acute kidney injury and mortality in very low birth weight infants. J Pediatr 2011;159:907-912.e1.
9. Stojanović V, Barišić N, Milanović B, Doronjski A. Acute kidney injury in preterm infants admitted to a neonatal intensive care unit. Pediatr Nephrol. 2014;29(11):2213-2220. doi: 10.1007/s00467-014-2837-0.
10. Cataldi L, Leone R, Moretti U, et al. Potential risk factors for the development of acute renal failure in preterm newborn infants: a case-control study. Arch Dis Child Fetal Neonatal Ed. 2005;90(6):F514-519. doi: 10.1136/adc.2004.060434.
11. Zappitelli M, Selewski DT, Askenazi DJ. Nephrotoxic medication exposure and acute kidney injury in neonates. Neoreviews 2012;13(7):e420-e427. doi: 10.1542/neo.13-7-e420
12. Ebru Özcan K. Yenidoğan yoğun bakım ünitesinde gözlenen olgularda akut böbrek hasarı ve pRIFLE kriterlerinin tanı ve prognozdaki önemi. TC. Başkent Üniversitesi Tıp Fakültesi, Çocuk Sağlığı ve Hastalıkları Anabilim Dalı, Uzmanlık Tezi, 2013.
13. Askenazi DJ, Koralkar R, Hundley HE, Montesanti A, Patil N, Ambalavanan N. Fluid overload and mortality are associated with acute kidney injury in sick near-term/term neonate. Pediatr

Nephrol. 2013;28(4):661-666. doi: 10.1007/s00467-012-2369-4.
14. Mathur NB, Agarwal HS, Maria A. Acute renal failure in neonatal sepsis. Indian J Pediatr. 2006;73(6):499-502. doi: 10.1007/BF02759894.
15. Bolat F, Comert S, Bolat G, et al. Acute kidney injury in a single neonatal intensive care unit in Turkey. World J Pediatr. 2013;9(4):323-329. doi: 10.1007/s12519-012-0371-3.
16. Koralkar R, Ambalavanan N, Levitan EB, McGwin G, Goldstein S, Askenazi D. Acute kidney injury reduces survival in very low birth weight infants. Pediatr Res. 2011;69(4):354-358. doi: 10.1203/PDR.0b013e31820b95ca.
17. Moghal NE, Embleton ND. Management of acute renal failure in the newborn. Semin Fetal Neonatal Med. 2006;11(3):207-213. doi: 10.1016/j.siny.2006.01.007.
18. Viswanathan S, Manyam B, Azhibekov T, Mhanna MJ. Risk factors associated with acute kidney injury in extremely low birth weight (ELBW) infants. Pediatr Nephrol. 2012;27(2):303-11. doi: 10.1007/s00467-011-1977-8.

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Fibromyalgia syndrome in mothers of children with cerebral palsy and its relationship with caregiver burden: a cross-sectional study

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ABSTRACT

Objectives: Fibromyalgia syndrome (FMS) is a stress-related disease. The birth of a child with cerebral palsy (CP) is an important stress factor for the mother. This study aims to investigate the incidence of FMS in mothers of children with CP by comparing it with the control group and determining the factors affecting the severity of FMS.

Methods: The study included 112 children with CP (age: 38-216 months), their caregiver mother (age: 23-50 years) (Group 1) and 52 non-disabled children (age: 40-180 months), their caregiver mothers (age: 27-50 years) (Group 2). Children were evaluated with the Gross Motor Function Classification System (GMFCS) and the functional independence scale for children (WeeFIM). The mothers were evaluated according to the 2010 ACR FMS diagnostic criteria. The FMS Impact Questionnaire (FIQ), the Hospital Anxiety and Depression Scale (HADS), and the Bakas Caregiving Outcomes Scale (BCOS) were applied.

Results: In Group 1, mothers had higher anxiety-depression scores and caregiving burden than Group 2 ($P<0.05$). FMS rate was %31.3 in Group 1 and %5.7 in Group 2. Mothers' anxiety-depression scores, widespread pain index, symptom severity score, pain, and caregiver burden were higher in Group 1 than in Group 2 ($P<0.05$). Factors affecting the severity of FMS are the number of siblings of children with CP, the number of siblings with CP, GMFCS, dependence level, anxiety-depression levels of mothers, and caregiver burden. The most influential factor is the caregiver burden.

Conclusions: Long-term heavy caregiver burden in mothers of children with CP may be effective in developing FMS.

Keywords: Caregiver burden, cerebral palsy, fibromyalgia, mothers, pain

Cerebral palsy (CP) is one of the most common causes of physical and developmental disability in childhood [1]. Abnormal muscle tone, loss of voluntary motor control, sensory and coordination disorders and muscle weakness are the most important characteristics of the disease. Cognitive-behavioral abnormalities, speech, vision problems, epilepsy, urinary system problems and nutritional problems may also accompany [2]. This situation brings along the dependency of children with CP on

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their caregivers at different rates in their daily living activities. Mothers bear the most responsibility for caring and often lack carer support [3].

The birth of a disabled child is an important stress factor for the family and mothers are affected the most. Mothers suffer from feelings of guilt about the child's condition. CP requires lifelong care and adversely affects parents' physical and psychological health [4]. Caring for a child with CP creates a financial burden on the family and causes limitations in social and cultural activities. Also, families are exposed to social stigma. Incidents of domestic violence are also common [3, 5]. Studies have shown that caregivers have high anxiety and depression and that caregiving impairs their quality of life [3, 6]. In addition, physical problems such as insomnia, intense physical activity-related musculoskeletal pain and hypertension have also been reported. The mother cannot find time to rest [3, 7].

Fibromyalgia is a stress-related disorder characterized by widespread chronic pain, profound fatigue, and sleep disturbances. It is counted among the central sensitivity syndromes [8, 9]. In the literature, the prevalence of fibromyalgia has been reported between 0.2% and 6.6% in the general population, and between 2.4% and 6.8% in women [10]. Emotional, physical, and environmental stressful events are frequently observed in patients with fibromyalgia [9]. Studies have shown that depression and anxiety are more common in fibromyalgia patients than healthy individuals, and that the presence of depression and anxiety increases pain severity and worsens quality of life [11]. In addition, studies have shown the relationship between posttraumatic stress disorder and fibromyalgia [9, 12]. In a study where the majority of the participants were women, 90% of the participants exhibited somatic symptom disorder. It is claimed that fibromyalgia patients may have neuropathic pain triggered by stress rather than a mental somatic symptom disorder. This is explained by the stress-response system dysfunction in fibromyalgia patients and the presence of abnormal connections between the stress response system and pain-transmitting nerves within the dorsal root ganglia [9].

In the light of this information, we thought that the emotional, physical and environmental chronic stress created by the burden of care for the child with CP may increase the incidence of fibromyalgia in mothers who often have to undertake the responsibility of care alone. However, according to our research, we could not find a study in this direction.

Objectives of our study: (1) To investigate the incidence of fibromyalgia in mothers of children with CP in comparison with the control group, (2) Comparison of mothers with and without fibromyalgia in terms of caregiving burden, depression, anxiety levels, and "children's functional status and independence levels," (3) To determine the factors affecting the severity of fibromyalgia in mothers of children with CP.

METHODS

The cross-sectional, observational clinical study was conducted in a training and research hospital's physical medicine and rehabilitation clinics (from May 2019 to March 2020). The study was planned under the Helsinki Declaration rules, and local ethics committee approval was received (2011-KAEK-25 2019 / 04-03). All participants were informed about the study and signed a written consent form.

Study Population

In this study, the study group consisted of mothers who cared for a child diagnosed with Cerebral Palsy aged 3 to 18 years (Group 1; n=140). The control group consisted of mothers of children aged 3 to 18 years who applied to the clinic with other diagnoses (Group 2; n=60).

Exclusion criteria: (1) not having sufficient cognitive function to evaluate the questionnaire questions, (2) having diseases such as infection, chronic systemic disease (cardiac, renal, endocrine diseases, etc.), rheumatological disease, neurological disease, psychotic disease, malignancy, etc., (3) pregnancy and breastfeeding.

In addition, children whose caregivers were not mothers were also excluded from the evaluation.

Intervention

The demographic data (age, body mass index [BMI kg/m^2] of children with CP, children in the control group, and their mothers were recorded.

Children

A physiatrist examined children with cerebral palsy, and the diagnosis was confirmed based on med-

ical records. CP type was determined (spastic diplegic, spastic quadriplegic, spastic hemiplegic, dyskinetic, ataxic) [13].

The Gross Motor Function Classification System (GMFCS) was used to evaluate the motor development of children with CP [14]. GMFCS assesses the gross motor development of children with CP at five levels: (1) They walk without difficulty. There is difficulty in more advanced gross motor skills. (2) They walk without assistive devices. There are restrictions on walking outside the home and in the community. (3) They walk with mobility aids. There are restrictions on walking outside the home and in the community. (4) Self-movement is limited. Children are carried on laps or use powered mobility devices outside the home and in the community. (5) Self-mobility is severely limited, even with the use of assistive technology.

The functional independence scale for children (WeeFIM) [15] was used to assess the functional status of children with CP. WeeFIM is examined under three subheadings: self-care, mobility, and cognitive function. A total of 18 questions are scored between 1 and 7. The lowest score is 18, and the highest score is 126.

Mothers

The mothers of the children were evaluated according to 2010 the American College of Rheumatology (ACR) FMS diagnostic criteria [16]. Mothers were questioned for pain at 19 specific body points. Sensitivity for FMS was assessed by applying a pressure of 4 kg/cm² to 19 specific body points. Thus, the patients' widespread pain index (WPI) was determined. Mothers were also questioned in terms of fatigue, waking unrefreshed, cognitive symptoms, and somatic symptoms according to 2010 ACR FMS diagnostic criteria, and symptom severity score (SSS) was determined. Mothers who defined generalized pain for more than three months were diagnosed with FMS according to the 2010 ACR FMS diagnostic criteria if the WPI was ≥7, SSS was ≥5, or WPI was 4-6, SSS was ≥9.

The pain intensity of the patients was evaluated using a visual analog scale (VAS) (On a 10 cm analog scale, 0 means no pain, ten means unbearable pain.), and functional status was evaluated using the FMS Impact Questionnaire (FIQ).

The FIQ is a valid and reliable method for evaluating the impact of FMS on daily life [17]. It measures ten characteristics: physical function, feeling unwell, absenteeism, difficulty at work, pain, fatigue, morning fatigue, stiffness, anxiety, and depression. The maximum score for the FIQ is 100, with higher scores indicating higher disease severity. A total FIQ score of 0-<39 indicates low impact, ≥9<59 indicates moderate impact, and≥59-100 indicates severe impact.

Mothers' anxiety and depression levels were evaluated using the Hospital Anxiety and Depression Scale (HAD). The scale consists of 14 questions, with each question being scored between 0-3. Seven questions (the odd-numbered items) measure anxiety (HAD-A), and the other seven (evenly numbered) measure depression (HAD-D). The score range for each anxiety and depression subscale is 0-21. 0-7 points indicate normal levels, 8-10 points indicate borderline abnormal levels, and 11-21 points indicate abnormal levels [18].

Mothers' caregiving burdens were evaluated with the Bakas Caregiving Outcomes Scale (BCOS) [19]. This scale was used to evaluate how the caregivers' lives changed with the start of the caregiving process. The scale shows both positive and negative effects. The scale consists of 15 questions scored between +3 (in the best direction) and -3 (in the worst direction). It is a Likert-type scale ranging from 1 to 7. A minimum of 15 and a maximum of 105 points can be obtained from the scale.

First, mothers of children with CP and mothers of children in the control group were compared in terms of evaluation parameters, and FMS rates of both groups were determined. The evaluation parameters of mothers of children with CP with and without FMS were compared in the next step. Factors affecting FMS severity were determined.

Statistical Analysis

IBM SPSS 23.0 statistical software was used to analyze the data. Descriptive statistical methods (frequency, percentage, median, and min-max) were used while evaluating the study's data. The data's compliance with a normal distribution was assessed using Shapiro-Wilk tests. Where the data showed normal distribution, a t-test was used. Otherwise, Wilcoxon Test and Mann-Whitney U test were used to make intergroup comparisons. A Chi-square test was used to compare categorical data. The relationships between variables were assessed using Spearman's rho corre-

lation test. Univariate and multivariate logistic regression analyses investigated the factors affecting FMS severity.

RESULTS

The study included one hundred twelve children with CP and their caregiver mothers (Group 1). The median age of the children was 106.5 months (range: 38-216 months), and the median age of the mothers was 35 years (range: 23-50 years). In the control group, 52 children and their mothers were included in the study (Group 2) (Fig. 1). The median age of the children was 108 months (range: 40-180 months), and the median age of the mothers was 34 years (range: 27-50 yeras).

In Table 1, the results of the evaluation parameters of the mothers of children with CP and the mothers in the control group are given: There was no statistically significant difference between the two groups in terms of demographic data. Mothers of children with CP had statistically significantly higher scores in anxiety and depression scores, WPI, SSS, and pain severity (VAS) than mothers in the control group (P<0.001). Mothers of children with CP had statistically significantly lower scores than mothers in the control group regarding caregiving burden (P<0.001). Mothers of children with CP received statistically significantly more care-

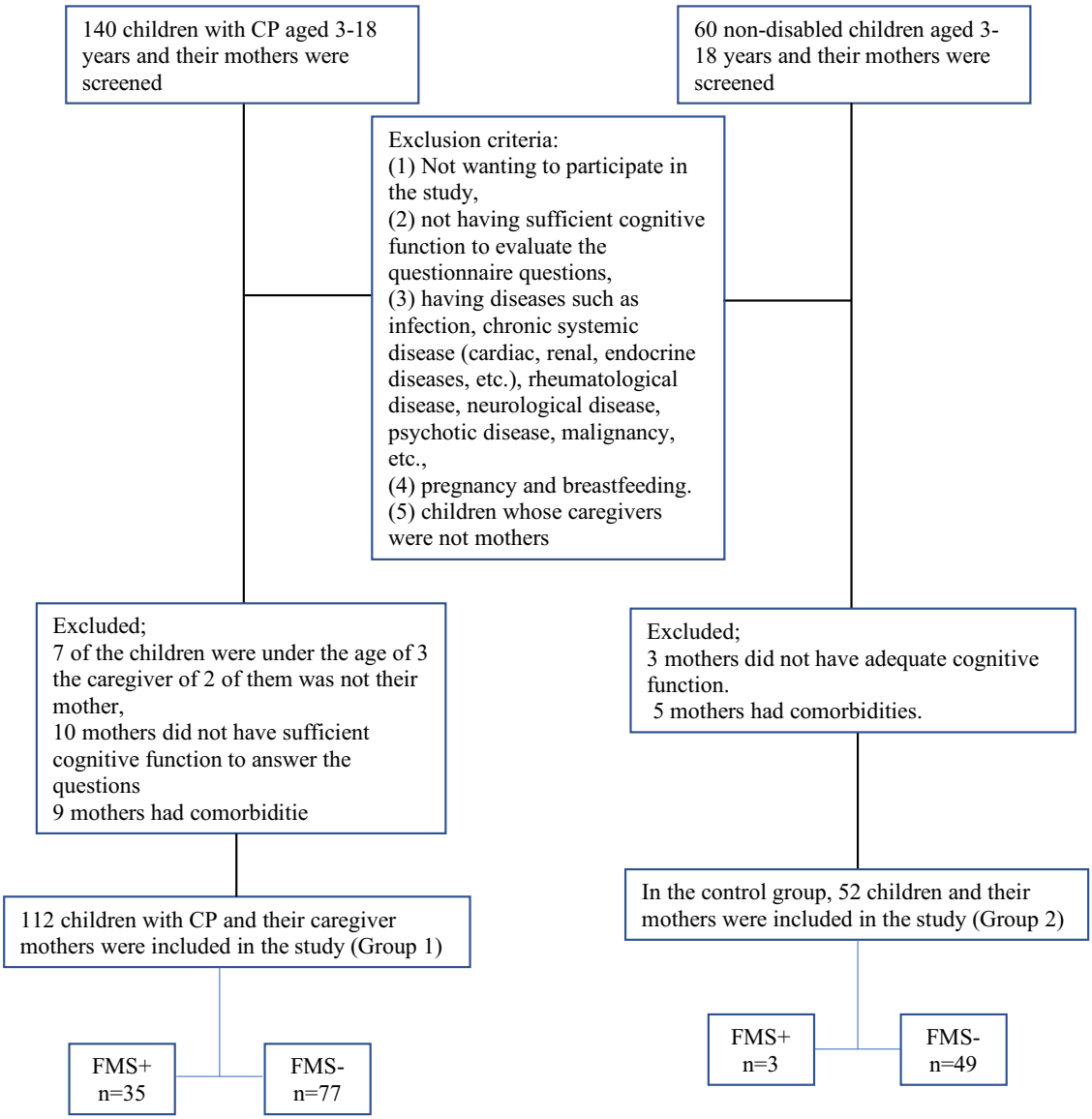

Fig. 1. Flow chart.

giver support than the control group (P<0.001). FMS was detected in 31.3% of Group 1. The FMS rate of Group 2 was 5.7%.

Demographic characteristics and clinical and functional status of children with CP and children in the control group are given in Table 2: The BMI (kg/m^2) values of the children with CP were significantly lower than the children in the control group (P<0.001).

The comparison of mothers of children with CP with and without FMS in terms of evaluation parameters is given in Table 3: Mothers with FMS of children with CP exhibited statistically significantly higher anxiety and depression scores; perceived caregiver burden was also statistically significantly higher. Children with CP of mothers with FMS had statistically significantly older, lower BMI, and more siblings and siblings with CP than those without FMS. Children with CP of mothers with FMS had a statistically significant higher GMFCS score and lower WeeFIM sub-parameters and total scores compared to those without FMS.

Table 4 shows the relationships between maternal FMS severity, pain severity, psychological status, care-

Table 1. Demographic characteristics of the mothers of children with CP, FMS, psychological status assessment, and caregiving burden

		Mothers of children with CP (n=112)	Control group (n=2)	P value
Age (year)		35 (23-50)	34 (27-50)	0.897
BMI (kg/m^2)		25.2 (18.8-37.3)	26.6 (19.7-32.4)	0.192
Marital status, n (%)	Married	103 (92%)	50 (94.3%)	0.584
	Single	9 (8%)	3 (5.7%)	
Working status, n (%)	Working	13 (11.6%)	8 (15.1%)	0.532
	Not working	99 (88.4%)	45 (84.9%)	
Social insurance, n (%)		110 (98.2%)	53 (100%)	0.329
Perceived income, n (%)	Income < expenses	54 (48.2%)	30 (56.6%)	0.260
	Income = expenses	47 (42%)	20 (37.7%)	
	Income > expenses	11 (9.8%)	3 (5.7%)	
Education n (%)	Primary education	65 (58%)	27 (50.9%)	0.450
	High school	33 (29.5%)	19 (35.8%)	
	University	14 (12.5%)	7 (13.2%)	
Caregiver assistance n (%)		38 (33.9%)	2 (3.8%)	**<0.001**
HADS-A		11 (4-21)	6 (4-13)	**<0.001**
HADS-D		12 (5-20)	7 (4-13)	**<0.001**
BCOS		53.50 (25-65)	66 (53-76)	**<0.001**
WPI		1 (0-12)	0 (0-8)	**<0.001**
SSS		5 (2-12)	3 (2-6)	**<0.001**
Pain (VAS)		4 (0-10)	0 (0-7)	**<0.001**
FMS (+), n (%)		35 (31.3%)	3 (5.7%)	**<0.001**

Data are shown as median (minimum-maximum) or n (%). CP=Cerebral palsy, FMS=Fibromyalgia syndrome, HADS-A=Hospital anxiety scale, HADS-D=Hospital depression scale, VAS=Visual analog scale, FIQ=FMS Impact Questionnaire, BCOS=Bakas Caregiving Outcomes scale, WPI=Widespread pain index, SSS=symptom severity scale, VAS=Visual analog scale

giving burden, and functional status of the child with CP: There was a statistically significant positive correlation between the FIQ scores and the total number of siblings of the children with CP, the number of siblings with CP, the GMFCS score, and the anxiety and depression scores of their mothers (P<0.05). There was a negative correlation between FIQ scores and WeeFIM self-care, mobility, cognitive function subscores, WeeFIM total score, and BCOS scores of children with CP (P<0.05).

Univariate and multivariate logistic regression analyses were performed to determine the factors affecting fibromyalgia severity (Table 5). In the multivariate analysis, only four of the ten variables with statistically significant odds ratios (OR) in the univariate regression analysis remained statistically significant. Outcomes that had statistical significance in Univariate regression analysis were number of siblings, number of siblings with CP, GMFCS level, Wee-FIM self-care, Wee-FIM mobility, Wee-FIM cognitive functions, Wee-FIM total, maternal anxiety and depression levels, and mother's caregiver burden. Outcomes that had statistical significance in the multivariate analysis were (in order of importance): mother's caregiver burden, mother's depression levels, GMFCS level of the child with CP, and a number of siblings with CP.

DISCUSSION

Our study showed that mothers of children with CP

Table 2. Demographic characteristics, clinical and functional status of children with CP and control group

		Children with CP (n=112)	Control group (n=52)	P value
Age (month)		106.5 (38-216)	108 (40-180)	0.536
Gender	Female	60 (53.6%)	35 (66%)	0.131
	Male	52 (46.4%)	18 (34%)	
BMI (kg/m^2)		17.20 (14.10-20.80)	18.90 (16.50-22)	**<0.001**
Number of siblings		1 (0-5)	1 (0-3)	0.875
Siblings with CP		11 (9.8%)	-	
Clinical type of SP n (%)	Spastic diplegia	29 (25.9%)	-	
	Spastic quadriplegia	44 (39.3%)	-	
	Spastic hemiplegia	32 (28.6%)	-	
	Dyskinetic & Ataxic	7 (6.3%)	-	
GMFCS n (%)	GMFCS 1	14 (12.5%)	-	
	GMFCS 2	43 (38.4%)	-	
	GMFCS 3	21 (18.8%)	-	
	GMFCS 4	18 (16.1%)	-	
	GMFCS 5	16 (14.3%)	-	
GMFCS, Median (min-max)		2 (1-5)		
WeeFIM self-care		24 (5-42)	-	
WeeFIM mobility		20.50 (5-35)	-	
WeeFIM cognitive function		28 (5-35)	-	
WeeFIM total		83.50 (18-126)	-	

Data are shown as median (minimum-maximum) or n (%). CP=Cerebral palsy, GMFCS=Gross Motor Function Classification System, WeeFIM=Functional independence scale for children

Tablo 3. Comparison of mothers with and without FMS of children with CP

		FMS (+) (n=35)	FMS (-) (n=77)	P value
Mothers of children with CP				
Age (year)		37 (26-50)	34 (23-49)	0.121
BMI (kg/m^2)		27.10 (18.80-37.30)	24.70 (19.70-32)	0.130
Marital status n (%)	Married	31 (88.5%)	71 (92.2%)	0.375
	Single	4 (11.5%)	6 (7.8%)	
Working status n (%)	Yes	4 (11.5%)	8 (10.3%)	0.968
	No	31 (88.5%)	69 (89.7%)	
Education n (%)	Primary	25 (71.4%)	40 (52.0%)	0.062
	High school	7 (20%)	25 (32.4%)	
	University	3 (8.6%)	12 (15.6%)	
Perceived income n (%)	Income<expenditure	19 (54.3%)	38 (49.3%)	0.300
	Income=expenditure	14 (40%)	33 (42.8%)	
	Income>expenditure	2 (5.7%)	6 (7.9%)	
HADS-A		12 (6-21)	11 (4-17)	**<0.001**
HADS-D		15 (9-20)	11 (5-20)	**<0.001**
Pain (VAS)		7 (4-10)	-	
FIQ		69.99 (60.32-94.26)	-	
BCOS		47 (25-60)	55 (38-65)	**<0.001**
Children with CP				
Age (month)		117 (41-216)	99 (38-214)	**0.038**
Gender	Female	18 (51.4%)	42 (54.5%)	0.760
	Male	17 (48.6%)	35 (45.5%)	
BMI (kg/m^2)		16.60 (14.10-19.90)	17.60 (14.10-20.80)	**0.004**
Number of siblings		1 (0-3)	1 (0-5)	**0.006**
Siblings with CP n (%)		8 (22.8%)	3 (3.8%)	**0.002**
Recipients of caregiver assistance		11 (31.4%)	27 (35.1 %)	0.708
Clinical type of Cp n (%)	Spastic diplegia	8 (22.9%)	21 (27.3%)	0.286
	Spastic quadriplegia	21 (60%)	23 (29.9%)	
	Spastic hemiplegia	2 (5.7%)	30 (39%)	
	Dyskinetic & Ataxic	4 (11.4%)	3 (3.9%)	
GMFCS n (%)	GMFCS 1	1 (2.9%)	13 (16.9%)	**<0.001**
	GMFCS 2	5 (14.3%)	38 (49.4%)	
	GMFCS 3	4 (11.4%)	17 (22.1%)	
	GMFCS 4	13 (37.1%)	5 (6.5%)	
	GMFCS 5	12 (34.3%)	4 (5.2%)	
WeeFIM self-care		12 (5-37)	27 (6-42)	**<0.001**
WeeFIM mobility		10 (5-35)	25 (5-35)	**<0.001**
WeeFIM cognitive function		17 (5-35)	29 (5-35)	**0.001**
WeeFIM total		57 (18-110)	93 (18-126)	**<0.001**

Data are shown as median (minimum-maximum) or n (%). CP=Cerebral palsy, FMS=Fibromyalgia syndrome, HADS-A=Hospital anxiety scale, HADS-D=Hospital depression scale, VAS=Visual analog scale, FIQ=FMS Impact Questionnaire, BCOS=Bakas Caregiving Outcomes scale, GMFCS=Gross Motor Function Classification System, WeeFIM=Functional independence scale for children

Table 4. The relationship between maternal FMS severity, psychological status, functional status, dependency level of the child with CP, and caregiving burden

	FIQ	
	R	P value
Children with CP		
Age (year)	0.168	0.077
Gender	0.013	0.893
Number of siblings	0.249	**0.008**
Siblings with CP	0.300	**<0.001**
Clinical type of CP	-0.106	0.268
GMFCS	0.555	**<0.001**
WeeFIM self-care	-0.468	**<0.001**
WeeFIM mobility	-0.426	**<0.001**
WeeFIM cognitive function	-0.360	**<0.001**
WeeFIM total	-0.482	**<0.001**
Mothers of children with CP		
Recipients of caregiver assistance	-0.610	0.523
HADS-A	0.360	**<0.001**
HADS-D	0.532	**<0.001**
BCOS	-0.589	**<0.001**

CP=Cerebral palsy, FMS=Fibromyalgia syndrome, HADS-A=Hospital anxiety scale, HADS-D=Hospital depression scale, VAS=Visual analog scale, FIQ=FMS Impact Questionnaire, BCOS=Bakas Caregiving Outcomes scale, GMFCS=Gross Motor Function Classification System, WeeFIM=Functional independence scale for children

had higher levels of anxiety, depression, and caregiving burden and higher FMS rates than the control group. Almost a quarter of mothers of children with CP had FMS. It was found that mothers with FMS of children with CP had higher anxiety, depression, and caregiving burden than those without FMS. Children with CP of mothers with FMS had a higher number of siblings and siblings with CP, advanced age, lower motor functions, and higher dependency levels than those without FMS. This study determined that the factors affecting FMS severity were the number of siblings of children with CP, the number of siblings with CP, GMFCS level, dependency level, mothers' anxiety and depression levels, and mother's caregiver burden. The most influential factor in FMS severity was the mother's caregiver burden. This was followed in order of importance by the mother's depression, the GMFCS level, and the number of the sibling with CP.

CP is the most disabling disease of childhood and requires lifelong care. In this process, parents' physical and psychological health is adversely affected [1, 4]. Previous studies have reported that musculoskeletal pain is more common in mothers of children with cerebral palsy than in mothers of healthy children [20, 21]. More commonly, spinal pain has been investigated [20-22]. In a study evaluating back pain and underlying causes in mothers of children with CP, only 20.67% of mothers did not complain of pain [22]. In the literature, the prevalence of FMS in women has been reported as 2.4% - 6.8% [10]. In the current study, 31.3% of mothers of children with CP were diagnosed with FMS. This rate was significantly different from the control group.

It has been found that the incidence and intensity of musculoskeletal pain in mothers of children with CP primarily depend on the child's functional status and dependency level, body weight, age, and intense physical activity load created by caregiving [3, 22]. In contrast, Byrne *et al*. [23] reported that CP severity did not affect parents' health status (including musculoskeletal pain). In addition, having more than one child with CP and maternal depression levels have been identified as risk factors for musculoskeletal pain in mothers [20]. Caring for a child with CP is a one-way, dependent, and long-term process. Therefore, as the child's age increases, the caregiver burden increases [20, 24]. In the current study, children with CP of mothers with FMS were older and had more siblings and siblings with CP, consistent with the literature. Contrary to the literature, the children of mothers with FMS had lower BMI. The developmental delay in the child may have caused the mothers to feel inadequate about feeding. Feelings of inadequacy have been significantly associated with anxiety and depression [25].

The quality of life of mothers of children with CP is adversely affected by various factors such as caregiver burden, fatigue, and psychological symptoms [26]. There are studies stating that the severity of CP negatively affects the mother's quality of life, as well as studies that do not support this [21, 23]. Caregivers of children with cerebral palsy suffer from a significant psychosocial burden, and this burden was found

Tablo 5. Evaluation of the factors affecting the severity of FMS in mothers of children with CP

	Univariate analysis 95% CI for OR				Multivariate analysis 95% CI for OR			
	OR	Lower	Upper	P value	OR	Lower	Upper	*p value*
Children with CP								
Age	1.009	0.999	1.018	0.073				
Gender	1.372	0.648	2.903	0.408				
Number of siblings	1.591	1.098	2.305	**0.014**				
Siblings with CP	8.585	2.459	269.966	**0.001**	6.974	1.108	43.886	**0.039**
Recipients of caregiver assistance	1.596	0.700	3.641	0.266				
Clinical type of CP	0.835	0.526	1.324	0.443				
GMFCS	3.171	2.046	4.916	**<0.001**	3.169	1.568	6.406	**0.001**
WeeFIM self-care	0.936	0.909	0.964	**<0.001**				
WeeFIM mobility	0.902	0.859	0.947	**<0.001**				
WeeFIM cognitive function	0.935	0.900	0.971	**<0.001**				
WeeFIM total	0.970	0.956	0.983	**<0.001**				
Mothers of children with CP								
HADS-A	1.473	1.265	1.715	**<0.001**				
HADS-D	1.701	1.417	2.042	**<0.001**	1.439	1.183	1.750	**<0.001**
BCOS	0.782	0.720	0.850	**< .001**	0.801	0.734	0.873	**<0.001**

CP=Cerebral palsy, FMS=Fibromyalgia syndrome, HADS-A=Hospital anxiety scale, HADS-D=Hospital depression scale, VAS=Visual analog scale, FIQ=FMS Impact Questionnaire, BCOS=Bakas Caregiving Outcomes scale, GMFCS=Gross Motor Function Classification System, WeeFIM=Functional independence scale for children

to be higher than that of healthy childcare [3]. Studies have identified the most important predictors of the caregiver burden on parents as the child's degree of disability, parents' anxiety and depression levels, and sense of efficacy [6, 27-29]. Depression and anxiety levels of mothers of children with CP were found to be higher than mothers of healthy children [30, 31]. However, one study found no difference between the anxiety levels of mothers of children with CP and mothers of healthy children [32]. In addition, a positive relationship has been reported between measures of caregiver burden and the severity of anxiety and depression in parents of children with CP [27, 28]. Although there are studies that reached the opposite conclusion [31], other studies reported a direct correlation between the disability and dependency level of the child with CP and the anxiety and stress levels of the mother [33]. Stressful situations associated with the daily care of a child with CP have been found to, directly and indirectly, affect parents' health, especially mothers [1, 4]. Anxiety and depression are more common in FMS than in the normal population and negatively affect the quality of life [11]. In the current study, although mothers of children with CP needed more caregiver assistance than mothers in the control group, they still had a higher caregiver burden. They had higher levels of depression and anxiety. These results support the results of other studies [3, 30, 31]. Also, in the current study, mothers with FMS of children with CP exhibited higher depression and anxiety scores than those without FMS, and the caregiver burden was higher. The functional level of the children of mothers with FMS was lower, and the dependence level was higher. In addition, there was a direct correlation between the severity of fibromyalgia and the severity of cerebral palsy disease, and the burden of

caregiving. The most important factor affecting FMS severity was caregiver burden, followed by maternal depression levels, child's functional status, and the number of siblings with CP. We thought that the heavy burden of providing lifelong care to a child with CP, anxiety, and depression could be triggering factors in the development of FMS by creating physical, environmental, and psychological stressors. Moreover, the fact that children with CP have a high number of siblings have siblings with CP, have low motor functions, and have high dependency levels may have affected the development of FMS by increasing the burden of caregiving.

Limitations

The strength of our study is that it is the first study to investigate FMS in mothers of children with CP. The study's limitations are that it does not reflect the general population, as it is a single-centered cross-sectional study. Nevertheless, we think that it is an essential step in drawing attention to FMS in mothers of children with CP. There is a need for multicenter prospective studies in this area to reflect the general population.

CONCLUSION

This study showed that mothers of children with CP had higher anxiety, depression, and caregiving burden and higher FMS rates than the control group. Children with CP in the group with FMS had a higher number of siblings, a number of siblings with CP, age and dependency levels, and lower motor functions than the children of those without FMS. In addition, this study determined the factors affecting the severity of FMS in mothers of children with CP. These factors are a total number of siblings and number of siblings with CP; decrease in motor functions and increase in dependency levels of children with CP; mothers' anxiety-depression levels; and the increase in caregiver burden. Independent variables are caregiver burden, mothers' depression level, children's GMFCS, and the number of siblings with CP. In the light of these results, we think that Fibromyalgia syndrome can also be kept in mind in the musculoskeletal system pain of mothers of children with CP. Reducing the burden on the mother, who is obliged to take care of her disabled child for life with psychosocial and physical support, may negatively affect the development of FMS by reducing the stress on mothers. In addition, accurate diagnosis and effective treatment (pharmacological and/or non-pharmacological) can improve mothers' quality of life. Comprehensive studies are needed on this subject.

Authors' Contribution

Study Conception: İAK, MKA, MY; Study Design: İAK, MKA, MY; Supervision: İAK, MKA, MY; Funding: İAK, MKA, MY; Materials: İAK, MKA, MY; Data Collection and/or Processing: İAK, MKA, MY; Statistical Analysis and/or Data Interpretation: İAK, MKA, MY; Literature Review: İAK, MKA, MY; Manuscript Preparation: İAK, MKA, MY and Critical Review: İAK, MKA, MY.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. Michael-Asalu A, Taylor G, Campbell H, Lelea LL, Kirby RS. Cerebral palsy: Diagnosis, epidemiology, genetics, and clinical update. Adv Pediatr. 2019;66:189-208. doi: 10.1016/j.yapd.2019.04.002.
2. Aisen ML, Kerkovich D, Mast J, et al. Cerebral palsy: clinical care and neurological rehabilitation. Lancet Neurol. 2011;10(9):844-852. doi: 10.1016/S1474-4422(11)70176-4.
3. Vadivelan K, Sekar P, Sruthi SS, Gopichandran V. Burden of caregivers of children with cerebral palsy: an intersectional analysis of gender, poverty, stigma, and public policy. BMC Public Health. 2020;20(1):645. doi: 10.1186/s12889-020-08808-0.
4. Khayatzadeh MM, Rostami HR, Amirsalari S, Karimloo M. Investigation of quality of life in mothers of children withcerebral palsy in Iran: association with socio-economic status,marital satisfaction and fatigue. Disabil Rehabil. 2013;35(10):803-808. doi: 10.3109/09638288.2012.708818.
5. Park EY, Nam SJ. Time burden of caring and depression among parents of individuals with cerebral palsy. Disabil Rehabil. 2019;41(13):1508-1513. doi: 10.1080/09638288.2018.1432705.
6. Marrón EM, Redolar-Ripol D, Boixadós M, et al. Burden on caregivers of children with cerebral palsy: predictors and related factors. Univ Psychol. 2013;12(3):767-777. doi: 10.11144/Javeriana.UPSY12-3.bccc

7. Ramezani M, Eghlidi J, Pourghayoomi E, Mohammadi S. Caring-related chronic low back pain and associated factors among mothers of children with cerebral calsy. Rehabil Res Pract. 2020;2020:8854435. doi: 10.1155/2020/8854435.
8. Siracusa R, Paola RD, Cuzzocrea S, Impellizzeri D. Fibromyalgia: pathogenesis, mechanisms, diagnosis and treatment options update. Int J Mol Sci. 2021;22(8):3891. doi: 10.3390/ijms22083891.
9. Martínez-Lavín M. Fibromyalgia in women: somatisation or stress-evoked, sex-dimorphic neuropathic pain? Clin Exp Rheumatol. 2021;39(2):422-425. doi: 10.55563/clinexprheumatol/0c7d6v.
10. Marques AP, Santo ASDE, Berssaneti AA, Matsutani LA, Yuan SLK. Prevalence of fibromyalgia: literature review update. Rev Bras Reumatol Engl Ed. 2017;57(4):356-363. English, Portuguese. doi: 10.1016/j.rbre.2017.01.005.
11. Aguglia A, Salvi V, Maina G, Rossetto I, Aguglia E. Fibromyalgia syndrome and depressive symptoms: comorbidity and clinical correlates. J Affect Disord. 2011;128(3):262-266. doi: 10.1016/j.jad.2010.07.004.
12. Amital D, Fostick L, Polliack ML, et al. Posttraumatic stress disorder, tenderness, and fibromyalgia syndrome: are they different entities? J Psychosom Res. 2006;61(5):663-669. doi: 10.1016/j.jpsychores.2006.07.003.
13. Mutch L, Alberman E, Hagberg B, Kodama K, Perat MV. Cerebral palsy epidemiology: where are we now and where are we going? Dev Med Child Neurol. 1992;34(6):547-551. doi: 10.1111/j.1469-8749.1992.tb11479.x.
14. Palisano R, Rosenbaum P, Walter S, Russell D, Wood E, Galuppi B. Development and reliability of a system to classify gross motor function in children with cerebral palsy. Dev Med Child Neurol. 1997;39(4):214-223. doi: 10.1111/j.1469-8749.1997.tb07414.x.
15. Ottenbacher KJ, Msall ME, Lyon NR, Duffy LC, Granger CV, Braun S. Interrater agreement and stability of the Functional Independence Measure for Children (WeeFIM): use in children with developmental disabilities. Arch Phys Med Rehabil. 1997;78(12):1309-1315. doi: 10.1016/s0003-9993(97)90302-6.
16. Wolfe F, Clauw DJ, Fitzcharles MA, et al. The American College of Rheumatology preliminary diagnostic criteria for fibromyalgia and measurement of symptom severity. Arthritis Care Res (Hoboken). 2010;62(5):600-610. doi: 10.1002/acr.20140.
17. Bennett RM, Bushmakin AG, Cappelleri JC, Zlateva G, Sadosky AB. Minimal clinically important difference in the fibromyalgia impact questionnaire. J Rheumatol. 2009;36(6):1304-1311. doi: 10.3899/jrheum.081090.
18. Zigmond AS, Snaith RP. The hospital anxiety and depression scale. Acta Psychiatr Scand. 1983;67(6):361-370. doi: 10.1111/j.1600-0447.1983.tb09716.x.
19. Bakas T, Champion V, Perkins SM, Farran CJ, Williams LS. Psychometric testing of the revised 15-item Bakas Caregiving Outcomes Scale. Nurs Res. 2006;55(5):346-355. doi: 10.1097/00006199-200609000-00007.
20. Terzi R, Tan G. Musculoskeletal system pain and related factors in mothers of children with cerebral palsy. Agri. 2016;28(1):18-24. doi: 10.5505/agri.2015.74436.
21. Kaya K, Unsal-Delialioglu S, Ordu-Gokkaya NK, et al. Musculo-skeletal pain, quality of life and depression in mothers of children with cerebral palsy. Disabil Rehabil. 2010;32(20):1666-1672. doi: 10.3109/09638281003649912.
22. Czupryna K, Nowotny-Czupryna O, Nowotny J. Back pain in mothers of cerebral palsied children. Ortop Traumatol Rehabil. 2014;16(5):497-505. doi: 10.5604/15093492.1128840.
23. Byrne MB, Hurley DA, Daly L, Cunningham CG. Health status of caregivers of children with cerebral palsy. Child Care Health Dev. 2010;36(5):696-702. doi: 10.1111/j.1365-2214.2009.01047.x.
24. Rapp CE Jr, Torres MM. The adult with cerebral palsy. Arch Fam Med. 2000;9(5):466-472. doi: 10.1001/archfami.9.5.466.
25. Ketelaar M, Volman MJ, Gorter JW, Vermeer A. Stress in parents of children with cerebral palsy: what sources of stress are we talking about? Child Care Health Dev. 2008;34(6):825-829. doi: 10.1111/j.1365-2214.2008.00876.x.
26. Farajzadeh A, Maroufizadeh S, Amini M. Factors associated with quality of life among mothers of children with cerebral palsy. Int J Nurs Pract. 2020;26(3):e12811. doi: 10.1111/ijn.12811.
27. Gugała B. Caregiver burden versus intensity of anxiety and depression symptoms in parents of children with cerebral palsy as well as factors potentially differentiating the level of burden: a cross-sectional study (Poland). BMJ Open. 2021;11(6):e036494. doi: 10.1136/bmjopen-2019-036494.
28. Boztepe H, Çınar S, Ay A, Kerimoğlu Yıldız G, Kılıç C. Predictors of caregiver burden in mothers of children with leukemia and cerebral palsy. J Psychosoc Oncol. 2019;37(1):69-78. doi: 10.1080/07347332.2018.1489441.
29. Unsal-Delialioglu S, Kaya K, Ozel S, Gorgulu G. Depression in mothers of children with cerebral palsy and related factors in Turkey: a controlled study. Int J Rehabil Res. 2009;32(3):199-204. doi: 10.1097/MRR.0b013e32832607b6.
30. Sajedi F, Alizad V, Malekkhosravi G, Karimlou M, Vameghi R. Depression in mothers of children with cerebral palsy and its relation to severity and type of cerebral palsy. Acta Med Iran. 2010;48(4):250-254.
31. Gugała B, Penar-Zadarko B, Pięciak-Kotlarz D, et al. Assessment of anxiety and depression in Polish primary parental caregivers of children with cerebral palsy compared to a control group, as well as identification of selected predictors. Int J Environ Res Public Health. 2019;16(21):4173. doi: 10.3390/ijerph16214173.
32. Ones K, Yilmaz E, Cetinkaya B, Caglar N. Assessment of the quality of life of mothers of children with cerebral palsy (primary caregivers). Neurorehabil Neural Repair. 2005;19(3):232-237. doi: 10.1177/1545968305278857.
33. Kavlak E, Kara G, Tekin F, Altuğ F, Ök N, Şenol H. Stress and depression levels of mothers who give care to children with cerebral palsy and mental retardation: a comparison study. Pam Med J. 2018;11(1):25-31. doi: 10.5505/ptd.2017.27676

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Effects of sarcopenia on in-hospital results and mid-term follow-up in patients with coronary artery disease and COVID-19

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ABSTRACT

Objectives: Sarcopenia is associated with atherosclerosis, vascular dysfunction, and poor in-hospital prognosis in the general COVID-19 population. Coronary artery disease (CAD) is also associated with poor prognosis in patients with COVID-19, however, the influencing factors in this association have not yet been fully documented. This study aimed to evaluate the effect of sarcopenia on both in-hospital acute-term and mid-term follow-up clinical results in patients with CAD and COVID-19.

Methods: The study population was selected from the general COVID-19 population. It consisted of 50 patients with CAD (group I) and 80 age- and gender-matched patients without CAD (group II). In-hospital acute term endpoints were determined as intensive care unit (ICU) admission, intubation, mortality, and its combination. Mid-term follow-up was also made for three-month. Sarcopenia was assessed by indexed skeletal muscle mass at T12 vertebrae level (T12-SMI) on initial chest computed tomography. Multivariable logistic regression analysis was used to detect independently related factors to endpoints.

Results: Group I had more severe COVID-19 disease and a higher rate of hospitalization, ICU admission, intubation as well as mortality compared to group II in acute-term. T12-SMI was lower and sarcopenia was more frequent in group I than in group II. During the three-month mid-term follow-up period, no additional adverse results occurred in both groups. In multivariate regression analysis; sarcopenia was independently related to in-hospital combined endpoint.

Conclusions: Sarcopenia is associated with in-hospital combined endpoint in patients with CAD during acute-term of COVID-19. However, it has no effect on three-month mid-term follow-up.

Keywords: COVID-19, coronary artery disease, sarcopenia

Coronavirus disease 2019 (COVID-19) caused by a new severe acute respiratory syndrome coronavirus 2 (SARS-Cov-2) has led to a worldwide pandemic in 2019 [1]. Unfortunately, it has caused millions of death. Its clinical course largely varies from patient to patient. Some patients can face life-threatening clinical disease that requires admission to the intensive care unit (ICU), intubation, and even death, while others may experience minimal symptoms or an asymptomatic situation. The presence

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of coronary artery disease (CAD) has been identified as one of the main determinants of poor prognosis in patients with COVID-19 [2-4]. However, the mechanism of this relationship has not been clearly understood yet.

Sarcopenia is a skeletal muscle disease that reflects the presence of low muscle quantity or quality, low muscle strength, and low physical performance [5]. Computed tomography (CT) is one of the most commonly used imaging methods to evaluate sarcopenia [6]. Currently, chest CT imaging is widely used as an initial evaluation tool in patients with COVID-19 that allows to assessment of sarcopenia by evaluating cross-sectional muscle areas at the level of T12 (thoracic 12) vertebrae. Clinically, it is associated with atherosclerosis and cardiovascular disease, lowered quality of life, increased hospitalization rate, and even death in the general population [7-13]. Recently, some studies evaluated the relationship between sarcopenia and in-hospital adverse clinical results in an unselected general population of COVID-19 have been published [14-16]. However, there is no data about the effect of sarcopenia on selected patient populations such as CAD.

Sarcopenia and CAD share some common pathophysiological mechanisms such as atherosclerosis, arterial stiffness, and vascular dysfunction. Therefore, sarcopenia may play a role in the development of prominent worse results in patients diagnosed with COVID-19 who have CAD. In addition, sarcopenia is a relatively chronic situation that may have a longer-term effect on the patients' results. Thus, in this study, we sought the effects of sarcopenia on both in-hospital acute term results and three-month mid-term follow-up in patients diagnosed with COVID-19 who have CAD that may suggest an etiopathological mechanism for poor prognosis.

METHODS

Patients Selection

The study population was prospectively selected among the patients diagnosed with COVID-19 by re-

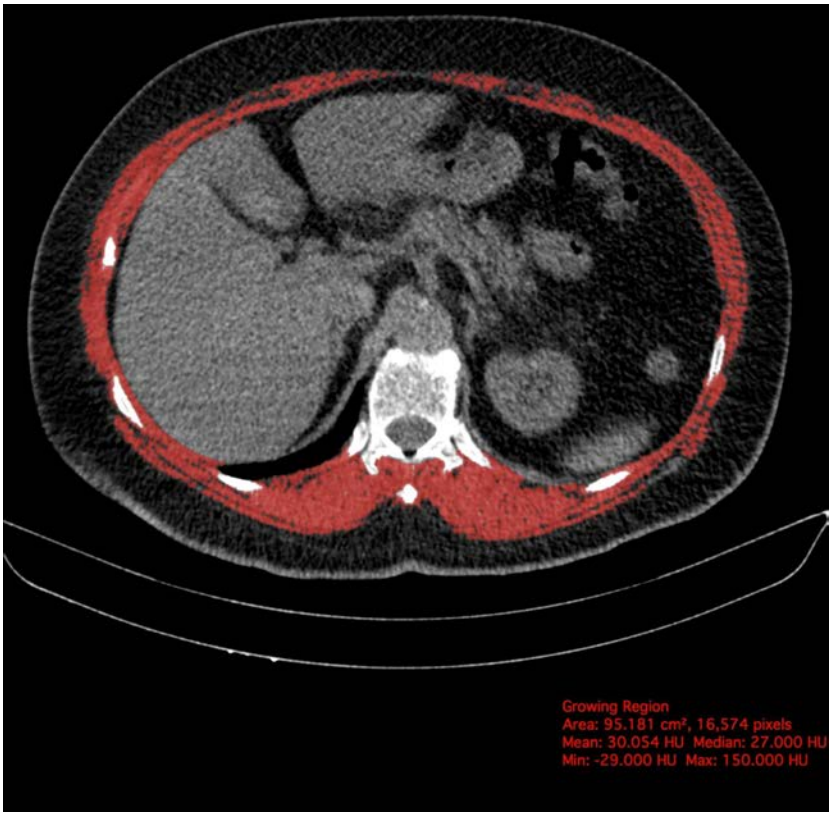

Fig. 1. T12-SMA measurement on chest CT image at the T12 vertebra level. Red zone indicates the cross-sectional skeletal muscle area identified using a threshold of -29 to +150 HU (T12-SMA: 95.18 cm² , T12-SMI: 34.99 cm²/m²).

verse transcription-polymerase chain reaction (RT-PCR) test. Group I consisted of 50 consecutive patients with CAD (previous coronary artery bypass graft operation, percutaneous coronary intervention, or known 50% stenosis of at least one epicardial coronary artery). Group II included 80 age- and gender-matched patients without CAD who served as a control subjects. Patients who underwent unenhanced chest CT examination during the initial clinical examination were included in the study but those with no or inadequate CT imaging were excluded from the study. In addition, patients who had heart failure (left ventricular ejection fraction <50%) and significant valvular heart disease, and the acute coronary syndrome were also excluded from the study. Hypertension (HT), diabetes mellitus (DM) and chronic obstructive pulmonary disease (COPD) were diagnosed by the presence of previous history and/or drug use. All laboratory values were obtained during the initial hospital presentation. The study was conducted by the guidelines in the Declaration of Helsinki. The Local Ethics Committee approved the study protocol. Written informed consent was obtained from all of the study participants.

Assessment and Definition of Sarcopenia

Sarcopenia was assessed by chest CT imaging obtained during the initial evaluation of patients with COVID-19. Chest CT images were acquired using a 64-slice multi-detector CT scanner (Somatom Sensation, Siemens, Germany). Initially, cross-sectional area (CSA, in cm^2) of all skeletal muscles at the level of the T12 (rectus abdominis, external and internal obliques, psoas, transverse abdominalis, rectus abdominalis, quadratus lumborum, and the erector spinae) were measured by using OsiriX Lite software (version 7.0.2, Pixmeo SARL, Bernex, Switzerland). The Hounsfield Units (HU) of -29 to 150 were used to isolate the skeletal muscle. Thus, the exact muscle area (T12-SMA, cm^2) was obtained based on HU, excluding vasculature and fat infiltration (Fig. 1). Then, CSA measurements were normalized to patient size by dividing CSA with height in square meters to provide the skeletal muscle index (T12-SMI; cm^2/m^2) [14, 15]. All T12-SMI values were divided into quartiles and stratified by gender. Finally, the patients in the first quartile of each gender that had the lowest SMI values were accepted as sarcopenia [14].

Disease Severity and End-Points

Patients were classified into four groups according to their clinical symptoms, signs, and chest imaging manifestations as being mild, moderate, severe, or critical COVID-19 cases [17]:

1. Mild cases: mild or minimal clinical symptoms, no sign of pneumonia on chest imaging
2. Moderate cases: fever and respiratory symptoms, pneumonia on chest imaging
3. Severe cases: severe respiratory distress and/or increased respiratory rate ≥30 breaths/min and/or decreased oxygen saturation (SpO_2) on room air with ≤93 % and/or arterial partial pressure of oxygen (PaO_2)/ fraction of inspired oxygen (FiO2)≦300 mmHg
4. Critical cases: respiratory failure requiring mechanical ventilation and/or septic shock and/or other organ failure requiring ICU admission.

In addition, the severity of COVID-19 disease was evaluated by chest CT severity score (CTSS). Involvement of each lung segment was assessed separately then a total score was computed by a predefined method [18]. According to this method, two lungs which were divided into 20 regions were evaluated for ground glass opacities. Each region was scored as 0 point for no parenchymal involvement, 1 point for ≤50% opacification and 2 point for >50% opacification. Finally, chest CT severity score was obtained by summing of points from each region.

In the study, there were two time-dependent end-points. First; short-term in-hospital adverse results included ICU admission, intubation, mortality, and their combined end-point. Second; thromboembolic event, hospitalization, and mortality at three-month mid-term follow-up.

Statistical Analysis

Categorical variables are expressed as numbers and percentages. The normal distribution of continuous variables was evaluated by using the Kolmogorov-Smirnov test and histogram. Then, continuous variables are expressed as mean±standard deviation for variables with normal distribution and as median (25^{th}-75^{th} quartiles) for variables without normal distribution. Continuous variables were analyzed by independent-sample t-test or Mann-Whitney U test according to normal distribution. Chi-square and Fisher's exact tests were used for categorical variables.

Table 1. The baseline demographic properties of study population

	Group I (n=50)	Group II (n=80)	P value
Age (years)	68 (60-76)	63 (58-70)	0.06
Gender (female), n (%)	30 (60)	49 (61.3)	0.89
Diabetes Mellitus, n (%)	25 (50)	29 (36.3)	0.12
Hypertension, n (%)	30 (60)	40 (50)	0.27
COPD, n (%)	5 (10)	6 (7.5)	0.78
T12-SMA (cm^2)	85.9±24.9	93.5±18.5	**0.03**
T12-SMI (cm^2/m^2)	30.9±7.3	34.3±5.5	**0.006**
Sarcopenia, n (%)	31 (62)	35 (43.8)	**0.04**

Data are shown as mean±standart deviation or median (25^{th}-75^{th} quartiles) or n (%). COPD=chronic obstructive pulmonary disease, T12-SMA=T12 skeletal muscle area, T12-SMI=T12 skeletal muscle index

Multivariable logistic regression analysis was used to detect independent variables of end-points. A *p* - value of less than 0.05 was considered statistically significant. All statistical analyses were carried out by the SPSS 21 statistical software (SPSS Inc., Chicago, Illinois, USA).

RESULTS

A total of 130 patients were included in the study (50 in group I and 80 in group II). The baseline demographic parameters of the study population were shown in Table 1. There were no differences between the two groups for HT, DM, and COPD. However, T12-SMA and T12-SMI were lower and the rate of sarcopenia was higher in group I than in group II (85.9±24.9 vs 93.5±18.5, P=0.03; 30.9±7.3 vs 34.3±5.5, P=0.006; 62% vs 43.8%, P=0.04, respectively). Laboratory parameters of the study population were given in Table 2. Group I had higher hs (high sensitivity) cardiac troponin I and lower blood oxygen saturation (SpO2) levels than group II.

In-hospital clinical course and end-point data were presented in Table 3. The rate of hospitalization, lung involvement, and in-hospital end-points (ICU admission, intubation, mortality, and combined end-point) were higher in group I than in group II. In addition, the patients in group I had more severe diseases compared to those in group II. During the three-month

Table 2. Laboratory values of study population

	Group 1 (n=50)	Group 2 (n=80)	P value
Creatinine (mg/dL)	0.91 (0.7-1.3)	0.9 (0.8-1.2)	0.78
hs cardiac troponin I (ng/mL)	16.1 (7.3-25.1)	8.7 (4.7-13.4)	**0.001**
SpO_2 (%)	92 (88-96)	95.5 (92.3-97)	**0.006**
CRP, (mg/L)	42.25 (12.3-112.5)	24.7 (10.6-58.7)	0.09
WBC (K/µL)	6.9 (4.9-8.9)	5.9 (4.6-7.9)	0.14
Ferritin (ng/mL)	153.95 (70-426.5)	198.5 (93-390.8)	0.95
D-dimer (µg/mL)	0.8 (0.28-1.7)	0.7 (0.4-1.1)	0.48

Data are shown as median (25^{th}-75^{th} quartiles). hs Troponin I=High sensitivity cardiac troponin I, SpO_2=Arterial blood partial oxygen pressure, CRP=C-reactive protein, WBC=White blood count

Table 3. In-hospital clinical course and end-points of study population

	Group I (n=50)	Group II (n=80)	P value
Hospitalization, n (%)	38 (76)	39 (48.8)	**0.002**
Lung involvement, n (%)			
No	3 (6)	16 (20)	**0.002**
Unilateral	3 (6)	4 (5)	
Bilateral	44 (88)	60 (75)	
Chest CT severity score	14 (7-23.3)	11.5 (2.3-19.8)	0.07
Disease severity, n (%)			
Mild	3 (6)	16 (20)	**0.03**
Moderate	20 (40)	38 (47.5)	
Severe	18 (36)	20 (25)	
Critical	9 (18)	6 (7.5)	
In-hospital end points, n (%)			
ICU admission	17 (34)	14 (17.5)	**0.03**
Intubation	14 (28)	10(12.5)	**0.03**
Mortality	14 (28)	8 (10)	**0.008**
Combined end-point	18 (36)	14 (17.5)	**0.02**

Data are shown as median (25^{th}-75^{th} quartiles) or n (%). CT=Computed tomography, ICU=Intensive care unit, Combined end point=Combination of in-hospital mortality, intubation and ICU admission

mid-term follow-up, there were no additional thromboembolic events, hospitalization, and mortality.

Only one multivariate regression model was created for the in-hospital combined end-point in the acute term because of the absence of additional adverse events during mid-term follow-up. The results of the univariate and multivariate analysis were shown in Table 4. In univariable analysis; C-reactive protein, white blood count, D-dimer, sarcopenia, and CTSS were associated with in-hospital combined end-point. But, in the multivariable model; sarcopenia (OR: 3.648, 95% CI: 1.081-12.03, P=0.037) and CTSS (OR: 1.121, 95% CI: 1.049-1.198, P=0.001) were independently associated with in-hospital combined end-point.

Table 4. The predictors of in-hospital combined end-point in the study population

	Univariable analysis				Multivariable analysis			
	OR	95% CI		P value	OR	95% CI		P value
		Upper	lower			Upper	lower	
CRP	1.016	0.008	1.023	**<0.001**	0.993	0.980	1.005	0.25
WBC	1.213	1.068	1.378	**0.003**	1.144	0.989	1.323	0.07
D-dimer	1.695	1.248	2.301	**0.001**	1.435	1.004	2.051	**0.05**
Sarcopenia	8.169	2.898	23.030	**<0.001**	3.648	1.081	12.307	**0.037**
CTSS	1.131	1.078	1.188	**<0.001**	1.121	1.049	1.198	**0.001**

CRP=C-reactive protein, WBC=white blood count, CTSS=Computed tomography severity score

DISCUSSION

In this study, we assessed the effect of sarcopenia on acute-term in-hospital results and three-month follow-up in a selected population of COVID-19. We found that the patients with CAD had a higher rate of sarcopenia, hospitalization, and more severe disease as well as more frequent in-hospital end-points compared to those without CAD. In addition, sarcopenia was independently associated with acute-term in-hospital combined end-point in this population. There were no additional events during the three-month mid-term follow-up that reflects it has no long-term effect in this population.

The COVID-19 pandemic has led to millions of cases and death around the world. The clinical picture during COVID-19 varies from asymptomatic situations to life-threatening clinical diseases such as respiratory collapse and thromboembolic events. Unfortunately, there is no effective and specific treatment for quickly controlling the disease. Therefore, predicting the patients at risk and detecting the factors associated with poor prognosis are critical to patient management. CAD has reported as an important contributing clinical situation to more severe disease and poor prognosis in COVID-19 [2-4]. However, the mechanism of this relationship and influential factors have not been clearly understood yet.

Sarcopenia has found in associated with increased morbidity and mortality in different populations during the last decade [7-13]. During the pandemic, its impact on outcomes for the general COVID-19 population has been evaluated in several recent studies [14-16]. Some of them have reported that sarcopenia is a contributing factor to poor prognosis in this population, while others have not found any relationship. In fact, sarcopenia and CAD share some common pathophysiological mechanisms such as atherosclerosis, arterial stiffness, and chronic inflammation. Therefore, we thought that sarcopenia may have a role in the development of poor results in patients with CAD who were diagnosed with COVID-19. Furthermore, sarcopenia may have a long-lasting effect on the results of patients with COVID-19 because it is a relatively chronic clinical entity. Therefore, in this study, we intended to evaluate the effect of sarcopenia on in-hospital acute-term and three-month mid-term follow-up results in this population. We found that sarcopenia is an independent predictor for in-hospital acute adverse outcomes, but it has no long-term effect in this population.

Vascular effects of sarcopenia can be proposed to explain this relationship. It is known that sarcopenia is associated with atherosclerosis and vascular dysfunction detected by several indicators such as arterial stiffness, carotid-intima media thickness, flow-mediated dilatation, endothelial progenitor cell counts [19, 20]. In this study, the group I had higher hs-cardiac troponin I compared to group II which reflects myocardial damage at in-hospital stage of COVID-19. We did not detect any additional adverse result during follow-up. Therefore, we thought that sarcopenia and its vascular effects are a predisposing factor for adverse results only active phase of COVID-19 disease characterized by a hyperinflammatory response. After an active inflammatory phase of COVID-19, chronic vascular effects of sarcopenia do not associate with poor results.

To our knowledge, this is the first study that evaluated the effect of sarcopenia on acute and mid-term results of patients with CAD and COVID-19. Our results have some clinical implications; first, the patients with sarcopenia in this population are at risk for poor prognosis in the acute term. Second, sarcopenia-related vasculopathy may be an etiopathological mechanism for this poor prognosis. Third, the presence of sarcopenia may be used as a criterion in the initial risk assessment of the patients with CAD during the acute term of COVID-19, but not follow-up.

Limitations

This study has some limitations. First, our study population was relatively small. Second, this was not a randomized study. However, it should be accepted that doing a randomized study has some ethical issues during the COVID-19 pandemic. Thus, we tried to eliminate this limitation using age- and gender-matching of groups. Third, we could not assess the vascular function of the study population by an objective method such as arterial stiffness. However, it was unlikely to be done in the pandemic setting, especially in intensive care patients who were intubated. Therefore, further studies are needed to investigate this issue.

CONCLUSION

Sarcopenia is independently associated with acute term in-hospital combined end-points including ICU admission, intubation, and mortality in patients with CAD and COVID-19. But, it does not affect results at a three-month mid-term follow-up.

Authors' Contribution

Study Conception: ME, İZ; Study Design: ME, İZ; Supervision: ME, İZ; Funding: ME, İZ; Materials: ME, İZ; Data Collection and/or Processing: ME, İZ; Statistical Analysis and/or Data Interpretation: ME, İZ; Literature Review: ME, İZ; Manuscript Preparation: ME, İZ and Critical Review: ME, İZ.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

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REFERENCES

1. Zhu N, Zhang D, Wang W, et al A Novel Coronavirus from Patients with Pneumonia in China, 2019. N Engl J Med. 2020;382(8):727-733. doi: 10.1056/NEJMoa2001017.
2. Scoccia A, Gallone G, Cereda A, et al. Impact of clinical and subclinical coronary artery disease as assessed by coronary artery calcium in COVID-19. Atherosclerosis. 2021;328:136-143. doi: 10.1016/j.atherosclerosis.2021.03.041.
3. Angeli F, Marazzato J, Verdecchia P, et al. Joint effect of heart failure and coronary artery disease on the risk of death during hospitalization for COVID-19. Eur J Intern Med. 2021;89:81-86. doi: 10.1016/j.ejim.2021.04.007.
4. Zuin M, Rigatelli G, Bilato C, Rigatelli A, Roncon L, Ribichini F. Preexisting coronary artery disease among coronavirus disease 2019 patients: a systematic review and meta-analysis. J Cardiovasc Med (Hagerstown). 2022;23(8):535-545. doi: 10.2459/JCM.0000000000001343.
5. Cruz-Jentoft AJ, Bahat G, Bauer J, et al. Sarcopenia: revised European consensus on definition and diagnosis. Age Ageing. 2019;48(1):16-31. doi: 10.1093/ageing/afy169.
6. Beaudart C, McCloskey E, Bruyère O, et al. Sarcopenia in daily practice: assessment and management. BMC Geriatr. 2016;16(1):170. doi: 10.1186/s12877-016-0349-4.
7. Bahat G, Ilhan B. Sarcopenia and the cardiometabolic syndrome: a narrative review. Eur Geriatr Med 2016;6:220-223. doi: 10.1016/j.eurger.2015.12.012
8. Bone AE, Hepgul N, Kon S, Maddocks M. Sarcopenia and frailty in chronic respiratory disease. Chron Respir Dis. 2017;14(1):85-99. doi: 10.1177/1479972316679664.
9. Beaudart C, Biver E, Reginster JY, et al. Validation of the SarQoL®, a specific health-related quality of life questionnaire for Sarcopenia. J Cachexia Sarcopenia Muscle. 2017;8(2):238-244. doi: 10.1002/jcsm.12149.
10. De Buyser SL, Petrovic M, Taes YE, et al. Validation of the FNIH sarcopenia criteria and SOF frailty index as predictors of long-term mortality in ambulatory older men. Age Ageing. 2016;45(5):602-608. doi: 10.1093/ageing/afw071.
11. Montano-Loza AJ, Meza-Junco J, Prado CM, et al. Muscle wasting is associated with mortality in patients with cirrhosis. Clin Gastroenterol Hepatol. 2012;10(2):166-673. doi: 10.1016/j.cgh.2011.08.028.
12. Gu DH, Kim MY, Seo YS, et al. Clinical usefulness of psoas muscle thickness for the diagnosis of sarcopenia in patients with liver cirrhosis. Clin Mol Hepatol. 2018;24(3):319-330. doi: 10.3350/cmh.2017.0077.
13. Cawthon PM, Lui LY, Taylor BC, et al. Clinical Definitions of Sarcopenia and Risk of Hospitalization in Community-Dwelling Older Men: The Osteoporotic Fractures in Men Study. J Gerontol A Biol Sci Med Sci. 2017;72(10):1383-1389. doi: 10.1093/gerona/glw327.
14. Kim JW, Yoon JS, Kim EJ, et al. Prognostic Implication of Baseline Sarcopenia for Length of Hospital Stay and Survival in Patients With Coronavirus Disease 2019. J Gerontol A Biol Sci Med Sci. 2021;76(8):e110-e116. doi: 10.1093/gerona/glab085.
15. Moctezuma-Velázquez P, Miranda-Zazueta G, et al. Low Thoracic Skeletal Muscle Area Is Not Associated With Negative Outcomes in Patients With COVID-19. Am J Phys Med Rehabil. 2021;100(5):413-418. doi: 10.1097/PHM.0000000000001716.
16. Feng Z, Zhao H, Kang W, et al. Association of Paraspinal Muscle Measurements on Chest Computed Tomography With Clinical Outcomes in Patients With Severe Coronavirus Disease 2019. J Gerontol A Biol Sci Med Sci. 2021;76(3):e78-e84. doi: 10.1093/gerona/glaa317.
17. China National Health Commission. Diagnosis and Treatment of Pneumonitis Caused by New Coronavirus Avaiable at: http://en.nhc.gov.cn/2020-03/29/c_78469.htm.Accessed May 30,2020.
18. Younus S, Maqsood H, Sattar A, Younas A, Shakeel HA. A novel chest CT severity score in COVID-19 and its correlation with severity and prognosis of the lung disease: A retrospective cohort study. Ann Med Surg (Lond). 2022;82:104692. doi: 10.1016/j.amsu.2022.104692.
19. Amarasekera AT, Chang D, Schwarz P, Tan TC. Does vascular endothelial dysfunction play a role in physical frailty and sarcopenia? A systematic review. Age Ageing. 2021;50(3):725-732. doi: 10.1093/ageing/afaa237.
20. Dvoretskiy S, Lieblein-Boff JC, Jonnalagadda S, Atherton PJ, Phillips BE, Pereira SL. Exploring the Association between Vascular Dysfunction and Skeletal Muscle Mass, Strength and Function in Healthy Adults: A Systematic Review. Nutrients. 2020;12(3):715. doi: 10.3390/nu12030715.

The European Research Journal 2024;10(1):77-83 Original Article

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Use of perioperative thermal camera for the assessment of sympathectomy effectiveness

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ABSTRACT

Objectives: Primary Hyperhidrosis (HH) is the excessive sweating condition in one or more parts of the body. The definitive treatment method is surgical. The most commonly applied surgical method is endoscopic thoracic sympathectomy (ETS). We aimed to investigate the effectiveness of patients with hyperhidrosis by making temperature measurements.

Methods: We analyzed the findings of 30 patients who were diagnosed with HH between January 1, 2019 and November 1, 2022 in Kahramanmaraş Sütçü İmam University Thoracic Surgery Clinic. Preoperative, intraoperative and postoperative hand temperatures of the patients who underwent bilateral endoscopic thoracic sympathectomy were examined by measuring hand temperatures with a thermal camera.

Results: After the evaluation of the examination, it was revealed that there was an increase in the hand temperature measurement values before sympathectomy and after the sympathectomy operation.

Conclusions: We believe that endoscopic thoracic sympathectomy performed by measuring temperature with a thermal camera will increase the comfort and safety of the surgery both on the part of the physician and the patient.

Keywords: Sympathectomy, sweating, thoracal surgery, thermal camera

Primary hyperhidrosis is involuntary excessive sweating condition in one or more parts of the body in case of psychological situations, under stress, and in some seasons. Sweating does not occur during sleeping at nights. The incidence of this condition varies between 1% and 3% in the society [1, 2]. It is observed equally in both sexes, regardless of gender. It has been reported that the incidence of Primary Hyperhidrosis increases in adolescence and young adulthood [2]. It tends to start in early childhood, its severity increases at puberty, and its incidence decreases gradually in elder ages [3, 4].

Although there are different medical and surgical options in its treatment, medical treatment provides temporary relief in many patients. The definitive treatment method for hyperhidrosis is surgical. Efforts to minimize surgical trauma and facilitate a faster healing process have led to the emergence of minimally invasive surgery [5]. With the developing techniques, the most applied surgical treatment is endoscopic thoracic sympathectomy [6].

In this article, we aimed to share the thermal camera measurement results conducted before and after sympathectomy in patients to whom we applied ETS in the light of the literature.

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METHODS

This study was conducted in accordance with the Declaration of Helsinki. Before participating in the study, ethical approval was obtained from the Non-Invasive Clinical Research Ethics Committee of our University on 06.03.2019 (Session no: 2019/5, Decision no: 4).

Design

The findings of patients to whom ETS was applied with the diagnosis of primary hyperhidrosis between 01.05.2018 and 01.10.2022 in Thoracic Surgery Clinic of our University were analyzed. Posteroanterior (PA) and lateral chest radiography and electrocardiography (ECG) were routinely taken for all patients before the operation. After the patient was intubated, the measurements of the patient were made with a thermal temperature camera (FLIR TG 165-x Oregon, U.S.) before and after the sympathectomy is performed.

Surgical procedure

The patients were operated in the semi-sitting position in a way that both arms were in abduction at 90 degrees. The operation was performed under general anesthesia using a double-lumen endotracheal tube. After one lung ventilation, the thoracic cavity was entered with blunt dissection after 1.5 cm skin incision was applied from the mid-axillary line. A port was placed in the hemithorax for which the operation was made. According to the patient's complaint, if it is facial HH, the 2nd sympathetic nerve was resected by being burned with hook electrocautery, and if it is palmar HH, 3rd and 4th sympathetic ganglions were resected by being burned with hook electrocautery, and if it is axillary HH, the 4th and 5th sympathetic ganglions were resected by being burned with hook electrocautery. After applying sympathectomy, it was aimed to prevent recurrence development due to alternative sympathetic nerve connections such as the kuntz nerve, and the inner surface of the rib at the operation level was drawn with a cautery about 2-3 cm from the sympathetic nerve level to the lateral. Into the pleural-thoracic cavity and after placing the other end in a container filled with saline, the lung was ventilated and it was continued until the air outlet ceased. After the air outlet ceased, the nelaton catheter was withdrawn and the hemithorax inlet was sutured. The same process was applied to the other hemithorax. The operation was ended without placing a chest tube. Patients were evaluated by taking postoperative chest X-ray in the operating room, and they were taken to the hospital service.

Statistical Analysis

The data obtained were analyzed using Graphpad Prism version 5 (GraphPad Software Inc., San Diego, CA, USA). After analysis of variance, Student's t-test was performed. Data were presented as mean ± standard error. P-values <0.05 were considered significant.

RESULTS

Fifteen of 30 patients were male, and 15 of them were female. The mean age of patients operated for palmar HH was 21. 8 (range: 14-33) years, 2 of them were operated for axillary HH, and 20 of them were operated for axillary and palmar HH. All patients except one patient underwent bilateral sympathectomy, and totally 58 ETSs were applied in the same session.

Average operation time was 20 minutes. The hospital stay of the patients was 0.93 days. All patients except one were taken to the thoracic surgery service without a chest tube. While 5 patients were discharged on the same day, 24 patients were discharged on the next day after the PA chest X-ray. A 30-year-old male patient was discharged on the 3rd day. No mortality was observed in the patients. While the mean preoperative hand temperature measurement values were 28.59±2.304 ºC for the right hand before the operation, the temperature value for the left hand was measured to be 28.61±2.246 ºC. After intubation and before applying sympathectomy (perioperative), the mean hand temperature measurements were 28.472±0.287 ºC for the right hand and it was measured to be 28.41±2.267 ºC for the left hand. After the sympathectomy operation (postoperative), while the mean temperature of the right hand was measured to be 29.50±2.519 ºC, the mean temperature of the left hand was 29.51±2.525 ºC.

The female body mass index was calculated to be 22.39±2.74 (range: 19.96-29.76) kg/m^2 and body mass index of the males was calculated to be 23.95±2.67 (range: 20.52-31.40) kg/m^2. There was no statistical difference between the two groups. In a patient with a high body mass index (31.97 kg/m^2), the operation could not be performed because the sympathetic nerve

could not be seen. Hand temperature measurements made throughout the entire intervention are shown in Table 1.

Although the postoperative temperature measurements were higher than preoperative measurements, no statistical difference was found between them (P>0.05). In addition, no statistical significance was found between the right- and left-hand temperature measurements of the patients (P>0.05) (Table 1).

Table 1. Hand temperature measurement values

n=30	Preoperative right hand (ºC)	Preoperative left hand (ºC)	Perioperative right hand (ºC)	Perioperative left hand (ºC)	Postoperative right hand (ºC)	Postoperative left hand (ºC)
1.	27.2	27.3	27.0	27.1	27.7	27.8
2.	25.2	25.3	25.1	25.2	25.9	26
3.	29.3	29.5	29.0	29.1	30.1	30.1
4.	32.3	32.3	32.1	32.0	33.5	33.5
5.	32.9	32.7	32.9	32.6	34.2	34.5
6.	29.2	29.1	29.1	29.0	29.7	29.7
7.	25.8	25.9	25.4	25.6	26.6	26.6
8.	30.1	30.2	30.0	29.9	31.9	31.9
9.	32.3	32.1	32.2	32.0	33.5	33.4
10.	32.9	32.7	32.7	32.6	34.2	34.2
11.	29.2	29.3	29.0	29.1	29.7	29.7
12.	25.8	25.8	25.8	25.7	26.6	26.6
13.	31.4	31.2	31.3	30.9	32.1	32.2
14.	28.4	28.4	28.3	28.4	29.2	29.2
15.	27.3	27.4	27.1	27.2	28.0	27.9
16.	30.2	30.3	30.0	30.1	31.8	31.7
17.	25.7	25.9	25.7	25.6	26.4	26.4
18.	30.4	30.4	30.1	30.0	30.9	30.8
19.	26.3	26.4	26.3	26.1	26.9	26.9
20.	29.4	29.4	29.3	29.1	30.3	30.3
21.	25.9	25.9	25.9	25.8	26.8	26.8
22.	28.2	28.2	28.1	28.0	30.1	30.2
23.	30.3	30.3	30.1	30.1	31.4	31.5
24.	25.9	25.8	25.9	25.8	26.5	26.4
25.	26.4	26.5	26.4	26.3	27.2	27.2
26.	27.1	27.2	27.0	27.0	27.9	27.8
27.	26.8	26.8	26.7	26.0	27.6	27.7
28.	27.3	27.4	27.3	27.2	27.9	27.8
29.	30.1	30.0	30.0	30.0	31.2	31.3
30.	29.7	29.5	29.6	29.6	29.6	29.7
Mean±SD	28.59±2.304	28.61±2.246	28.472±0.287	28.41±2.267	29.50±2.519	29.51±2.525

SD=standard deviation

DISCUSSION

Thermoregulation, and thus sweat production, is regulated by the preoptic region of the anterior hypothalamus, cerebral cortical structures, and the sympathetic nervous system. Thermal receptors are found in the internal organs and hypothalamus in the body as well as in all parts of the body such as brain stem, spinal cord, and skin. Afferent nerve fibers carry signals to the hypothalamus via pathways in the lateral part of the medullaspinalis. Efferent sympathetic sudomotor pathways for thermoregulation pass from the cerebral cortex to the hypothalamus, from the hypothalamus to the medulla oblongata, and proceed along the medulla spinalis cornu lateralis. From here, it passes into the intermediolateral cell nuclei of the paravertebral sympathetic ganglion of the medulla spinalis, and then ends with the stimulation of unmyelinated, postganglionic sympathetic class C nerve fibers and postsynaptic muscarinic receptors of the eccrine sweat gland [7, 8].

Sweating is a complex and physiological process. Both emotional sweating and thermoregulatory sweating are triggered by sympathetic cholinergic nerves. Emotional sweating is regulated by the limbic system Cingulate cortex's anterior and the hypothalamus. The parts that control emotional sweating are more commonly the armpits, palms, soles, forehead, and scalp. Existence of excess eccrine sweat glands, especially in the hands, feet and armpits, as a result of stimuli originating from the central nervous system is called hyperhidrosis. Eccrine sweat glands are believed to be responsible for primary HH. Eccrine sweat glands are the only sweat glands found in the palms [8, 9].

In our study, the affected parts of patients with HH were palm and axilla. The rates of coming to the hospital of female and male patients were equal in our study, and the patients came to our clinic most commonly due to palmar HH (28 patients) (93%).

Hyperhidrosis not only makes the life of the individual difficult, but also has negative effects on the social life of the individual. In its treatment; drugs, botox application and iontophoresis, and surgical treatment methods are used. Surgical treatment is the definitive treatment method. Today, the most frequently applied surgical method is ETS [6]. In our study, ETS was applied to 30 patients who came to our clinic with the complaint of HH. However, only for 1 patient, the desired result was not obtained.

In the classical management of anesthesia in minor endoscopic surgery procedures, a double-lumen tube (DLT) or endobronchial blocker (EBB) is used as an airway device to provide single-lung ventilation. Although ETS, which has a high efficiency and chance of success, in one lung ventilation with DLT and EBB; damage to tracheobronchial structures, bleeding, malposition of the tube, dependent and non-dependent lung barotrauma, volutrauma, atelectotrauma and bio-

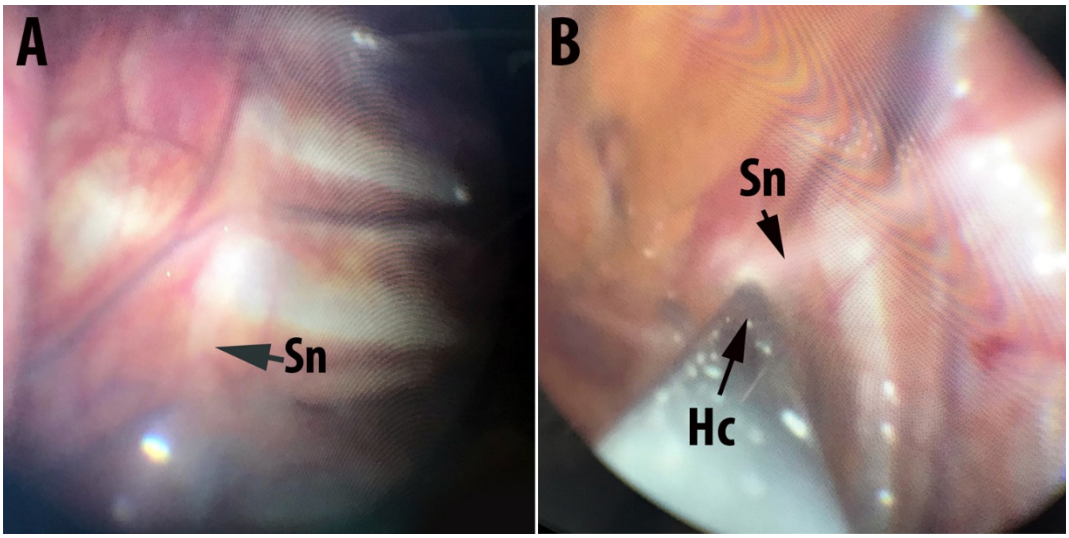

Fig. 1. (A) Image of the Sympathetic Nerve on the Chest Wall, (B) The moment of cauterization of the sympathetic nerve (Sn=Sympathetic nerve, Hc=Hook Electrocautary).

trauma and postoperative respiratory complications may occur [10-13]. No trauma related to intubation was seen in our study. All patients were extubated and taken to the thoracic surgery service without any problem.

The fact that ETS is a minimally invasive method has made it a standard, well-tolerated option in the treatment of the disease [14, 15]. ETS surgery, which was initially performed with the use of more than one port, has been applied as a single port in recent years, with the popularity of the single port approach in other surgical fields. In our clinic, sympathectomy, which was initially performed by using double port, has recently started to be performed by using the single port, and the single port was applied to all patients in this study.

In ETS, there are different applications in terms of the type of blockage of the sympathetic nerve as well as the way of entering the thorax [16, 17]. Despite these differences, in a study conducted; it was found that there was no significant difference between applications such as burning, cutting and clipping in terms of postoperative complications and patient satisfaction [18]. The thoracic sympathetic chain extends below the parietal pleura on both sides of the spine and consists of 10-12 ganglions. ETS is performed at certain levels by interrupting this ganglion and the pathways between them [16, 17]. In our clinic, the operations were completed by interrupting the sympathetic ganglion using ETS hook electrocautery (Fig. 1). Although there are debates and conflicting opinions about the best level for sympathetic chain resection, it is recommended to remove the T3 or T3-4 ganglion for palm sweating, T4 for armpit sweating, and T2 ganglion for facial sweating [19]. In our study, ablation was applied by making operation to T3 and T4 ganglions for palmar HH, and to T4-T5 ganglions for axillary HH.

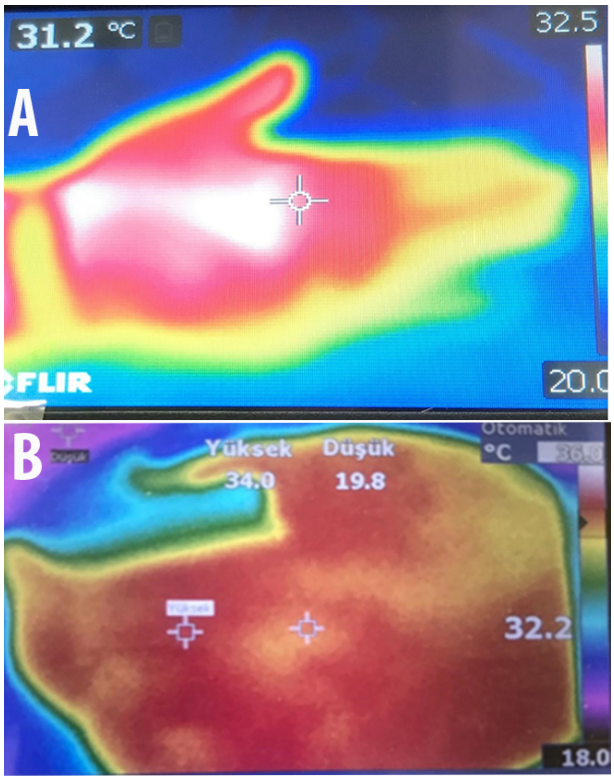

Fig. 2. (A) Images of hand temperature measurement before sympathectomy and (B) after sympathectomy.

Preferring the thoracoscopic approach instead of traditional surgery and avoiding additional port incision cause less muscle tissue and intercostal nerve damage. This helps to feel less postoperative pain, increase patient satisfaction, and help the patient to return to social life more quickly [20, 21]. In particular, hand sweating is a condition that negatively affects social life, but can be treated with low morbidity and high success rates. Success rates are reported to be between 90-100% for hand sweating [22-24]. In our study, it was seen that the satisfaction rate in patient feedback information was 96%. We think that the fact that patients return to social and business life early by being discharged on the next day or on the same day is effective in the high patient satisfaction rates.

Before ETS surgery, patients should be informed in detail about possible complications and their consent should be obtained [25].

The most common complication of ETS is compensatory sweating, and its incidence varies between 3% and 98% among all different surgical approaches in the literature [2, 26]. However, it was determined that the complaints of all patients disappeared 6 months after the operation. All patients had a low body mass index except for 1 patient we operated. Sympathectomy could not be performed in the patient who was not weak, since the adipose tissue did not allow us to see the sympathetic nerve.

In a study, an increase in palm temperature was observed, which was detected by cutting the sympathetic chain during performing the operation [27]. In parallel with this study, a significant increase in hand temperature values was observed after sympathectomy in the study we conducted in our clinic (Fig. 2).

CONCLUSION

We believe that endoscopic sympathectomy, which is a practical and reliable method with a short discharge time and returning to normal life on the same day as well as being more cautious in patients with a high body mass index and performing the operation by measuring the temperature with a thermal camera will increase even more the patient satisfaction and the reliability as well as the prevalence of this method in addition to increasing the comfort of the physician in surgery.

Authors' Contribution

Study Conception: AA; Study Design: AY; Supervision: AA; Funding: AY; Materials: AA; Data Collection and/or Processing: AA, AY; Statistical Analysis and/or Data Interpretation: AA; Literature Review: AA; Manuscript Preparation: AA and Critical Review: AA.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

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REFERENCES

1. Acıpayam A, Sayan M, Tokur M. Single-port endoscopic thoracic sympathectomy in cases of primary hyperhidrosis: single-center experience. Eur Arch Med Res. 2022;38(3):178-182. doi: 10.4274/eamr.galenos.2021.28199.
2. Cerfolio RJ, De Campos JR, Bryant AS, et al. The Society of Thoracic Surgeons' expert consensus for the surgical treatment of hyperhidrosis. Ann Thorac Surg 2011;91(5):1642-1648. doi: 10.1016/j.athoracsur.2011.01.105.
3. Shargall Y, Spratt E, Zeldin RA. Hyperhidrosis: what is it and why does it occur? Thorac Surg Clin. 2008;18(2):125-32, v. doi: 10.1016/j.thorsurg.2008.01.001.
4. Solish N, Wang R, Murray CA. Evaluating the patient presenting with hyperhidrosis. Thorac Surg Clin. 2008;18(2):133-140. doi: 10.1016/j.thorsurg.2008.01.002.
5. Barutcu AG, Klein D, Kilian M, et al. Long-term follow-up after single-incision laparoscopic surgery. Surg Endosc. 2020;34(1):126-132. doi: 10.1007/s00464-019-06739-5.
6. Gürkök S, Genç O. [Hyperhidrosis (Endoscopic Thoracal Sympathectomy)]. Turkiye Klinikleri J Thor Surg-Special Topics. 2009;2(2):62-9. [Article in Turkish]
7. Chen X, Gasecka P, Formanek F, Galey JB, Rigneault H. In vivo single human sweat gland activity monitoring using coherent anti-Stokes Raman scattering and two-photon excited autofluorescence microscopy. Br J Dermatol. 2016;174(4):803-12. doi: 10.1111/bjd.14292.
8. Nawrocki S, Cha J. The etiology, diagnosis, and management of hyperhidrosis: a comprehensive review: etiology and clinical work-up. J Am Acad Dermatol. 2019;81(3):657-666. doi: 10.1016/j.jaad.2018.12.071.
9. Eisenach JH, Atkinson JL, Fealey RD. Hyperhidrosis: evolving therapies for a well-established phenomenon. Mayo Clin Proc. 2005;80(5):657-666. doi: 10.4065/80.5.657. Erratum in: Mayo Clin Proc. 2005;80(6):828.
10. Knoll H, Ziegeler S, Schreiber JU, et al. Airway injuries after one-lung ventilation: a comparison between double-lumen tube and endobronchial blocker: a randomized, prospective, controlled trial. Anesthesiology. 2006;105(3):471-477. doi: 10.1097/00000542-200609000-00009.
11. Lohser J, Slinger P. Lung injury after one-lung ventilation. Anesth Analg. 2015;121(2):302-318. doi: 10.1213/ANE.0000000000000808.
12. Soto R. Incidence and risk factors for postoperative residual neuromuscular blockade. Curr Anesthesiol Rep 2020;10:81-84. doi: 10.1007/s40140-020-00386-6.
13. van de Pas JM, van der Woude MC, Belgers HJ, Hulsewé KW, de Loos ER. Bronchus perforation by EZ-BlockerTM endobronchial blocker during esophageal resection after neoadjuvant chemoradiation -a case report. Korean J Anesthesiol. 2019;72(2):184-187. doi: 10.4097/kja.d.18.00237.
14. Kaplan T, Ekmekçi P, Koçer B, Han S. Bilateral sympathicotomy for hyperhidrosis without using single-lung ventilation. Turk J Med Sci. 2015;45(4):771-774. doi: 10.3906/sag-1405-51.
15. Krasna MJ, Demmy TL, McKenna RJ, Mack MJ. Thoracoscopic sympathectomy: the U.S. experience. Eur J Surg Suppl. 1998;(580):19-21. doi: 10.1080/11024159850191085.
16. Hornberger J, Grimes K, Naumann M, et al; Multi-Specialty Working Group on the Recognition, Diagnosis, and Treatment of Primary Focal Hyperhidrosis. Recognition, diagnosis, and treatment of primary focal hyperhidrosis. J Am Acad Dermatol. 2004;51(2):274-286. doi: 10.1016/j.jaad.2003.12.029.
17. Ro KM, Cantor RM, Lange KL, Ahn SS. Palmar hyperhidrosis: evidence of genetic transmission. J Vasc Surg. 2002 Feb;35(2):382-386. doi: 10.1067/mva.2002.119507.
18. Whitson BA, Andrade RS, Dahlberg PS, Maddaus MA. Evolution of clipping for thoracoscopic sympathectomy in symptomatic hyperhidrosis. Surg Laparosc Endosc Percutan Tech. 2007;17(4):287-290. doi: 10.1097/SLE.0b013e318074c350.
19. Dewey TM, Herbert MA, Hill SL, Prince SL, Mack MJ. One-year follow-up after thoracoscopic sympathectomy for hyperhidrosis: outcomes and consequences. Ann Thorac Surg. 2006;81(4):1227-1232; discussion 1232-1233. doi: 10.1016/j.athoracsur.2005.11.006.
20. Chen YB, Ye W, Yang WT, et al. Uniportal versus biportal video-assisted thoracoscopic sympathectomy for palmar hyperhidrosis. Chin Med J (Engl). 2009;122(13):1525-1528.
21. Shanthanna H, Aboutouk D, Poon E, et al. A retrospective study of open thoracotomies versus thoracoscopic surgeries for

persistent postthoracotomy pain. J Clin Anesth. 2016;35:215-220. doi: 10.1016/j.jclinane.2016.07.029.
22. Görür R, Yıldızhan A, Türüt H, et al. [Analysis of 530 sympathectomy operations performed for palmar hyperhidrosis and long-term results]. Turk Gogus Kalp Damar Cerrahisi Derg. 2009;17(1):28-32. [Article in Turkish]
23. Sugimura H, Spratt EH, Compeau CG, Kattail D, Shargall Y. Thoracoscopic sympathetic clipping for hyperhidrosis: long-term results and reversibility. J Thorac Cardiovasc Surg. 2009;137(6):1370-376; discussion 1376-1377. doi: 10.1016/j.jtcvs.2009.01.008.
24. Weksler B, Blaine G, Souza ZB, Gavina R. Transection of more than one sympathetic chain ganglion for hyperhidrosis increases the severity of compensatory hyperhidrosis and decreases patient satisfaction. J Surg Res. 2009;156(1):110-115. doi: 10.1016/j.jss.2009.04.015.
25. Currie AC, Evans JR, Thomas PR. An analysis of the natural course of compensatory sweating following thoracoscopic sympathectomy. Int J Surg. 2011;9(5):437-439. doi: 10.1016/j.ijsu.2011.04.006.
26. Li X, Chen R, Tu YR, et al. Epidemiological survey of primary palmar hyperhidrosis in adolescents. Chin Med J (Engl). 2007;120(24):2215-2217.
27. Dural K, Özpolat B, Günal N, Zorlu E, Kür S. [The relationship between thoracic sympathectomy effectiveness and palm heat measurement]. KÜ Tıp Fak Derg. 2020;22(3):288-292. doi:10.24938/kutfd.658166. [Article in Turkish]

The European Research Journal 2024;10(1):84-91 Original Article

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Evaluating the cross-sectional area of the internal jugular vein in Turkish adults using ultrasonography

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ABSTRACT

Objectives: To assess the cross-sectional area (CSA) of the right and left internal jugular veins (IJVs) in the adult Turkish population.

Methods: The CSA of the IJVs was quantified at three anatomical landmarks: below the angle of the mandible, at the level of the cricothyroid membrane, and in the supraclavicular region. Measurements were taken under three conditions: at rest, during a deep breath hold, and throughout the Valsalva maneuver.

Results: The study encompassed 321 volunteers with a mean age of 30.40±7.75 years. At the anatomical landmarks of the angle of the mandible, cricothyroid, and supraclavicular regions, the CSA of the IJV in men was consistently larger than in women during rest, deep breath hold, and the Valsalva maneuver. During both the deep breath hold and the Valsalva maneuver at these landmarks, the right CSA of the IJV in both genders was greater than the left CSA. In both males and females, the CSA of the IJV at the supraclavicular location was superior to that at both the angle of the mandible and the cricothyroid regions. The CSA at the cricothyroid regions surpassed that at the angle of the mandible.

Conclusions: The CSA of the IJV was found to be the largest in the right supraclavicular region during the Valsalva maneuver in both genders. By accurately measuring the CSA of the IJV at the angle of the mandible, cricothyroid, and supraclavicular anatomical landmarks during a deep breath hold and the Valsalva maneuver, potential interventional and surgical risks can be mitigated.

Keywords: Internal jugular vein, ultrasonography, cross-sectional area

The internal jugular vein (IJV) is a major vein responsible for draining blood from the head and neck. Due to its potential as a large and superficial vein, it is frequently used for central venous catheter insertion to deliver intravenous medications, facilitate hemodialysis, volume resuscitation, and administer drugs and blood products [1]. It serves as a significant anatomical landmark in the head and neck region, especially during the dissection of the cervical lymph nodes in oncological surgeries [2]. In recent years, the diameter of the IJV has been recognized as a potential predictor of central venous pressure [3-5].

Ultrasonography (USG) has become the primary imaging modality for vascular diseases due to its non-invasive nature, ease of application, cost-effectiveness, and patient tolerance. It also aids in the catheterization process [6]. Recently, the use of ultrasound guidance has made it possible to insert a catheter safely and easily into the IJV. However, in emergency situations or in settings without access to an ultrasound machine,

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the procedure relies on traditional methods based on external anatomical landmarks [7]. Moreover, many surgeons prefer using anatomical landmarks over ultrasound guidance, as three-dimensional, anatomy-based procedures play a pivotal role in the training process for surgeons, especially for the insertion of percutaneous central venous catheters [8].

A larger vein diameter is associated with a higher success rate of catheter procedures on the first attempt. It is well-documented that the size of the internal jugular vein changes in response to respiratory variations [9]. An increased cross-sectional area (CSA) of the IJV has been linked to enhanced cannulation success and reduced mechanical complications [10].

Blood flow alterations, attributable to different respiratory phases, maneuvers, and breathing patterns, can be effectively studied using ultrasonography. When assessing jugular vein diameters, it is more accurate to measure using USG which accounts for respiratory changes [11].

This study aimed to assess the CSA of the right and left IJV at three anatomical landmarks below the angle of the mandible, at the level of the cricothyroid membrane, and in the supraclavicular region. under three conditions: at rest, during a deep breath hold, and throughout the Valsalva maneuver, within the adult Turkish population sample.

METHODS

Study Design and Setting

This single-center prospective observational study was conducted with volunteers between 1 September and 1 November 2023, following the approval of the local Ethics Committee (date: 13.09.2023, no:2023-537) and in line with the Declaration of Helsinki. Each participant provided written informed consent after receiving a comprehensive explanation of the study's objectives and protocol.

Study Population

Healthy volunteers aged 18 and older were included in the study. Exclusion criteria encompassed individuals with known chronic diseases, abnormal neck anatomy, a history of neck puncture or surgery, pregnancy, a history of alcohol abuse, or those who were unable to communicate effectively. The study co-

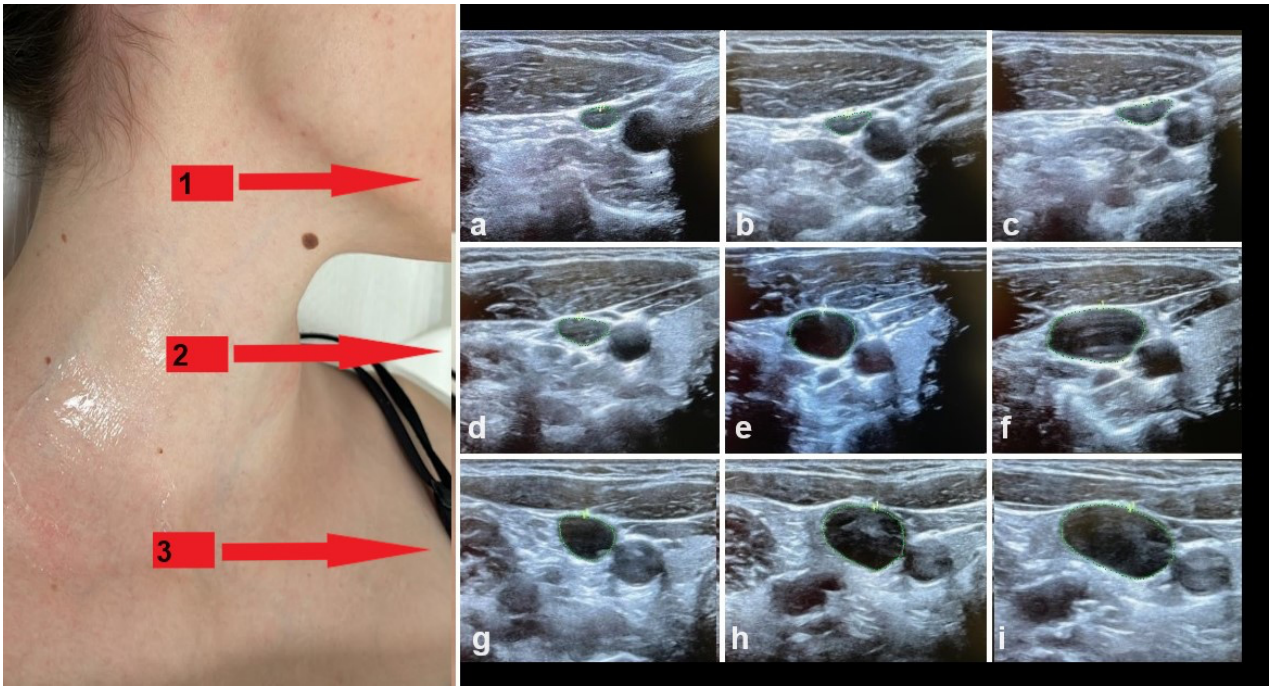

Fig. 1. The measurement of the cross-sectional area at three anatomical landmarks: below the angle of the mandible (1), at the level of the cricothyroid membrane (2), and in the supraclavicular region (3) are taken under three conditions: at rest (a, d, g), during a deep breath hold (b, e, h), and throughout the Valsalva maneuver (c, f, i).

hort comprised hospital staff, as well as spouses and relatives of patients undergoing treatment at our center.

Study Protocol and Measurements

The age (years), height (centimeters), and weight (kilograms) of the volunteers were documented. The body mass index (BMI) was computed using the formula: weight (in kilograms) divided by the square of height (in meters^2).

The CSA of the right and left IJV of the volunteers was assessed using a LOGIQ E10 ultrasound device

Table 1. The evaluation of the volunteer's cross-sectional area of the internal jugular vein is based on the anatomical locations in their necks and their responses to respiratory changes and the Valsalva maneuver

Anatomic landmark	Condition	Gender	Right (mean±SD)	Left (mean±SD)	P value
Angle of the mandible	Rest	Female	2.09±0.15	2.00±0.11	**<0.001**
		Male	2.17±0.14	2.08±0.20	**<0.001**
		P value	**<0.001**	**<0.001**	
	Deep breath - hold	Female	2.69±0.24	2.56±0.26	**<0.001**
		Male	2.75±0.21	2.62±0.24	**<0.001**
		P value	**<0.001**	**<0.001**	
	Valsalva maneuver	Female	2.86±0.42	2.64±0.48	**<0.001**
		Male	3.27±0.20	3.06±0.21	
		P value	**<0.001**	**<0.001**	**<0.001**
Cricothyroid	Rest	Female	2.44±0.23	2.30±2.24	**<0.001**
		Male	2.67±0.28	2.59±0.29	**<0.001**
		P value	**<0.001**	**<0.001**	
	Deep breath - hold	Female	2.77±0.23	2,68±0.25	**<0.001**
		Male	2.93±0.16	2.85±0.21	**<0.001**
		P value	**<0.001**	**<0.001**	
	Valsalva maneuver	Female	3.03±0.27	2.88±0.27	**<0.001**
		Male	3.11±0.19	2.94±0.24	**<0.001**
		P value	**<0.001**	**<0.001**	
Supraclavicular	Rest	Female	2.73±0.35	2.44±0.25	**<0.001**
		Male	3.20±0.22	2.83±0.33	**<0.001**
		P value	**<0.001**	**<0.001**	
	Deep breath - hold	Female	2.90±0.21	2.80±0.18	**<0.001**
		Male	3.24±0.16	3.05±0.21	**<0.001**
		P value	**<0.001**	**<0.001**	
	Valsalva maneuver	Female	3.18±0.26	3.03±0.27	**<0.001**
		Male	3.45±0.20	3.25±0.19	**<0.001**
		P value	**<0.001**	**<0.001**	

SD=standard deviation

(GE Healthcare) equipped with a 7 MHz linear probe operating at a frequency range of 4.0-20 MHz. During the examination, the volunteers' heads were rotated 15° contralateral to the cervical region being examined, ensuring clinical stability. Care was taken to apply the ultrasound probe gently to prevent distortion of the underlying low-pressure venous structures. A ten-year, experienced radiologist conducted the assessment of the IJV's CSA.

Consistent with existing literature, the CSA of the right and left IJV (m^2) was measured at three anatomical landmarks: below the angle of the mandible, at the level of the cricothyroid membrane, and in the supraclavicular region. Measurements were taken under three conditions: at rest, during a deep breath hold, and throughout the Valsalva maneuver, with the volunteers in a supine position [12] (Fig. 1).

Statistical Analysis

Analyses were conducted using IBM SPSS version 20 (Chicago, IL, USA). Descriptive statistics for continuous data were presented as mean±standard deviation, while categorical data were expressed as numbers and percentages. Comparisons of the CSA of the IJV across gender, side, region, and respiratory conditions were made using a two-way analysis of variance (ANOVA). The relationship between the CSA of the IJV with age and BMI was examined using Pearson's correlation coefficient. A P-value of less than 0.05 was considered statistically significant.

RESULTS

The study encompassed 321 volunteers with an average age of 30.40±7.75 years, ranging from 18 to 46 years. The mean BMI was 21.77±1.51 kg/m^2, with a range of 18.7 to 26 kg/m2. At the anatomical landmarks of the angle of the mandible, cricothyroid, and supraclavicular regions, during conditions of rest, deep breath hold, and the Valsalva maneuver, the CSA of the IJV in men was consistently larger than in women (P< 0.001 for all conditions). Specifically, at the angle of the mandible, the left CSA of the IJV in men was greater than in women during rest, deep breath hold, and the Valsalva maneuver (P<0.001, P<0.001 and P<0.001, respectively). In the cricothyroid region, the left CSA of the IJV in men was larger than in women during these conditions (P<0.001, P<0.001 and P<0.001, respectively). Similarly, in the supraclavicular region, the left CSA of the IJV in men exceeded that of women during all three conditions (P<0.001 for each) (Table 1).

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In both genders, during rest and deep breath hold, the CSA of the IJV in the right and left supraclavicular regions was found to be higher than both the right and left angle of the mandible and the right and left cricothyroid regions (P<0.001 for all). Additionally, the CSA in the right and left cricothyroid regions was greater than that of the right and left angles of the mandible (P<0.001 for all). (Table 2).

There was no significant difference in the CSA of the right and left IJV across all anatomical locations during rest, deep breath hold, and the Valsalva maneuver with respect to age and BMI (P>0.05 for all) (Table 3).

DISCUSSION

The knowledge of the CSA of IJV plays a pivotal role in patient management and treatment across various medical fields. This knowledge is particularly vital for radiologists, emergency physicians, surgeons, and anesthetists due to its clinical implications. General asymmetry between the right and left sides of the body is a well-recognized phenomenon, and this extends to the diameters of certain arteries and veins, which can also exhibit side-to-side variations [13]. Factors such as age, demographics, and respiratory changes might contribute to the diverse measurements reported in sci-

Table 2. The volunteer's cross-sectional area of the internal jugular vein is evaluated based on their gender, anatomical locations in the neck, and their responses to respiratory changes and the Valsalva maneuver.

Condition	Localization	Gender	Anatomic landmark			P value
			Angle of the mandible (mean±SD)	Cricothyroid (mean±SD)	Supraclavicular (mean±SD)	
Rest	Right	Female	2.09±0.15	2.44±0.23	2.73±0.35	**<0.001**
		Male	2.17±0.14	2.67±0.28	3.20±0.22	**<0.001**
		P value	**<0.001**	**<0.001**	**<0.001**	
	Left	Female	2.00±0.11	2.30±2.24	2.44±0.25	**<0.001**
		Male	2.08±0.20	2.59±0.29	2.83±0.33	**<0.001**
		P value	**<0.001**	**<0.001**	**< 0.001**	
Deep breath - hold	Right	Female	2.69±0.24	2.77±0.23	2.90±0.21	**<0.001**
		Male	2.75±0.21	2.93±0.16	3.24±0.16	**<0.001**
		P value	**<0.001-**	**<0.001**	**<0.001**	
	Left	Female	2.56±0.26	2.68±0.25	2.80±0.18	**<0.001**
		Male	2.62±0.24	2.85±0.21	3.05±0.21	**<0.001**
		P value	**<0.001**	**<0.001**	**<0.001**	
Valsalva maneuver	Right	Female	2.86±0.42	3.03±0.27	3.18±0.26	**<0.001**
		Male	3.27±0.20	3.11±0.19	3.45±0.20	**<0.001**
		P value	**<0.001**	**<0.001**	**<0.001**	
	Left	Female	2.64±0.48	2.88±0.27	3.03±0.27	**<0.001**
		Male	3.06±0.21	2.94±0.24	3.25±0.19	**<0.001**
		P value	**<0.001**	**<0.001**	**<0.001**	

SD=standard deviation

entific literature [9]. Recognizing these potential variations is crucial, especially when considering adult groups within the healthy Turkish population.

Yoon *et al*. [14] observed that the mean CSA of the right and left IJV were 165±81 mm2 and 119±57 mm2, respectively, highlighting a relatively larger CSA for the right IJV compared to the left. Similarly, Botero *et al*. [15] reported a greater CSA for the right IJV than the left. Such asymmetry between the right and left IJVs can be attributed to the differential blood drainage patterns through the dural venous sinuses, often resulting in the right IJV being larger than its left counterpart [16]. As the IJVs descend towards the thorax, the cumulative blood flow increases, leading to the largest CSA observed at the lower cervical levels. Furthermore, the CSA of the right IJV has been consistently reported to be significantly larger than the left across all cervical levels [17]. Consistent with these findings, our study also determined that the CSA of the right IJV was significantly larger than the left at all examined levels.

Although the IJV is often represented as having a cylindrical shape, some studies suggest that it assumes a conical form, decreasing in size from the subclavian vein towards the cranial vault [17, 18]. However, in a study by Jeon *et al*. [7], where the CSA of the right and left IJV was measured using computed tomography at the levels of the hyoid bone, cricoid cartilage, and the first thoracic vertebra, the IJV was observed to have a rhomboid shape. This shape was larger at the middle level and tapered both above and below [7]. It's noteworthy that in this study, the median age of the

Table 3. Pearson correlation coefficients for age and body mass index variables

Anatomic landmark	Condition	Localization	Age		Body mass index	
			r*	P value	r*	P value
Angle of the mandible	Rest	Right	0.054	0.106	0.109	0.133
	Rest	Left	0.057	0.109	0.058	0.301
	Deep breath - hold	Right	0.029	0.601	0.109	0.133
	Deep breath - hold	Left	0.025	0.661	0.104	0.063
	Valsalva maneuver	Right	0.090	0.106	0.108	0.053
	Valsalva maneuver	Left	0.047	0.108	0.113	0.137
Cricothyroid	Rest	Right	0.071	0.214	0.066	0.239
	Rest	Left	0.054	0.110	0.019	0.735
	Deep breath - hold	Right	0.036	0.658	0.065	0.249
	Deep breath - hold	Left	0.031	0.558	0.012	0.825
	Valsalva maneuver	Right	0.070	0.212	-0.074	0.189
	Valsalva maneuver	Left	0.015	0.786	-0.070	0.214
Supraclavicular	Rest	Right	0.064	0.145	-0.059	0.290
	Rest	Left	0.067	0.264	0.018	0.743
	Deep breath - hold	Right	0.063	0.261	-0.018	0.742
	Deep breath - hold	Left	0.082	0.144	0.064	0.255
	Valsalva maneuver	Right	0.020	0.723	-0.094	0.094
	Valsalva maneuver	Left	0.045	0.420	-0.053	0.344

patients was 65.0, many had chronic illnesses, a history of chemotherapy, and not all patients were in the same physiological conditions. Moreover, the measurements were taken during full inspiration. In our study, we found that the CSA of the IJV in the supraclavicular region was higher than at the angle of the mandible and cricothyroid, irrespective of gender. Another CT-based study reported a larger CSA in males compared to females, though the difference was not statistically significant. Contrarily, in our study, across all anatomical landmarks, the CSA of IJV values for males were statistically higher than those for females. Zamboni *et al*. [19] investigated the impact of respiration on intracranial venous flow and demonstrated that deep inspiration, in comparison to regular breathing, augments the blood return from the brain. Judickas *et al*. [19] observed a significant increase in the CSA of both the RIJV and LIJV during a deep breath hold. Both studies observed similar results, with an even more pronounced increase in CSA, during the Trendelenburg maneuver. Furthermore, hypothesized that the elevated intra-abdominal pressure, pushing the diaphragm upwards, leads to increased intrathoracic and right intracardiac pressures. This rise in intra-abdominal pressure was thought to be associated with a larger CSA [10]. In our study, the CSA of the IJV was elevated during the Valsalva maneuver across all anatomical landmarks.

Both age and BMI have been identified as significant factors influencing the size of blood vessels [7]. Research by Belov *et al*. [20] indicated that the CSA of the IJV in the lower neck tends to increase with age in both men and women. Furthermore, as BMI rises, there's a corresponding expansion of the IJVs, a trend that persists irrespective of the individual's age [20]. Another study by Magnano *et al*. [17] highlighted that after adjusting for factors related to cardiovascular health, the IJV's CSA showed a notable increase with age. This growth was more distinct in the right IJV than its left counterpart and was more pronounced in males. Studies postulated that as people age, the gradual increase in BMI might be a contributing factor to

the enhanced CSA observed in the elderly [17]. In contrast, this study did not observe any notable variations in the IJV's CSA across different anatomical points during various respiratory conditions when considering age or BMI. This discrepancy might be due to our participants being generally younger and having a lower average BMI.

Limitations

A significant strength of this study is the inclusion of a large number of healthy volunteers. However, our study does have certain limitations. While the USG utilized offers the advantage of delivering real-time structural images and showcasing hemodynamics, its accuracy, and reproducibility largely hinge on the operator's expertise. Additionally, the absence of standardized pressure application on the USG probe presents another limitation to our research.

CONCLUSION

In conclusion, our study offers valuable insights into the variations in the CSA of the IJV among the adult population. Notably, both male and female participants exhibited the largest CSA in the right supraclavicular region during the Valsalva maneuver. This observation underscores the dynamic nature of the IJV's morphology in response to physiological changes. Furthermore, our findings emphasize the importance of understanding these variations, especially when considering anatomical landmarks such as the angle of the mandible, cricothyroid, and supraclavicular regions. By accurately identifying and distinguishing the CSA of the IJV during procedures like deep breath holds and the Valsalva maneuver, clinicians can make more informed decisions, potentially reducing interventional and surgical risks. As the medical community continues to prioritize patient safety and procedural efficacy, such knowledge becomes indispensable, paving the way for improved clinical outcomes.

Ethics Committee Approval

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Etlik City Hospital Clinical Research Ethics Committee (Ankara, Türkiye) (date: 13.09.2023, no:2023-537)

Authors' Contribution

Study Conception: DÖK, NK; Study Design: DÖK, NK; Supervision: DÖK, NK; Funding: DÖK, NK; Materials: DÖK, NK; Data Collection and/or Processing: DÖK, NK; Statistical Analysis and/or Data Interpretation: DÖK, NK; Literature Review: DÖK; Manuscript Preparation: DÖK, NK and Critical Review: DÖK.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. Du Y, Wang J, Jin L, Li C, Ma H, Dong S. Ultrasonographic assessment of anatomic relationship between the internal jugular vein and the common carotid artery in infants and children after ETT or LMA insertion: a prospective observational study. Front Pediatr. 2020;8:605762. doi: 10.3389/fped.2020.605762.
2. Cvetko E. Unilateral fenestration of the internal jugular vein: a case report. Surg Radiol Anat. 2015;37(7):875-877. doi: 10.1007/s00276-015-1431-x.
3. Parenti N, Bastiani L, Tripolino C, Bacchilega I. Ultrasound imaging and central venous pressure in spontaneously breathing patients: a comparison of ultrasound-based measures of internal jugular vein and inferior vena cava. Anaesthesiol Intensive Ther. 2022;54(2):150-155. doi: 10.5114/ait.2022.114469.
4. Chawang HJ, Kaeley N, Bhardwaj BB, et al. Ultrasound-guided estimation of internal jugular vein collapsibility index in patients with shock in emergency department. Turk J Emerg Med. 2022;22(4):206-212. doi: 10.4103/2452-2473.357352.
5. Vaidya GN, Kolodziej A, Stoner B, et al. Bedside ultrasound of the internal jugular vein to assess fluid status and right ventricular function: The POCUS-JVD study. Am J Emerg Med. 2023;70:151-156. doi: 10.1016/j.ajem.2023.05.042.
6. Rafailidis V, Huang DY, Yusuf GT, Sidhu PS. General principles and overview of vascular contrast-enhanced ultrasonography. Ultrasonography. 2020;39(1):22-42. doi: 10.14366/usg.19022.
7. Jeon JC, Choi WI, Lee JH, Lee SH. Anatomical morphology analysis of internal jugular veins and factors affecting internal jugular vein size. Medicina (Kaunas). 2020;56(3):135. doi: 10.3390/medicina56030135.
8. Seçici S. Landmark guided internal jugular vein catheterization

in infants undergoing congenital heart surgery. Eur Res J 2021;7(4):375-379. doi: 10.18621/eurj.748292.
9. Kosnik N, Kowalski T, Lorenz L, Valacer M, Sakthi-Velavan S. Anatomical review of internal jugular vein cannulation. Folia Morphol (Warsz). 2023. doi: 10.5603/FM.a2023.0008.
10. Judickas Š, Gineitytė D, Kezytė G, Gaižauskas E, Šerpytis M, Šipylaitė J. Is the Trendelenburg position the only way to better visualize internal jugular veins? Acta Med Litu. 2018;25(3):125-131. doi: 10.6001/actamedica.v25i3.3859.
11. Laganà MM, Pirastru A, Ferrari F, et al. Cardiac and respiratory influences on intracranial and neck venous flow, estimated using real-time phase-contrast MRI. Biosensors (Basel). 2022;12(8):612. doi: 10.3390/bios12080612.
12. Iankovitch A, Ledley JS, Almabrouk T, Al-Jaberi N, Coey J. Anatomical variations of the internal jugular vein in the context of central line placement: a visual approach to data processing. Clin Anat. 2023;36(2):172-177. doi: 10.1002/ca.23939.
13. Salari M, Sasani MR, Masjedi M, Pourali A, Aghazadeh S. The association of diameter and depth of internal jugular and subclavian veins with hand dominancy. Electron Physician. 2018;10(7):7115-7119. doi: 10.19082/7115.
14. Yoon HK, Lee HK, Jeon YT, Hwang JW, Lim SM, Park HP. Clinical significance of the cross-sectional area of the internal jugular vein. J Cardiothorac Vasc Anesth. 2013;27(4):685-689. doi: 10.1053/j.jvca.2012.10.007.
15. Botero M, White SE, Younginer JG, Lobato EB. Effects of trendelenburg position and positive intrathoracic pressure on internal jugular vein cross-sectional area in anesthetized children. J Clin Anesth. 2001;13(2):90-93. doi: 10.1016/s0952-8180(01)00220-3.
16. Saiki K, Tsurumoto T, Okamoto K, Wakebe T. Relation between bilateral differences in internal jugular vein caliber and flow patterns of dural venous sinuses. Anat Sci Int. 2013;88(3):141-150. doi: 10.1007/s12565-013-0176-z.
17. Magnano C, Belov P, Krawiecki J, Hagemeier J, Beggs C, Zivadinov R. Internal Jugular Vein Cross-Sectional Area Enlargement Is Associated with Aging in Healthy Individuals. PLoS One. 2016;11(2):e0149532. doi: 10.1371/journal.pone.0149532.
18. Giordano CR, Murtagh KR, Mills J, Deitte LA, Rice MJ, Tighe PJ. Locating the optimal internal jugular target site for central venous line placement. J Clin Anesth. 2016;33:198-202. doi: 10.1016/j.jclinane.2016.03.070.
19. Zamboni P, Menegatti E, Pomidori L, et al. Does thoracic pump influence the cerebral venous return? J Appl Physiol (1985). 2012;112(5):904-10. doi: 10.1152/japplphysiol.00712.2011.
20. Belov P, Magnano C, Krawiecki J, et al. Age-related brain atrophy may be mitigated by internal jugular vein enlargement in male individuals without neurologic disease. Phlebology. 2017;32(2):125-134. doi: 10.1177/0268355516633610.

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Peritonitis in patients on peritoneal dialysis: a 12-year experience from a large medical center in Bursa

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ABSTRACT

Objectives: Despite all technical advances, Peritonitis remains the most important peritoneal dialysis (PD) complication. Peritonitis causes complications such as additional hospitalization, technical failure, peritoneal membrane damage, consequent transition to hemodialysis, and death in this patient group. Early diagnosis, isolation of the causative pathogen with appropriate methods as soon as possible, and determination of antibiotic susceptibilities play a crucial role in solving the problem of treating peritonitis in PD. This study evaluated the frequency of peritonitis, culture positivity rate, and prognosis in peritoneal dialysis patients in our unit for 12 years.

Methods: A total of 171 patients (80 F, 91 M; mean age: 51.9±15.3 years; mean PD duration 36.5±36.4 months) who were followed up in our department between January 2009 and July 2021 were included in the study. Patient records were retrospectively analyzed.

Results: Peritonitis never occurred in 105 of the 171 patients included in the study. Of the remaining 66 patients, 43 had one peritonitis attack, and 23 had more than two. The mean peritonitis rate was 1.68±1.36. One hundred eleven episodes of peritonitis were detected in 66 of the patients. Bacterial growth was observed in 63.06% of the culture samples obtained from the 93 peritonitis episodes. The peritoneal catheter was withdrawn in 14 (21.21%) cases.

Conclusions: In our unit, the rate of culture positivity was 63.06%, and the peritonitis attack rate was 0.017 per patient-month and 0.211 per patient-year over a period of twelve years, with a mean of 57.1 patient months of peritonitis.

Keywords: Peritoneal dialysis, peritonitis, infections

Peritonitis is a common and serious complication of peritoneal dialysis (PD) and causes significant morbidity, mortality, and healthcare expenses. It also significantly limits the use of this critical dialysis method. Recurrent episodes of peritonitis are the most important causes of technical inadequacy of dialysis and transition to hemodialysis. The incidence of peritonitis varies according to age, race, education level, dialysis type, and environmental factors, while the course of peritonitis depends on the causative microorganism [1]. PD-associated peritonitis is a leading cause of mortality in over 15% of peritoneal dialysis patients. Single or multiple episodes of severe peritonitis can reduce the efficiency of peritoneal ultrafiltration and are the most common cause of conversion in long-term hemodialysis [2]. After

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each episode of peritonitis, a root cause analysis is recommended to identify the cause and possible interventions to reduce the risk of recurrence. The PD catheter should be considered for replacement after recurrent or repeated peritonitis [3]. The International Society for Peritoneal Dialysis (ISPD) recommends monitoring the overall rate of peritonitis, the rate of peritonitis due to specific organisms, the percentage of patients free of peritonitis per year, and the extent of antibiotic resistance [3].

This study aimed to analyze the clinical features of peritonitis associated with continuous ambulatory peritoneal dialysis (CAPD) characteristics, causative organisms, and antibiotic susceptibilities.

METHODS

Study Population and Baseline Characteristics

A total of 171 patients (80 F, 91 M; mean age 51.9 ± 15.3 years; mean PD duration 36.5±36.4 months) who were followed up in our department between January 2009 and July 2021 were included in the study. Patient records were retrospectively analyzed.

Baseline demographics included sex, age, body mass index (BMI), cause of end-stage renal disease (ESRD), PD modality (automated PD or continuous ambulatory PD), duration of PD at study entry, presence of diabetes mellitus (DM), and residual urine volume. Clinical features included fever, jaundice, abdominal pain, previous peritonitis, extraperitoneal tuberculosis, and time from diagnosis to treatment initiation (days). Laboratory parameters included peritoneal dialysis effluent characteristics with white blood ceel (WBC) count, including neutrophil and lymphocyte percentage, erythrocyte count, polymerase chain reaction (PCR), and peritoneal fluid culture. Blood analysis included WBC count, hemoglobin (Hb), platelet (PLT) count, C-reactive protein (CRP), blood urea nitrogen (BUN), creatinine, and albumin.

Peritonitis Diagnosis

The following criteria were used to diagnose peritonitis: (1) Gram stain of the peritoneal dialysis fluid or culture for micro-organisms; (2) The number of white blood cells in the peritoneal dialysis fluid exceeds 100 cells/mm3. The percentage of neutrophils is greater than 50%; and (3) There are signs of inflammation of the peritoneum. All patients were tested for tuberculosis and fungal infection if peritoneal fluid cultures were negative for microorganisms. Patients' peritoneal fluid samples were analyzed at least three times using the alcohol-resistant bacilli (AARB) staining technique, and mycobacteria and fungi were cultured. Lowenstein Jensen and Sabouroud dextrose were used for mycobacterial culture, and agar medium was used for fungal culture. Tuberculosis was diagnosed by microscopic examination or detection of Mycobacterium tuberculosis in culture.

Peritonitis Treatment

After obtaining the appropriate microbiological specimens, empirical antibiotic therapy was initiated following ISPD recommendations [3]. The fundamental principle ensures sufficient coverage of Gram-positive and Gram-negative organisms, including Pseudomonas species. The current guidelines suggest vancomycin or first-generation cephalosporin for Gram-positive organism coverage and third-generation cephalosporin or aminoglycoside for Gram-negative organism coverage. The decision to use vancomycin or a first-generation cephalosporin relied on the results of the patient's previous peritoneal fluid culture. Vancomycin was administered if Methicillin-resistant Staphylococcus aureus (MRSA) was detected in the last culture, whereas a first-generation cephalosporin was used otherwise.

Statistical Analysis

Statistical analysis was performed using SPSS 19.0 software (SPSS Inc., Chicago, IL, USA). The Shapiro-Wilk test was used to assess the normal distribution of numerical variables. Descriptive statistics were presented as mean ± standard deviation and median (range) for numerical variables and as numbers and percentages for categorical data.

RESULTS

The mean age was 51.9±15.3 years. Ninety-one (43.3%) patients were male, and 80 (46.7%) were female. Of the 105 patients diagnosed with peritonitis, 36 (54.5 %) were male and 30 (45.5%) females, and their mean age was 49.9±16.2 years.

Cloudiness of the peritoneal fluid was observed in

Table 1. Patients' symptoms and signs during peritonitis episodes

Symptom and Sign	n	%
Cloudy fluid	50	75
Abdominal pain	38	57.5
Fever	3	4.5
Nausea	7	10.6
Vomiting	2	3
Diarrhea	2	3

50 (75.7%) patients. In addition, 38 (57.5%) of patients reported abdominal pain, 3 (4.5%) had fever, and 2 (3.0%) had nausea. Seven (10.6%) of patients had vomiting, and 2 (3.0%) had diarrhea (Table 1 and 2).

During a period of 6329 patient months (or 527.4 patient-years), 111 peritonitis attacks were observed. The attack rates were 0,017 per patient-month and 0,211 per patient-year. On the mean, peritonitis was observed in 57.01 patient-month.

Peritonitis never occurred in 105 of the 171 patients included in the study. Of the remaining 66 patients, 43 had one peritonitis attack, and 23 had more than two. The mean peritonitis rate was 1.68±1.36. One hundred eleven episodes of peritonitis were detected in 66 of the patients. Bacterial growth was observed in 63.06% of the culture samples taken from the 93 attacks of peritonitis. In 38.46% of peritonitis episodes, no microbial growth was observed in culture. Gram-positive factors comprised 40.0% of the micro-organisms that grew, while gram-negative factors accounted for 10.8%. Staphylococci, responsible for 27.7%, were the most common bacteria. Among the gram-negative bacteria, Escherichia. coli was identified as the most common causative agent (7.7%) (Fig. 1).

Medical treatment was successful in 74.24% of cases, according to empirical and antibiogram results. In 14 (21.21%) patients, hemodialysis was performed after peritoneal catheter removal, and one patient died during the follow-up period.

Table 2. Patients laboratory findings during peritonitis episodes

Biochemistry	mean±SD
C-reactive protein (mg/L)	59.7±53.4
Sedimentation (%)	77.8±30.8
WBC (mm^3)	8652±3769
Albumin (g/L)	3.14±0.5
PF-WBC (mm^3)	3195±3434

WBC=white blood cell, PF-WBC=peritoneal fluid white blood cell, SD=standard deviation

DISCUSSION

Preventing and reducing peritonitis can improve longevity and quality of life in peritoneal dialysis patients. The incidence of peritonitis and positive culture rates in our peritoneal dialysis patients were comparable to those in previous studies. In our unit, the culture positivity rate was 63.06%, and the peritonitis attack rate was 0.017 per patient-month and 0.211 per patient-year over twelve years, with a mean of 57.1 patient-months of peritonitis. In the 2019 National Nephrology, Dialysis, and Transplantation Registry System Report, the frequency of peritonitis in PD patients was reported as 0.46 attacks/patient-year [4]. This registry was established with the results of 29 principal peritoneal dialysis centers in the country. Compared to our country's registration system, the peritonitis rates we found in our study were better. There may be many reasons related to patients and

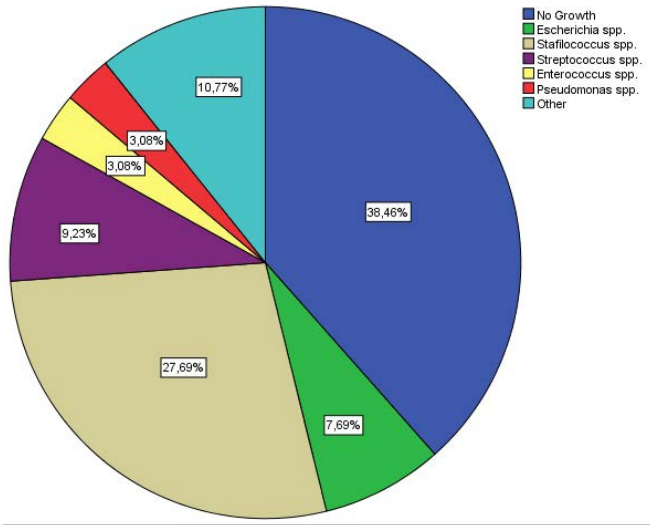

Fig. 1. Peritoneal fluids bacterial growth: distribution of pathogenic microorganisms in peritoneal fluid samples.

centers. There are several possible explanations for this finding. Disposable dialysate containers, the Y-connector as a single set, the routine use of disinfectants, and several technical enhancements have improved technical issues over time. However, the most important could be patient education in our unit. A study published in 2016 by Seker *et al*. [5] found an attack rate of 0.29 attacks/patient-year over 15 years (2000-2014). The study by Ozturk *et al*. [6] evaluated 65 patients between 2011 and 2017; the peritonitis attack rate was calculated as 0.224 attacks/patient-year (1 attack/53.57 patient-months). According to a systematic review, only 33 high- and middle-income countries monitor the peritonitis rate; within these countries, the global average peritonitis rate decreased from 0.6 episodes per patient-year in 1992 to 0.3 per patient-year in 2019. Asia-Pacific region had the highest rates, followed by Europe, the Middle East, and Africa. The Americas (including North, South, and Central America) had the lowest rate [7]. Consistent with our findings, peritonitis rates have also decreased worldwide.

Despite the ISPD recommendation that culture-negative peritonitis rates should be maintained at < 15%, most countries, including Canada (16.0%), Japan (21.0%), the United States (16.0%), and India (18.2%) failed to meet this target [3, 1]. While the culture negativity rate was 30.1% in the Seker *et al*.'s study [5], Ozturk *et al*. [6] reported 36.7% culture negativity rate [6]. In our study, the culture negativity rate was 36.07%. This is consistent with previous studies conducted in Turkey. Peritoneal fluid culture samples were collected by trained nurses for continuous ambulatory peritoneal dialysis (CAPD). A significant decrease was observed in the negativity rate. Our department's employing dedicated nurses to collect peritoneal fluid cultures has resulted in a low negative culture rate consistent with the literature.

In our study, gram-positive agents were the most frequently detected microorganisms in culture-positive cases, and staphylococci were the most common agents. This finding is in line with the literature. In a 2011 analysis of registries in Australia and New Zealand, Gram-positive organisms accounted for 53.4% of all episodes of peritonitis. The most common Gram-positive and -negative organisms were coagulase-negative staphylococci (27.2%) and E. coli (6.3%) [8]. Similar findings were reported in North America - Gram-positive organisms caused 62.0% of infections in the USA and 61.0 % in Canada [9]. An international 2020 study found lower rates of Gram-positive peritonitis in Australia and New Zealand (39.0%), the United States (37.0%), and Canada (45.0%) than reported in previous studies [10]. Studies reported from our country were similar to our findings; Seker *et al*. [5] reported 58.3%, and Ozturk *et al*. [6] reported 42.84% gram-positive bacteria, respectively.

Peritonitis outcomes vary widely between countries. These include medical cure (69.0-80.7%), catheter removal (10.8-20.4%), and mortality (1.8-6.0%) [11, 8, 9, 12]. In our study, the complete recovery rate was 74.24%, and the hemodialysis initiation rate after catheter removal was 21.21%. One patient died. Our mortality rate is relatively low, and our recovery and catheter removal rates are comparable to those reported in the literature. This may be due to our strict adherence to the ISPD recommendations for catheter removal.

CONCLUSION

In conclusion, although the incidence of peritonitis has decreased in recent years, early and accurate diagnosis of peritonitis is essential for a successful PD program. Successful treatment of peritonitis attacks is also necessary. Therefore, prevention and early diagnosis of peritonitis can be achieved through continuous patient education. In addition, culture positivity can be increased by using the correct technique for dialysate collection by experienced nurses.

Authors' Contribution

Study Conception: CBG; Study Design: CBG; Supervision: N/A; Funding: N/A; Materials: N/A; Data Collection and/or Processing: CBG; Statistical Analysis and/or Data Interpretation: CBG; Literature Review: CBG; Manuscript Preparation: CBG and Critical Review: CBG.

Conflict of interest

The author disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. Bello AK, Okpechi IG, Osman MA, et al. Epidemiology of peritoneal dialysis outcomes. Nat Rev Nephrol. 2022;18(12):779-793. doi: 10.1038/s41581-022-00623-7.
2. Szeto CC, Li PK. Peritoneal dialysis-associated peritonitis. Clin J Am Soc Nephrol. 2019;14(7):1100-1105. doi: 10.2215/CJN.14631218.
3. Li PK, Szeto CC, Piraino B, et al. ISPD Peritonitis Recommendations: 2016 Update on Prevention and Treatment. Perit Dial Int. 2016;36(5):481-508. doi: 10.3747/pdi.2016.00078.
4. Seyahi N, Ateş K, Süleymanlar G. Current Status of Renal Replacement Therapy in Turkey: A Summary of the 2019 Turkish Society of Nephrology Registry Report. Turkish J Nephrol. 2021;30(2):105-111. doi: 10.5152/turkjnephrol.2021.21436
5. Şeker A, Candan F, Hüzmeli C, Akkaya L, Kayataş M. [Evaluation of continuous ambulatory peritoneal dialysis-related peritonitis attacks]. Turk Neph Dial Transpl 2016;25(2):142-146. doi: 10.5262/tndt.2016.1002.03. [Article in Turkish]
6. Öztürk Y, Delikanlı Çorakçı B, Bilici M, Boraza A. [In our unit, peritonitis incidence and agents determined during six-year period]. Batı Karadeniz Tıp Derg. 2017;1(2):46-51. [Article in Turkish]
7. Marshall MR. A systematic review of peritoneal dialysis-related peritonitis rates over time from national or regional population-based registries and databases. Perit Dial Int. 2022;42(1):39-47. doi: 10.1177/0896860821996096.
8. Ghali JR, Bannister KM, Brown FG, et al. Microbiology and outcomes of peritonitis in Australian peritoneal dialysis patients. Perit Dial Int. 2011;31(6):651-62. doi: 10.3747/pdi.2010.00131.
9. Mujais S. Microbiology and outcomes of peritonitis in North America. Kidney Int Suppl. 2006;(103):S55-62. doi: 10.1038/sj.ki.5001916.
10. Perl J, Fuller DS, Bieber BA, et al. Peritoneal dialysis-related infection rates and outcomes: results from the Peritoneal Dialysis Outcomes and Practice Patterns Study (PDOPPS). Am J Kidney Dis. 2020;76(1):42-53. doi: 10.1053/j.ajkd.2019.09.016.
11. de la Espada Piña V, Ganga PLQ, Junquero JMG, et al. Two decades of analysis of peritonitis in peritoneal dialysis in Andalusia: epidemiological, clinical, microbiological and progression aspects. Nefrologia (Engl Ed). 2021;41(4):417-425. doi: 10.1016/j.nefroe.2021.10.004.
12. Htay H, Cho Y, Pascoe EM, et al. Center effects and peritoneal dialysis peritonitis outcomes: analysis of a national registry. Am J Kidney Dis. 2018;71(6):814-821. doi: 10.1053/j.ajkd.2017.10.017.

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Prognostic value of non-alcoholic fatty liver disease in patients with pulmonary embolism

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ABSTRACT

Objectives: Pulmonary embolism (PE) is an important disease due to its mortality and morbidity-related clinical conditions. Patients with a high risk of death within 30 days are discriminated against with the help of various clinical scores. Non-alcoholic fatty liver disease (NAFLD) has been found to be associated with atherosclerosis. We aimed to investigate the effect of NAFLD on disease severity and early death rate in patients with pulmonary embolism.

Methods: This retrospective study includes patients who applied to the emergency department with suspected pulmonary embolism and whose diagnosis was confirmed according to the results of the examination. In addition to confirming the diagnosis of PE, hepatic steatosis was detected and graded by tomographic examination of the liver and spleen. Disease severity was stratified by Simplified Pulmonary Embolism Severity Index (sPESI).

Results: A total of 165 patients (105 with sPESI≥1 and 60 with sPESI<1 controls) were included. The rate of mortality was 12% (n=13) in the sPESI≥1 group. The prevalence of NAFLD was 64% and the prevalence of hepatosteatosis was similar according to disease severity and prognosis (67% vs. 58%; P=0.28 and 69% vs. 63%; P=0.77). Besides the effect of disease severity; chronic lung disease (CLD) and chronic kidney disease (CKD) were independently associated with poor prognosis by multivariate analysis [3.71 (1.02-13.46); P=0.04 and 15.89 (2.57-98.35); P=0.003].

Conclusion: No association between disease severity and prognosis was observed with NAFLD in acute PE disease.

Keywords: Pulmonary embolism, simplified pulmonary embolism severity index, non-alcoholic fatty liver disease, hepatosteatosis

Deep vein thrombosis and pulmonary embolism (PE), with a prevalence of 1-2 per 1000 people in the world, are common causes of mortality, along with myocardial infarction and cerebrovascular events [1]. Disease-related early mortality is between 2% and 18%, and it has been shown that this risk may increase up to 30% in the long term [2]. The simplified PE severity index (sPESI) is a simplified and easier to calculate form of a previously defined index (PESI) [3]. It has as much accuracy as the original version to predict the risk of 30-day mortality in patients with acute symptomatic PE. In addition to

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these scoring methods, despite developments that positively affect disease-related diagnosis and treatment, the prognostic gain has not been at the expected level [4]. Determining the factors that negatively affect the severity of the disease may contribute positively to the prognosis of the disease and may lead to the development of new treatment strategies for PE patients.

Hepatic steatosis is defined as non-alcoholic fatty liver disease (NAFLD) in the absence of other causes for secondary hepatic fat accumulation. It is the uncontrolled storage of free fatty acids due to insulin resistance that is used in its pathogenesis [5]. Studies have reported that the prevalence of hepatosteatosis exceeds approximately 50% in the general population [6]. Recent studies have associated fatty liver disease with comorbid conditions, including an increased risk of cardiovascular disease, chronic kidney disease (CKD), and diabetes mellitus. [7]. Association of NAFLD with atherosclerosis and increased risk of thrombosis is claimed in the literature [8]. In addition to increased factor VIII activity but decreased protein C activity, the activity of various pro-coagulation factors has also been shown to be "increased in patients with NAFLD" [9, 10]. However, the relationship between clinical venous thrombo-embolism and NAFLD has not been studied much in the literature. Therefore, in this study, we aimed to evaluate the effect of NAFLD on disease severity and hospital prognosis in hospitalized PE patients.

METHODS

Data Collection

Between March 1, 2019 and September 30, 2020 patients who were examined in the emergency department for PE and whose diagnosis was confirmed after their tests were included in the study. Study eligibility required patients diagnosed with the International Classification of Diseases (ICD) should have symptomatic PE. Patients were diagnosed with PE by high-probability ventilation-perfusion scanning or spiral computed tomography (CT) according to the criteria of the Prospective Study of the Diagnosis of Pulmonary Embolism [11]. The presence of hepatic steatosis was detected and classified by defining the difference between CT imaging and liver-kidney hyperechogenicity intensities. Patient information was obtained by examining related hospitalization reports and clinical data. The patient's clinical characteristics were documented, including coronary heart disease (CAD), congestive heart failure, diabetes, chronic lung disease (CLD), CKD, and malignancy. Hospitalization details were also recorded, including referral departments and length of stay. An electronic information system was used to access the laboratory data of the patients.

Our group consisted of eligible people over the age of eighteen without covid 19 disease. Patients with missing patient registry data were excluded. Corticosteroid users, cirrhosis, or other documented chronic liver disease or imaging findings, and patients with a history of splenic diseases or recurrent venous thromboembolism or those patients with a known diagnosis of hereditary thrombophilia were excluded. Also, regular (moderate-to-severe) alcohol use of more than 2 drinks per day was another exclusion criterion.

This study was conducted in line with the principles of the Declaration of Helsinki and ethical approval has been obtained from the Local Institutional Ethics Committee.

Liver Density Evaluation

The density of different liver segments was measured in Hounsfield units (HU). Measurements of the hepatic lobular segments were made using a circular region of interest (ROI) with the maximum possible diameter per segment without including macroscopic vascular or biliary structures Previous studies have shown that the difference in liver density between different CT slices is small enough to allow valid measurement of liver fat using a single slice [12]. Spleen density was measured using HU and ROI with the maximum diameter possible without including macroscopic vascular structures. Usually, spleen density measurements were taken on the same slices used for liver density measurements (Fig. 1).

Definitions

Several non-contrast CT methods are used for the identification of hepatosteatosis: First, visual assessment attitudes of radiologists according to their professional experience [13]. Second, the liver density itself: Density <48 HU means a fatty liver and <40 HU was found to be associated with a macro-vesicular steatosis of at least 30% [14]. This measurement is a

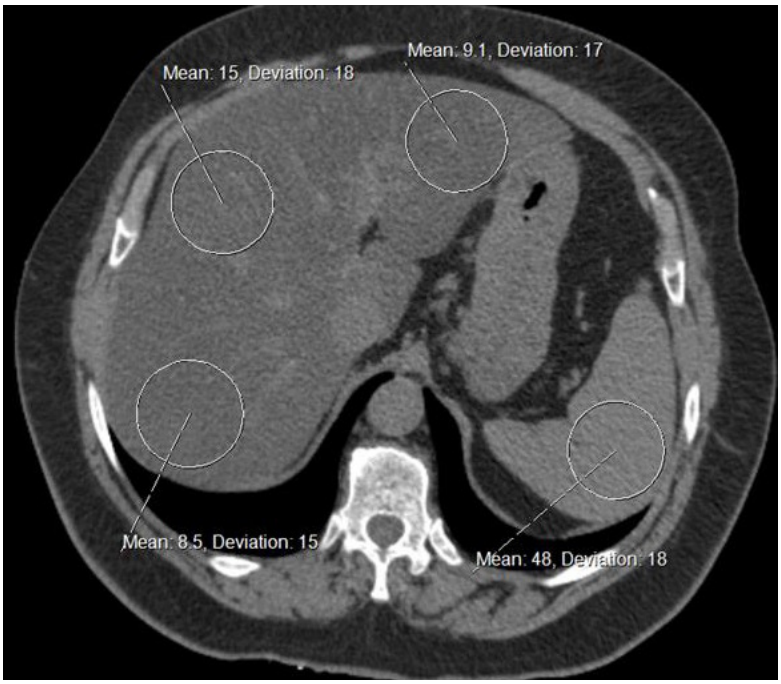

Fig. 1. Calculation of the HU values of the liver and spleen in patient with steatosis. Pay attention to the difference between the liver and the spleen HU.

strong indicator of the severity of steatosis at the histological level. Third, the ratio between liver and spleen densities (CTL/S): Using spleen density neutralizes the effect of the difference between CT scanners. A ratio of less than 0.8 between liver and spleen densities was found to be associated with at least 30% macro-vesicular steatosis [15]. Finally, the difference between liver and spleen densities (CTL-S): Normally, liver density is approximately 10 HU higher than that of the spleen. Setting the CTL-S<-9 threshold was found to have a sensitivity of 80 % and a specificity of 99 % in determining the level of high-grade hepatosteatosis [16]. This density difference method was chosen for this study and averaged over 3-segment measurements of more than nine HU inter-organ density differences defined as our dependent variable.

The sPESI, which stratifies high and low-risk patients with PE; also helps to discriminate patients who are at higher risk of death within a month. [3]. This index consists of six clinical variables including vital signs, age, and underlying comorbid conditions, each given an equal weight of one point. At least 1 point identifies high-risk patients. Low-risk patients who may benefit from early hospital discharge are also identified by this method [17].

Statistical Analysis

The Kolmogorov-Smirnov test was used to assess the normality of the distribution. Analysis of baseline features according to hepatic steatosis status, disease severity, and survival status were compared with Mann-Whitney U (for continuous variables) and Chi-square tests (for categorical parameters). Descriptive statistics were presented as median and interquartile range for continuous variables, number of cases, and (%) for categorical variables. Parameters that may have an effect on in-hospital mortality and more severe forms of disease were investigated by binary logistic regression analysis. As a result of univariate statistical analyses, the combined effects of risk factors (Age, CLD, CKD) on mortality were evaluated by multivariate regression analysis. Since we had a small number of subjects (n=13) in terms of mortality in our cohort. In order to maintain the statistical power of the regression analysis, 3 different models were created and analyzed. Chronic lung disease and CKD separately with age and CLD and CKD were evaluated together with multivariate logistic regression analysis, without including age. The SPSS 21.0 program (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.) was

used for performing statistical analysis. The level of significance was set as P<0.05.

RESULTS

A total of 165 patients who had been diagnosed with PE comprised the study cohort. Thus, the database was reviewed and a total of 105 (64%) patients were determined to have hepatosteatosis. Demographic, basic characteristics, laboratory, and prognostic parameters were not different between groups according to the presence of fatty liver. The more severe form of the disease was seen in men, while the prognosis was worse in elderly patients. Moreover, higher rates of hypertension, CAD, CLD, congestive heart failure, and malignancy were documented in the severe disease group. Contrary to other comorbid conditions, the presence of CLD and CKD significantly accompanied poor prognosis. The hepatic steatosis rate was also similar in both groups according to disease severity and prognosis (67% vs 58%; P=0.28 and 69% vs 63%; P=0.77). As expected Laboratory parameters related to prognosis differed significantly for all groups in our cohort (Tables 1 and 2).

The rate of mortality was 12% (n=13) in the sPESI≥1 group. Besides the effect of disease severity; CLD and CKD were independently associated with poor prognosis [3.71 (1.02-13.46), P=0.04 and 15.89 (2.57-98.35), P=0.003] (Table 3). Age was also associated significantly with mortality [1.13 (1.05-1.21), P=0.001]. When age was added to the multivariate regression analysis models, the significance of the presence of CKD persisted, while CLD lost its significance (for age; 1.20 (1.08-1.33), P=0.001; for CKD; 8.5 (154.7-2490.5 and for age; 1.12 (1.04-1.20), P=0.001; for CLD; 2.27 (0.62-8.35), P=0.22).

DISCUSSION

In this study, we demonstrated a high prevalence of NAFLD in a cohort of hospitalized patients with a diagnosis of PE. Interestingly while the frequency of comorbidities is higher in the presence of more severe disease, we did not detect any difference in the preva-

Table 1. Basic characteristics, laboratory investigations, and fatty liver status of our group according to disease severity

	Overall (n=165)	sPESI≥1 (n=105)	sPESI<1 (n=60)	P value
Age (years)	67 (56-78)	71 (64-80)	57 (40-68)	**0.0001**
Male, n (%)	91 (55)	65 (62)	26 (43)	**0.02**
Diabetes Mellitus, n (%)	42 (26)	30 (29)	12 (20)	0.22
Hypertension, n (%)	94 (57)	74 (71)	20 (33)	**0.0001**
CAD, n (%)	53 (32)	47 (45)	6 (10)	**0.0001**
Chronic lung disease, n (%)	68 (41)	61 (58)	7 (12)	**0.0001**
Chronic kidney disease, n (%)	6 (4)	5 (5)	1 (2)	0.16
Congestive heart failure, n (%)	29 (18)	25 (24)	4 (7)	**0.005**
Malignancy, n (%)	33 (20)	26 (25)	7 (12)	**0.04**
Peak troponin I (ng/dL)	38 (17-102)	80 (41-150)	15 (11-21)	**0.0001**
D-dimer (mg/L)	4.2 (1.7-10.4)	6.9 (4.2-17.4)	1.4 (1.1-2.1)	**0.0001**
Pro-BNP, ng/L	123 (42-781)	421 (123-2669)	34 (23-51)	**0.0001**
Creatinine (mg/dL)	1.0 (0.9-1.2)	1.1 (0.9-1.4)	0.9 (0.8-1.0)	**0.0001**
Fatty liver, n (%)	105 (64)	70 (67)	35 (58)	0.28

Continuous variables are presented as median (IQR), and nominal variables are presented as frequency (%). CAD=coronary artery disease, BNP=B-type natriuretic peptide

Table 2. Basic characteristics, laboratory investigations, disease severity, and fatty liver status of our group according to prognosis

	Overall (n=165)	Non-survivors (n=13)	Survivors (n=152)	P value
Age (years)	67 (56-78)	82 (80-87)	66 (54-77)	**0.0001**
Male, n (%)	91 (55)	6 (46)	85 (56)	0.49
Diabetes Mellitus, n (%)	42 (26)	5 (39)	37 (24)	0.32
Hypertension, n (%)	94 (57)	10 (77)	84 (55)	0.13
CAD, n (%)	53 (32)	4 (31)	49 (32)	0.92
Chronic lung disease, n (%)	68 (41)	9 (69)	59 (39)	**0.03**
Chronic kidney disease, n (%)	6 (4)	3 (23)	3 (2)	**0.007**
Congestive heart failure, n (%)	29 (18)	4 (31)	25 (16)	0.25
Malignancy, n (%)	33(20)	3 (23)	30 (20)	0.73
Peak troponin I (ng/dL)	38 (17-102)	451 (211-754)	32 (17-90)	**0.0001**
D-dimer (mg/L)	4.2 (1.7-10.4)	38.0 (21.4-42.0)	3.9 (1.7-7.0)	**0.0001**
Pro-BNP (ng/L)	123 (42-781)	6524 (3240-11600)	108 (38-463)	**0.0001**
Creatinine (mg/dL)	1.0 (0.9-1.2)	2.1 (1.3-3.9)	1.0 (0.9-1.2)	**0.0001**
sPESI≥1	105 (64)	13 (100)	92 (61)	**0.002**
Fatty liver, n (%)	105 (64)	9 (69)	96 (63)	0.77

Continuous variables are presented as median (IQR), and nominal variables are presented as frequency (%). CAD=coronary artery disease, BNP=B-type natriuretic peptide

lence of comorbid conditions other than chronic lung disease and CKD, in those who died and those who survived. No association was observed between NAFLD and disease severity and prognosis in acute PE disease.

Detection rates of hepatic steatosis by evaluation of the liver and spleen with CT vary according to the radiological criteria used [18]. According to the hepatosteatosis definition criteria in the literature, fatty liver rates vary and reach 80% [19, 20]. The prevalence of 64% hepatic steatosis detected in our study is in line with the literature. Recently, NAFLD was detected in 101 cases (27%) in a study in which 411 cases were evaluated by CT pulmonary angiography [6]. We think that the wide range of observed hepatosteatosis frequencies is affected by the difference in radiological criteria defined between studies. The relationship between idiopathic venous thromboembolism and NAFLD has also been investigated and it has been suggested that the presence of central obesity is particularly related to the development of PE [21, 22]. Zeina *et al*. [6], found NAFLD as a significant risk factor for PE independent of advanced age, immobilization/surgery, malignancy, obesity, diabetes, and tobacco use (HR = 4.339, P<0 .0001, and 95% CI=2.196–8.572). Also, the majority of NAFLD patients in that study had more vascular complications and other components of the metabolic syndrome. Although the incidence of malignancy was similar to that study, the NAFLD patients in our cohort were predominantly male and older, and the incidence of CAD was higher. The prevalence of diabetes was 26% in our patients, while it was 40% in this study. Our study was different from this study, in terms of its design and outcomes.

The literature suggests NAFLD as a pro-coagulant state and its contribution to the risk of thrombosis [9, 10]. Also, disturbances of hemostasis have been documented particularly in patients with hepatic steatosis and cirrhosis [23, 24]. Since the prognostic significance of NAFLD in PE disease is obscure, we find our findings valuable in terms of their scientific contribution to the literature in this context.

In parallel with our work, some clinical characteristics

Table 3. Univariate and multivariate regression analysis models for determining the predictors of in-hospital mortality

	Univariate Odds Ratio (95% CI)	P value	Multivariate Odds Ratio (95% CI)	P value
Chronic lung disease	3.55 (1.04-12.04)	**0.04**	3.71 (1.02-13.46)	**0.04**
Chronic kidney disease	14.90 (2.65-83.51)	**0.002**	15.89 (2.57-98.35)	**0.003**

CI=confidence interval

of patients with pulmonary embolism have been shown to be associated with the clinical severity of pulmonary embolism but do not affect mortality. [25, 26]. It has been found that a decrease in the estimated glomerular filtration rate (eGFR) increases the risk of in-hospital death due to venous thromboembolism by 7 times [27]. Previously published studies in the literature showed that, in addition to the negative effect of decreased eGFR on PE, advanced CKD may worsen the prognosis of patients presenting with acute PE [28]. In the literature, it has been suggested that the risk of mortality is increased in patients with PE in combination with other comorbid conditions, or in combination with other comorbidities independently of CKD [29, 30]. Similar to previous studies, the frequency of CKD was higher in non-survivors than in survivors in our study. Also, it is not surprising that CLD was more common in patients who died during the in-hospital follow-up period after acute PE [31]. However, when adjusted for age, only CKD remained as a confounding independent parameter for mortality. Elderly patients with pulmonary embolism had a higher 30-day mortality than non-elderly patients, and mortality gradually increased with age [32]. We found the effect of age on prognosis in PE similar to previous studies.

Lower serum levels of B-type natriuretic peptide (BNP), troponin, and D-dimer were associated with survival. Therefore, these indices are used to evaluate the prognosis of patients with PE [33]. PE may cause a sharp rise in pulmonary artery and right ventricular pressure. This pathophysiology affects serum levels of these parameters by increasing ventricular load and myocardial damage. It has been suggested that the combined determination of these indices improves the prognostic assessment of patients with acute PE [33]. Although these parameters show that the cardiorespiratory reserve is decreased in patients with PE, they do not affect the importance of other important clinical factors such as age, cancer, previous lung or heart disease on disease prognosis in the context of cause-effect relationship [34]. Therefore, although our findings are consistent with recent studies, these blood parameters are markers that appear early in the disease and can change in a short time. Since we are dealing with longer-term clinical conditions that may have an impact on disease severity and early prognosis, which is our main aim in the study, they were not included in the regression analysis in order not to affect the statistical power.

Limitations

Missing data and selection bias could not be eliminated due to the retrospective study design. Since we investigated early death rates, we think that our results were not affected much. Some indicators of PE severity (troponin I, BNP, D-dimer, and echocardiographic results) were not included in the final analysis. Since we think that these parameters indicate coexistence rather than causality, we think that they will not translate to a clinically significant effect on our results. Further prospective studies are needed to confirm our findings. NAFLD had no effect on disease severity and prognosis in patients with acute PE disease.

CONCLUSION

The present study demonstrated a high prevalence of NAFLD in hospitalized patients with a diagnosis of PE. Interestingly while the frequency of comorbidities is higher in the presence of more severe disease, we did not detect any difference in the prevalence of comorbid conditions other than chronic lung disease and

CKD, in those who died and those who survived. No association was observed between NAFLD and disease severity and prognosis in acute PE disease.

Authors' Contribution

Study Conception: NKK; Study Design: MK; Supervision: NKK; Funding: NÖŞ; Materials: ÖFD; Data Collection and/or Processing: MK; Statistical Analysis and/or Data Interpretation: NÖŞ; Literature Review: NKK; Manuscript Preparation: ÖFD and Critical Review: MK.

Conflict of interest

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EFERENCES

1. Beckman MG, Hooper WC, Critchley SE, Ortel TL. Venous thromboembolism: a public health concern. Am J Prev Med. 2010;38(4 Suppl):S495-501. doi: 10.1016/j.amepre.2009.12.017.
2. Jiménez D, de Miguel-Díez J, Guijarro R, et al; RIETE Investigators. Trends in the management and outcomes of acute pulmonaryeEmbolism: analysis from the RIETE registry. J Am Coll Cardiol. 2016;67(2):162-170. doi: 10.1016/j.jacc.2015.10.060.
3. Jiménez D, Aujesky D, Moores L, et al, Yusen RD; RIETE Investigators. Simplification of the pulmonary embolism severity index for prognostication in patients with acute symptomatic pulmonary embolism. Arch Intern Med. 2010;170(15):1383-1389. doi: 10.1001/archinternmed.2010.199.
4. Heit JA. Venous thromboembolism: disease burden, outcomes and risk factors. J Thromb Haemost. 2005;3(8):1611-1617. doi: 10.1111/j.1538-7836.2005.01415.x.
5. Torres DM, Williams CD, Harrison SA. Features, diagnosis, and treatment of nonalcoholic fatty liver disease. Clin Gastroenterol Hepatol. 2012;10(8):837-58. doi: 10.1016/j.cgh.2012.03.011.
6. Zeina AR, Kopelman Y, Mari A, et al. Pulmonary embolism risk in hospitalized patients with nonalcoholic fatty liver disease: a case-control study. Medicine (Baltimore). 2022;101(45):e31710. doi: 10.1097/MD.0000000000031710.
7. Armstrong MJ, Adams LA, Canbay A, Syn WK. Extrahepatic complications of nonalcoholic fatty liver disease. Hepatology. 2014;59(3):1174-1197. doi: 10.1002/hep.26717.
8. Sookoian S, Pirola CJ. Non-alcoholic fatty liver disease is strongly associated with carotid atherosclerosis: a systematic review. J Hepatol. 2008;49(4):600-607. doi: 10.1016/j.jhep.2008.06.012.7
9. Tripodi A, Fracanzani AL, Primignani M, et al. Procoagulant imbalance in patients with non-alcoholic fatty liver disease. J Hepatol. 2014 Jul;61(1):148-154. doi: 10.1016/j.jhep.2014.03.013.
10. Kotronen A, Joutsi-Korhonen L, Sevastianova K, et al. Increased coagulation factor VIII, IX, XI and XII activities in non-alcoholic fatty liver disease. Liver Int. 2011;31(2):176-183. doi: 10.1111/j.1478-3231.2010.02375.x.
11. Remy-Jardin M, Remy J, Wattinne L, Giraud F. Central pulmonary thromboembolism: diagnosis with spiral volumetric CT with the single-breath-hold technique--comparison with pulmonary angiography. Radiology. 1992;185(2):381-387.
12. Davidson LE, Kuk JL, Church TS, Ross R. Protocol for measurement of liver fat by computed tomography. J Appl Physiol (1985). 2006;100(3):864-8. doi: 10.1152/japplphysiol.00986.2005.
13. Lee SW, Park SH, Kim KW, et al. Unenhanced CT for assessment of macrovesicular hepatic steatosis in living liver donors: comparison of visual grading with liver attenuation index. Radiology. 2007;244(2):479-485. doi: 10.1148/radiol.2442061177.
14. Kodama Y, Ng CS, Wu TT, et al. Comparison of CT methods for determining the fat content of the liver. AJR Am J Roentgenol. 2007;188(5):1307-1312. doi: 10.2214/AJR.06.0992.
15. Park SH, Kim PN, Kim KW, et al. Macrovesicular hepatic steatosis in living liver donors: use of CT for quantitative and qualitative assessment. Radiology. 2006;239(1):105-112. doi: 10.1148/radiol.2391050361.
16. Lee SS, Park SH. Radiologic evaluation of nonalcoholic fatty liver disease. World J Gastroenterol. 2014;20(23):7392-7402. doi: 10.3748/wjg.v20.i23.7392.
17. Yamashita Y, Morimoto T, Amano H, et al. Validation of simplified PESI score for identification of low-risk patients with pulmonary embolism: From the COMMAND VTE Registry. Eur Heart J Acute Cardiovasc Care. 2020;9(4):262-270. doi: 10.1177/2048872618799993.
18. Doğan E, Bacaksızlar Sarı F. Is fat deposition of renal sinus a concomitant finding to fatty liver disease? The first study regarding the relationship between kidney and liver fat content with non-contrast computed tomography. Spartan Med Res J. 2022;7(1):32411. doi: 10.51894/001c.32411.
19. Yılmaz A, Yılmaz F, Beydilli İ, et al. Ultrasonographically detected hepatosteatosis independently predicts the presence and severity of coronary artery disease. Afr Health Sci. 2022;22(2):273-285. doi: 10.4314/ahs.v22i2.31.
20. Foschi FG, Bedogni G, Domenicali M, et al. Prevalence of and risk factors for fatty liver in the general population of Northern Italy: the Bagnacavallo Study. BMC Gastroenterol. 2018;18(1):177. doi: 10.1186/s12876-018-0906-8.
21. Abdel-Razik A, Mousa N, Shabana W, et al. De novo portal vein thrombosis in non-cirrhotic non-alcoholic fatty liver disease: a 9-year prospective cohort study. Front Med (Lausanne). 2021;8:650818. doi: 10.3389/fmed.2021.650818.
22. Di Minno MN, Tufano A, Rusolillo A, Di Minno G, Tarantino G. High prevalence of nonalcoholic fatty liver in patients with idiopathic venous thromboembolism. World J Gastroenterol. 2010;16(48):6119-6122. doi: 10.3748/wjg.v16.i48.6119.
23. Tripodi A, Anstee QM, Sogaard KK, Primignani M, Valla DC. Hypercoagulability in cirrhosis: causes and consequences. J Thromb Haemost. 2011;9(9):1713-1723. doi: 10.1111/j.1538-7836.2011.04429.x.
24. Stine JG, Niccum BA, Zimmet AN, et al. Increased risk of venous thromboembolism in hospitalized patients with cirrhosis due to non-alcoholic steatohepatitis. Clin Transl Gastroenterol.

2018;9(3):140. doi: 10.1038/s41424-018-0002-y.
25. Lüthi-Corridori G, Giezendanner S, Kueng C, et al. Risk factors for hospital outcomes in pulmonary embolism: A retrospective cohort study. Front Med (Lausanne). 2023;10:1120977. doi: 10.3389/fmed.2023.1120977.
26. Isath A, Shah R, Bandyopadhyay D, et al. Dispelling the saddle pulmonary embolism myth (from a comparison of saddle versus non-saddle pulmonary embolism). Am J Cardiol. 2023;201:341-348. doi: 10.1016/j.amjcard.2023.06.048.
27. Ģībietis V, Kigitoviča D, Vītola B, Strautmane S, Skride A. Glomerular filtration rate as a prognostic factor for long-term mortality after acute pulmonary embolism. Med Princ Pract. 2019;28(3):264-272. doi: 10.1159/000497436.
28. Rattazzi M, Villalta S, De Lucchi L, et al. Chronic kidney disease is associated with increased risk of venous thromboembolism recurrence. Thromb Res. 2017;160:32-37. doi: 10.1016/j.thromres.2017.10.011.
29. Cantu-Martinez O, Martinez Manzano JM, Tito S, et al. Clinical features and risk factors of adverse clinical outcomes in central pulmonary embolism using machine learning analysis. Respir Med. 2023;215:107295. doi: 10.1016/j.rmed.2023.107295.
30. Fabbian F, Gallerani M, Pala M, et al. In-hospital mortality for pulmonary embolism: relationship with chronic kidney disease and end-stage renal disease. The hospital admission and discharge database of the Emilia Romagna region of Italy. Intern Emerg Med. 2013;8(8):735-740. doi: 10.1007/s11739-012-0892-8.
31. Park SH. Pulmonary embolism is more prevalent than deep vein thrombosis in cases of chronic obstructive pulmonary disease and interstitial lung diseases. Springerplus. 2016;5(1):1777. doi: 10.1186/s40064-016-3475-8.
32. Zhang T, Xue PJ, Li YY, Peng M, Sun XF, Shi JH. [Clinical and prognostic analysis of acute pulmonary thromboembolism in the elderly]. Zhonghua Jie He He Hu Xi Za Zhi. 2022;45(6):539-545. doi: 10.3760/cma.j.cn112147-20211126-00839. [Article in Chinese]
33. Bi W, Liang S, He Z, et al. The prognostic value of the serum levels of brain natriuretic peptide, troponin I, and D-dimer, in addition to the neutrophil-to-lymphocyte ratio, for the disease evaluation of patients with acute pulmonary embolism. Int J Gen Med. 2021;14:303-308. doi: 10.2147/IJGM.S288975.
34. Jiménez D, Díaz G, Molina J, et al. Troponin I and risk stratification of patients with acute nonmassive pulmonary embolism. Eur Respir J. 2008;31(4):847-853. doi: 10.1183/09031936.00113307.

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Effects of insulin resistance on cardiovascular risk factors in obese and non-obese patients

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ABSTRACT

Objectives: Objectives: It is known that insulin resistance increases cardiovardiovascular risk. But it could not obviously be understood whether insulin resistance itself or the metabolic syndrome parameters, like obesity, that already exist in most of them, are responsible for this increased risk. Our aim is to determine cardiovascular risks in obese and non-obese insulin-resistant patients.

Methods: One hundred thirty-nine patients were included in the study. They were divided into 4 groups: Group 1 (having obesity and insulin resistance), Group 2 (having only insulin resistance but not obesity), Group 3 (having obesity but not insulin resistance), and Group 4 (having neither obesity nor insulin resistance). Patients having any systemic disease were excluded. Insulin resistance is calculated via Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) formula. Electrocardiographic, echocardiographic and lipid parameters of these patients were compared.

Results: High-density lipoprotein (HDL) levels of Group-4 were higher than Group 1 and Group 2. There was no statistical difference in HDL cholesterol levels between Group 3 and the others. Triglyceride and very low-density lipoprotein levels were higher in Group 1. There was no difference in P wave dispersion between the groups. In echocardigraphy, epicardial fat tissue thickness of Group 1 was significantly higher. Prevalance of diastolic dysfunction was higher in Group 1 compared to Group 4.

Conclusion: Insulin resistance itself is a risk factor for low HDL levels independent of obesity. When obesity is added to insulin resistance, other cardiovascular risk factors appear, like high triglyceride levels, increase in epicardial fat tissue thickness and presence of diastolic dysfunction. Early detection of insulin resistance may alert us to the risks of cardiovascular diseases.

Keywords: Insulin resistance, cardiovascular risk factors, obesity, high-density lipoprotein

Insulin resistance defines biological responsiveness to both endogenous and exogenous insulin in the body. In clinical practice, mostly the Homeostatic Model Assessment of Insulin Resistance (HOMA-IR) formula is used to determine insulin resistance. It may be accepted as insulin resistance when

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HOMA-IR is calculated above 2.7. [1, 2].

Metabolic syndrome (MS) is a collection of cardiometabolic risk factors that includes obesity, insulin resistance, hypertension, and dyslipidemia [3]. In many cases, metabolic syndrome or insulin resistance is accompanied by obesity. To determine obesity, mostly body mass index (BMI) and waist circumference measurements are used. BMI over 30 kg/m2 shows obesity.

It is well known that obesity and metabolic syndrome are important risk factors for cardiovascular diseases. The relation between metabolic syndrome and cardiovascular diseases is thought to be based on insulin resistance and the hyperinsulinemia following it [4]. However, insulin resistance and all the components of metabolic syndrome are accepted as cardiac risk factors.

There are many studies in the literature that shows an increased risk of hypertension, dyslipidemia, heart failure, coronary artery diseases, and arrhythmias in patients with metabolic syndrome or insulin resistance [5-10]. Also, diastolic and systolic functions of the heart are worse affected in those groups of patients.

In many of those studies, patients with insulin resistance or metabolic syndrome also have increased body mass index. As a result, it couldn't obviously be understood whether insulin resistance itself or obesity, which is seen in most of them, is responsible for cardiovascular risks in these patients. To our knowledge, there are no studies evaluating cardiac risks in obese and non-obese patients with insulin resistance.

Our aim in this study is to observe obese and non-obese insulin-resistant patients in terms of determining cardiovascular risks in this group.

METHODS

One hundred and thirty-nine patients, who were admitted to the outpatient clinic of Internal Medicine for some reason without acute inflammatory or infectious disease that could change blood sugar and insulin levels and known systemic diseases were included in the study. The demographic features of patients are shown in Table 1. These patients were divided into 4 groups: Group 1 (Patients that have both obesity and insulin resistance), Group 2 (Patients that only have insulin resistance but not obesity), Group 3 (Patients that only have obesity but not insulin resistance), and Group 4 (Patients without obesity and insulin resistance; control group). These groups were matched in terms of age and sex.

All the subjects were evaluated first for the presence of any systemic disease. Patients that have diabetes mellitus, hypertension, coronary artery disease, heart failure, kidney failure, renal failure, cardiac arrhythmias, hypothyroidism, hyperthyroidism, valvular heart disease, electrolyte imbalance, age under 20 and over 70 were excluded.

The patients underwent complete physical examination and laboratory tests including fasting blood glucose, insulin, glycated hemoglobin (HbA1c), urea, creatinine, hemogram, aspartate aminotransferase, alanine aminotransferase, total cholesterol, low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol, triglyceride, free t4, thyroid stimulating hormone, sodium, potassium, calcium, and magnesium. Insulin resistance is calculated via HOMA-IR formula. The formula HOMA-IR = fasting glucose(mg/dL) × insulin (U/mL) /405 was used to determine the HOMA-IR score. Patients having HOMA-IR values over 2.7 were accepted as insulin resistant. Body mass indexes (BMI) were calculated for each of the patients. The physical examinations and medical evaluations of the patients were done by the same doctor [1, 2].

Also, patients were evaluated by electrocardiography (ECG) and echocardiography. P wave dispersions in ECG were calculated. Echocardiography was performed by a cardiologist. In echocardiography, the left ventricular mass index, epicardial fat tissue thickness, myocardial performance index, sol atrial volume, and E/E' were the main parameters. Patients were also investigated whether they had diastolic dysfunction. Standard parasternal, apical, and subcostal views were used using a 3. 5 MHz transducer from the VIVID 5S GE Medical System in Istanbul, Turkey, for the two-dimensional echocardiography. Measurements were made in the parasternal long-axis view of the left atrium (LA), left ventricular end-diastolic diameter (LVEDD), and interventricular septum diameter (IVSD). Using a two-dimensional picture of the LV chamber during systole and diastole in the 4- and 2-chamber apical views, the left ventricular ejection fraction was determined using Simpson's formula. The diagnosis of left ventricular hypertrophy (LVH) was

Table 1. Distribution of demographical features

	Mean±SD	Min-Max
Age (year)	46.81±8.75	25-68
Height (cm)	162.28±7.85	146-186
Weight (kg)	78.77±16.63	49-133
BMI (kg/m^2)	29.89±5.87	19-47
Waist circumference (cm)	95.35±13.17	72-132
Gender	**n**	**%**
Female	113	81.3
Male	26	18.7

BMI=Body mass index, SD=standard deviation, Min=minimum, Max=maximum

made using the algorithm suggested by the American Society of Echocardiography (ASE) for predicting LV mass from 2D linear LV measures and indexed to body surface area (the cut-off values were LVMI >115 g/m^2 for men and >95 g/m^2 for women) [11]. The pulsed-wave Doppler gate was positioned in the LV at the level of the mitral valve margins, and mitral valve inflow was captured in the apical 4-chamber view. We measured the phase E deceleration time and the E/A ratio. The flow pattern via the aortic and mitral valves could be registered and the isovolumic diastolic time could be calculated simultaneously with the apical 5-chamber image. Mitral annular velocity was measured using tissue Doppler imaging in the apical views. The sample volume was adjusted as necessary (often 5-10 mm) to cover the longitudinal excursion of the mitral annulus during diastole, with the sample being placed at or within 1 cm of the septal insertion sites of the mitral leaflets. Additionally, the E/E' ratio and mitral septal annulus early diastolic velocity (septal E') were computed. LA volume measured by biplane area-length method: Orthogonal apical views, apical four and two-chamber views are obtained for determination of LA area and length. The length is determined from the middle of the plane of the mitral annulus to the posterior wall. Left atrial volume is calculated on the basis of the algorithm 0.85×A1×A2/L; where A1 and A2 are the areas of LA in four and 5 two-chamber views and L is the shortest of the lengths obtained from the orthogonal views and indexed to body surface area.

Epicardial adipose tissue (EAT) measurement by

Table 2. Age and gender distribution of groups

	Group 1 (Obese + IR) (n = 37)	Group 2 (IR) (n = 32)	Group 3 (Obese) (n = 32)	Group 4 (Control) (n = 38)	P value
Age (years)					
Mean±SD	46.97±9.11	46.63±8.03	47.63±10.59	46.13±7.44	0.913[a]
Median (Min-Max)	47 (28-68))	46.5 (31-64))	49 (25-65)	46.5 (29-61)	
Gender, n (%)					
Female	29 (78.4)	25 (78.1)	27 (84.4)	32 (84.2)	0.842[b]
Male	8 (21.6)	7 (21.9)	5 (15.6)	6 (15.8)	

IR=insulin resistance, SD=standard deviation, Min=minimum, Max=maximum. [a]One Way Anova Test, [b]Pearson Chi-Square Test

echocardiography is defined as an echo-free space above the right ventricular free wall by transthoracic echocardiography and measured the thickness from the anterior aspect of the right ventricular free wall through parasternal long axis window.

The study obtained approval from the local ethic committee (no: 89513307/1009/ 284 and date: 06.05.2014)nd it has been carried out as per the Helsinki Declaration. All the participants gave informed consent before they were included in the study.

Statistical Analysis

The statistical analysis was conducted using the NCSS (Number Cruncher Statistical System) 2007 Statistical Software (Utah, USA) application. The Oneway Anova test was used for the intergroup comparisons of parameters with normal distribution during the evaluation of the study data, regarding the comparisons of quantitative data as well as descriptive statistical methods (Mean, Standard deviation, median, frequency, and ratio), and the Tukey HSD test was used to determine the group causing the difference. When comparing parameters between groups that did not have a normal distribution, the Kruskal-Wallis test was utilized, and the Mann-Whitney U test was used to identify the group that caused the difference and to evaluate the performance of the two groups. For the comparison of qualitative data, Yates Continuity Correction and Pearson Chi-Square tests were applied. At a significance level of $P<0.05$, the data were assessed using a 95% confidence interval.

RESULTS

A total of 139 participants, 81.3% (n=113) female and 18.7% (n=26) male, aged between 25-68 years, were included in this study. Demographic parameters are shown in Table 1. The patients were divided into 4 groups according to the presence of insulin resistance and obesity. The age and sex distribution of the groups are shown in Table 2. Lipid profiles, P wave dispersion in ECG, and echocardiography parameters were compared between the groups.

Laboratory parameters were shown in Table 3, and lipid profile distributions of the groups were shown in Table 4. There was no statistically meaningful difference between the groups in LDL-cholesterol and total cholesterol levels ($P>0.05$). HDL-cholesterol levels of Group 4 were higher than Group 1 and Group 2

Table 3. Distribution of laboratory findings

	Mean±SD	Min-Max
Glucose (mg/dL)	95.60±10.00	76-121
HbA1c (%)	5.44±0.36	4.2-6.4
Insulin (µU/mL)	12.56±6.79	3.3-38.9
Urea (mg/dL)	27.65±6.79	13-51
Creatinine (mg/dL)	0.67±0.15	0.24-1.22
AST (U/L)	22.30±7.29	12-61
ALT (U/L)	24.83±20.71	6-209
Total cholesterol (mg/dL)	224.84±42.89	123-359
LDL cholesterol (mg/dL)	144.52±34.66	72-217
HDL cholesterol (mg/dL)	53.19±12.31	27-92
Triglyceride (mg/dL)	135.48±90.92	26 – 678
VLDL cholesterol (mg/dL)	26.96±17.94	6-136
HOMA-IR	2.99±1.72	0.74-11.10

HbA1c= glycated hemoglobin, AST= aspartate aminotransferase, ALT= alanine aminotransferase, HDL=high density lipoprotein, VLDL=very low density lipoprotein, HOMA-IR= Homeostatic Model Assessment of Insulin Resistance, SD=standard deviation, Min=minimum, Max=maximum

Table 4. **Lipid profile of groups**

		Group 1 (Obese + IR) (n=57)	Group 2 (IR) (n=36)	Group 3 (Obese) (n=17)	Group 4 (Control) (n=35)	P value	P value (1-2)	P value (1-3)	P value (1-4)	P value (2-3)	P value (2-4)	P value (3-4)
Total cholesterol (mg/dL)	Mean±SD	223.30±45.51	230.38±40.18	217.09±42.76	228.18±43.22	**0.607**[a]	0.904	0.933	0.961	0.607	0.997	0.706
	Median (Min-Max)	218 (154-359)	240.5 (139-291)	213 (123-290)	226 (147-326)							
LDL Cholesterol (mg/dL)	Mean±SD	138.47±33.14	151.03±37.43	143.47±32.86	145.97±35.62	**0.526**[a]	0.463	0.934	0.790	0.827	0,933	0.991
	Median (Min-Max)	140.5 (86-211)	152.5 (81-217)	141.5 (73-216)	152 (72-212)							
HDL Cholesterol (mg/dL)	Mean±SD	49.41±13.25	50.75±9.44	52.91±9.54	5918±13.69	**0.003**[a]	0.965	0.610	**0.003**	0.885	**0,018.**	0.124
	Median (Min-Max)	49 (27-83)	49 (36-69)	51.5 (37-78)	57.5 (37-92)							
Triglyceride (mg/dL)	Mean±SD	172.08±116.51	148.19±99.47	104.13±48.15	115.53±68.22	**0.004**[c]	0.273	**0.002**	**0.007**	**0.019***	0.075	0.934
	Median (Min-Max)	138 (49-678)	115.5 (59-481)	87.5 (30-234)	93 (26-289)							
VLDL cholesterol (mg/dL)	Mean±SD	34.46±23.38	29.06±18.8	20.84±9.72	23.05±13.60	**0.003**[c]	0.243	**0.002**	**0.006**	**0.021***	0.068	0.953
	Median (Min-Max)	28 (10-136)	23.5 (12-96)	17.5 (6-47)	18.5 (8-58)							

SD=standard deviation, Min=minimum, Max=maximum, [a]Oneway Anova Test (post hoc Tukey HSD), [c]Kruskal Wallis Test (post hoc Mann Whitney U test)

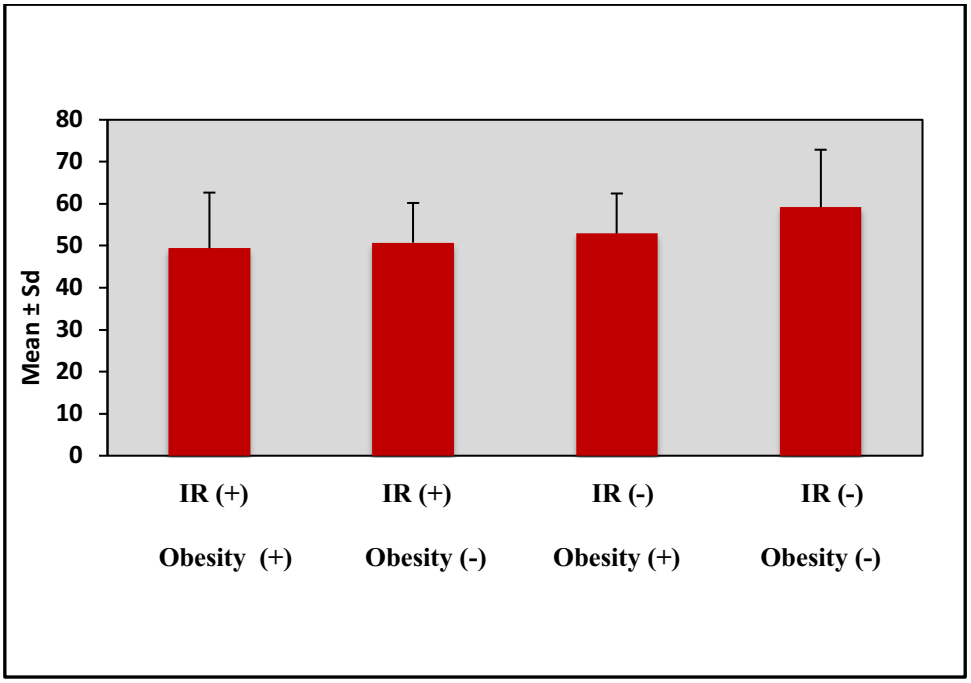

Fig. 1. HDL cholesterol level distribution of groups.

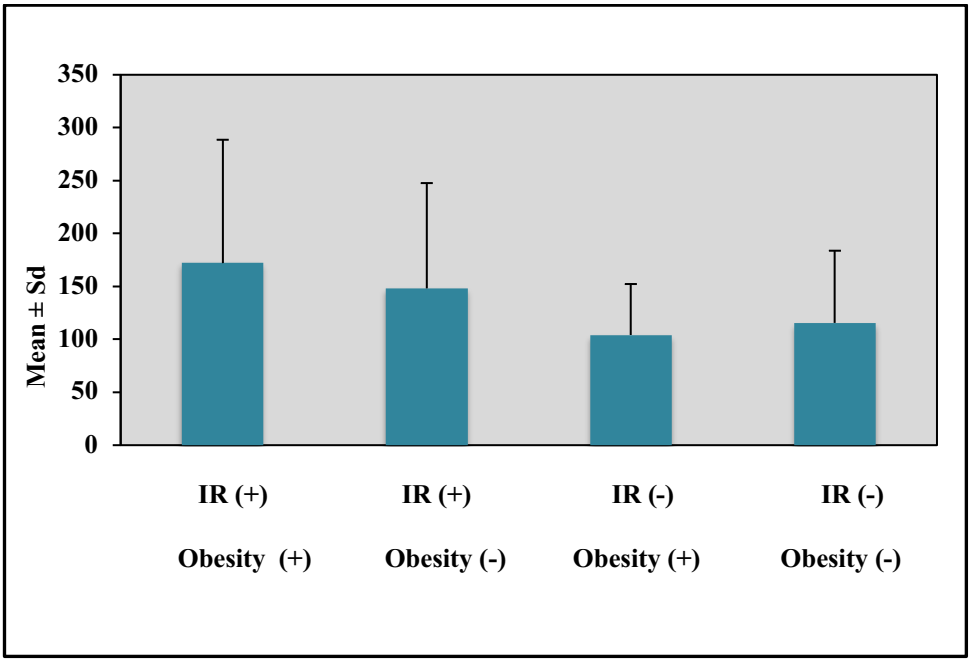

Fig. 2. Triglyceride level distribution of groups.

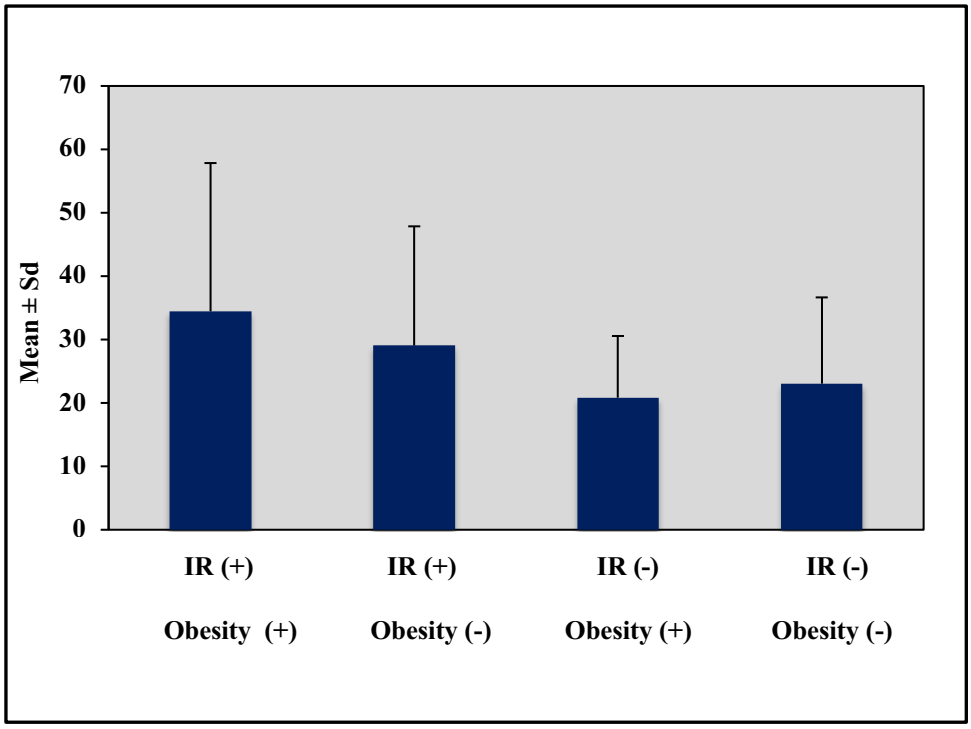

Fig. 3. VLDL cholesterol level distribution of groups.

(P<0.05). No statistical difference was detected in HDL cholesterol levels between Group 3 and the others (Fig. 1). Triglyceride and VLDL-cholesterol levels of Group 1 were higher than Group 3 and Group 4 (P<0.01). Triglyceride and VLDL-cholesterol levels of Group 2 were higher than Group 3 (P<0.05). (Figs. 2 and 3).

The results of echocardiography parameters were shown in Table 5. There was no difference in P wave dispersion, left ventricular mass index, myocardial performance index, and E/E ratio between the groups (P>0.05). Epicardial fat tissue thickness of Group 1 was significantly higher than Group 2, Group 3, and Group 4 (P<0.01) (Fig. 4). Left atrial volumes of Group 2 were statistically lower than Group 3 and Group 4 (P<0.05) (Fig. 5).

The prevalence of diastolic dysfunction was higher in Group 1 compared to Group 4 (P<0.01). (Fig. 6). The distribution of echocardiography parameters of all the groups were shown in Table 6

DISCUSSION

The leading cause of death in the world continues to be diseases of the cardiovascular system. Cardiovascular diseases (CVD) include hypertension, coronary heart disease, congestive heart failure, atherosclerosis, cerebrovascular diseases, peripheral artery diseases, and conditions that often occur in combination. In 2012 and 2013, CVD was estimated to result in 17.3 million deaths worldwide on an annual basis [12, 13].

Table 5. Distribution of echocardiography parameters

	Mean±SD	Min-Max
Left ventricular mass index (g/m^2)	71.40±15.13	37-134
Epicardial fat tissue thickness (mm)	2.39±1.22	1-6
Myocardial performance index	0.41±0.10	0.14-0.70
Left atrial volüme (mL/m^2)	17.49±4.42	7-30
E/E'	6.55±1.69	3.40-11.20
Diastolic dysfunction	**n**	**%**
No	82	59.0
Yes	57	41.0

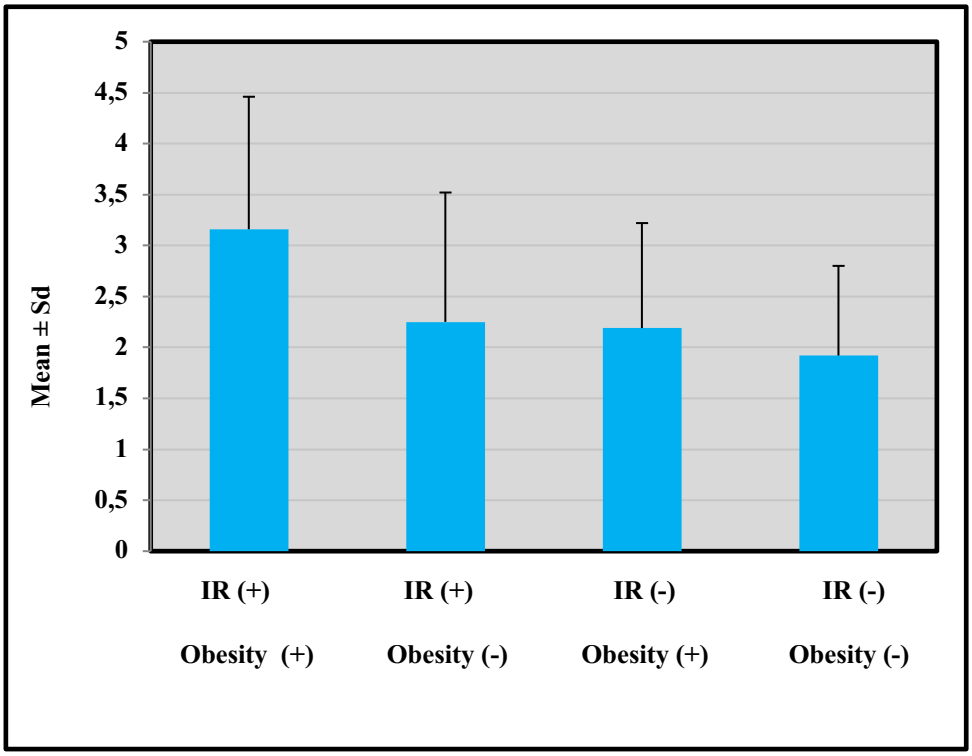

Fig. 4. Epicardial fat tissue thickness distribution of groups.

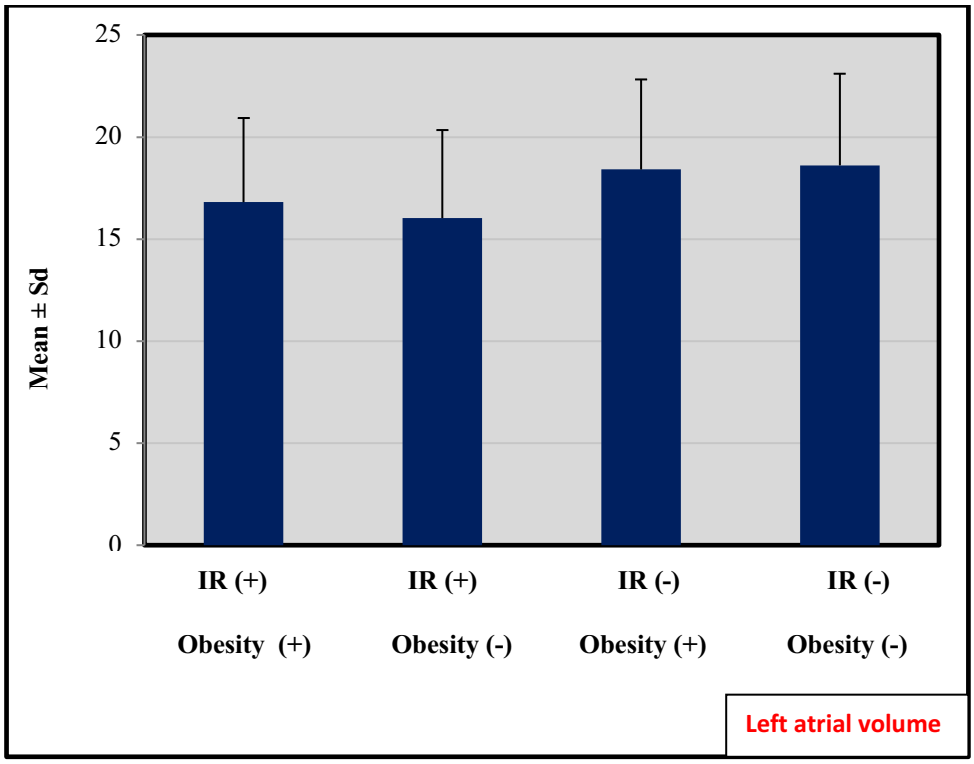

Fig. 5. Left atrial volume distribution of groups.

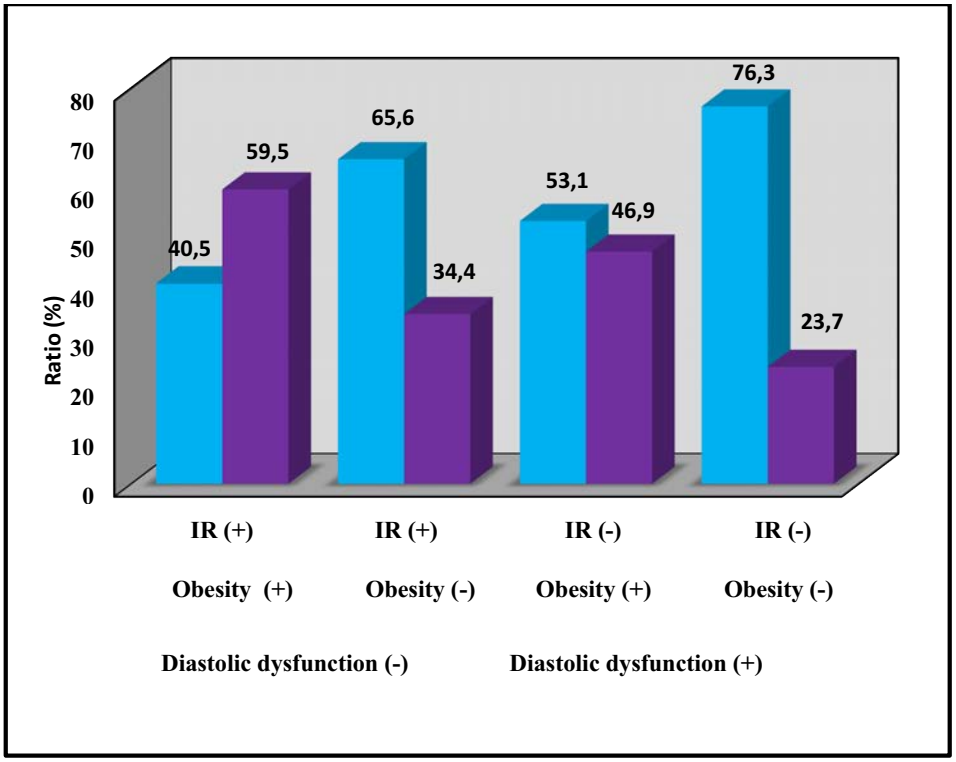

Fig. 6. Presence of diastolic dysfunction distribution of groups.

And this number of deaths is expected to reach 23 million by the year 2030. Many individuals in the general population have one or more risk factors for CVD. The five leading modifiable risk factors (hypercholesterolemia, diabetes, hypertension, obesity, and smoking) are estimated to be responsible for more than half of cardiovascular mortality [14]. The increase in risk when multiple risk factors are present has been noted in several studies [15, 16].

Many risk factors for CVD are modifiable by specific preventive measures. In this study we focused on insulin resistance that can later lead to diabetes mellitus, which is reported as a coronary heart disease risk equivalent, thereby elevating it to the highest risk category. It has also been suggested as an important risk factor in the development of metabolic syndrome, a cluster of abnormalities comprising glucose intolerance, dyslipidemia, high blood pressure, and impaired fibrinolysis activity that is associated with an increased risk of developing type 2 diabetes mellitus and CVD. For this reason, detecting insulin resistance, diagnosing patients in the prediabetic stage, and taking preventive measures by modifying environmental risk factors may reduce total cardiovascular mortality in the population. There are many studies that show increased cardiovascular risk in insulin-resistant patients [17, 18].

Obesity is the most important cause of insulin resistance and also a component of metabolic syndrome. It is well demonstrated that obesity is a risk factor for type 2 diabetes and CVD [19]. In the literature, studies made about insulin resistance and metabolic syndrome mostly contain obese individuals also. This close association between insulin resistance and obesity has made it difficult to establish whether insulin resistance, independent of obesity, is associated with cardiovascular risk. But it must be borne in mind that there are also non-obese insulin-resistant individuals.

Our study aimed to see the cardiovascular risks of non-obese insulin-resistant patients. For this purpose, we looked at the lipid profiles of these patients and performed ECG, and echocardiography on each of them. Then we compared the results with the control and the obese group.

In the lipid profile, we saw that obesity alone does not contribute to a decline in HDL levels. But insulin resistance alone, without obesity, triggers low HDL levels. In patients having both insulin resistance and

Table 6. Echocardiography parameters of groups

		Group 1 (Obese + IR) (n=37)	Group 2 (IR) (n=32)	Group 3 (Obese) (n=32)	Group 4 (Control) (n=38)	P value	P value (1-2)	P value (1-3)	P value (1-4)	P value (2-3)	P value (2-4)	P value (3-4)
Left ventricular mass index (g/m^2)	Mean±SD	76.38±17.47	68.91±14.11	71.69±15.53	68.42±12.18	**0.094**[a]	0.168	0.564	0.102	0.879	0.999	0.799
	Median (Min-Max)	76 (37-134)	66.5 (49-113)	69.5 (47-120)	46-101 (66)							
Epicardial fat tissue thickness (mm)	Mean±SD	3.16±1.30	2.25±1.27	2,19 ± 1,03	1.92±0.88	**0.001**[c]	**0.003**	**0.002**	**0.001**	0.955	0.382	0.318
	Median (Min-Max)	3 (1-6)	2 (1-6)	2 (1-5)	2 (1-4)							
Myocardial performance index	Mean±SD	0.4±0.10	0.45±0.10	0.4±0.13	0.4±0.08	**0.130**[a]	0.173	0.998	1.000	0.264	0.181	0.999
	Median (Min-Max)	0.39 (0.24-0.7)	0.4 (0.2-0.7)	0.39 (0.14-0.7)	0.4 (0.2-0.56)							
Left atrial volüme (mL/m^2)	Mean±SD	16.81±4.12	16.03±4.31	18.41±4.41	18.61±4.49	**0.041**[a]	0.878	0.425	0.281	**0.030**	**0.014**	0.997
	Median (Min-Max)	16 (9-27)	16 (7-25)	18.5 (11-27)	18 (8-30)							
E/E'	Mean±SD	6.85±1.56	6.25±1.99	7.00±1.79	6.14±1.34	**0.080**[a]	0.442	0.983	0.253	0.279	0.993	0.143
	Median (Min-Max)	6.5 (4.4-11)	5.7 (3.8-11.2)	6.75 (4.1-11)	6.05 (3.4-10)							
P wave dispersion (ms)	Mean±SD	31.89±11.98	34.38±13.66	34.38±9.14	34.74±13.7	**0.722**[c]	0.482	0.247	0.394	0.780	0.912	0.876
	Median (Min-Max)	40 (20-60)	40 (20-60)	40 (20-40)	40 (20-60)							
Diastolic dysfunction, n (%)	No	15 (40.5)	21 (65.6)	17 (53.1)	29 (76.3)	**0.012**[b]	0.066	0.422	**0.004**	0.445	0.471	0.074
	Yes	22 (59.5)	11 (34.4)	15 (46.9)	9 (23.7)							

SD=standard deviation, Min=minimum, Max=maximum, [a]One way Anova Test (post hoc Tukey HSD), [b]Pearson Chi-Square Test, [c]Kruskal Wallis Test (post hoc Mann Whitney U test)

obesity, HDL - cholesterol levels were lower than the control group. Neither insulin resistance nor obesity alone correlates with triglyceride levels. But in non-obese insulin-resistant patients, triglyceride levels are found higher than in the obese, insulin-sensitive group. When obesity and insulin resistance come together, triglyceride levels reach to significantly higher levels compared to control group.

Results detected in triglyceride were also valid for VLDL-cholesterol. There was no statistically meaningful difference between the groups in LDL-cholesterol and total cholesterol levels. These results were similar to the ones found in the study done by Piché *et al*. [20] in 2005. But differently, our study showed that insulin resistance is a risk factor for low HDL-cholesterol levels independent of obesity. Also, triglyceride levels in insulin-resistant non-obese patients were found higher than insulin-sensitive obese patients. This suggested that insulin resistance may be a more important risk factor for high triglyceride levels than obesity.

The diminished antilipolytic activity of insulin can be used to explain the independent role of insulin resistance in the variation in triglyceride concentrations. This causes a rise in the amount of free fatty acids in the blood and their flow to the liver, which can promote the synthesis of triglycerides [21]. Another study found that the dysregulation of intramyocellular fatty acid metabolism was related to insulin resistance in the skeletal muscle of healthy, young, thin, insulin-resistant offspring of people with type 2 diabetes. These changes in fatty acid metabolism might serve as a connection between insulin resistance and hypertriglyceridemia [22].

In a different study conducted in 2003 by Nieves *et al*. [23], they discovered that non-obese insulin-resistant patients had different lipid-lipoprotein profiles than non-obese insulin-sensitive individuals (higher concentrations of triglycerides, LDL cholesterol, and apo-B, and lower concentrations of HDL cholesterol). In the electrocardiography of patients, there was no meaningful difference between groups in terms of P wave dispersion. In echocardiography, we found that neither obesity nor insulin resistance alone has an independent risk for developing diastolic dysfunction. But when they are seen together, diastolic dysfunction prevalence increases. Carvalho *et al*. [24] discovered that people with increased insulin resistance had lower diastolic function measures in their study. In addition, Dinh *et al*. [25] showed that insulin resistance was a separate factor in the development of LVDD in a sample of chosen non-diabetic patients undergoing elective coronary angiography. In another study performed by Russo *et al*. [26], they demonstrated an independent association between LVDD and obesity.

Subclinical left ventricular diastolic dysfunction (LVDD) is widespread in the population and is known to be a significant indicator of long-term mortality and heart failure. The identification of these asymptomatic abnormalities in left ventricular function and the early detection of its primary risk factors are of particular importance, according to current heart failure recommendations [27]. According to certain research, changes in diastolic function, which are already present in pre-diabetic patients [28], may be linked to the condition of insulin resistance and occur before the onset of diabetes. MS, also known as insulin resistance syndrome, is a collection of risk factors for cardiovascular disease that have been demonstrated to work in concert to raise the chance of unfavorable cardiovascular events as well as to cause subclinical alterations in heart structure and function. Indeed, LVDD is more common among patients with metabolic syndrome [29].

In our study, like diastolic dysfunction, it is found that obesity or insulin resistance alone are not directly related to increased epicardial adipose tissue (EAT) thickness. But when they are seen together, this causes an increase in EAT thickness.

EAT is a part of the visceral fat deposit. It has endocrine, paracrine, vasocrine, and inflammatory effects and is related to metabolic syndrome, insulin resistance, coronary artery disease, and hypertension [30, 31]. EAT produce various cytokines and vasoactive peptides such as free fatty acids, interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), angiotensin II, and plasminogen activator inhibitor, all of which increase the cardiovascular risk [32]. In patients with significant coronary artery disease, higher levels of chemokines and cytokines were detected in epicardial fat tissue [33]. Also, a relationship between EAT thickness and coronary artery calcification score and severity of coronary heart disease was detected in a study by Picard *et al*. [34].

Limitations

Main limitations of this study is its low sample size and being unable to rule out contribution of genetic factors which are very important for cardiovascular risk determinants.

CONCLUSION

In conclusion, early detection of insulin resistance may alert us to the risks of cardiovascular diseases. Insulin resistance itself is a risk factor for low HDL-cholesterol levels independent of obesity. We should also consider cardiovascular risk factors in cases of insulin resistance not accompanied by obesity and we should screen these patients for dyslipidemia. When obesity is added to insulin resistance, other cardiovascular risk factors appear, like high triglyceride levels, an increase in epicardial fat tissue thickness, and the presence of diastolic dysfunction. Future studies will evaluate whether administering medications that boost insulin sensitivity can enhance the structure and function of the myocardium, particularly diastolic function, or have a positive impact on the lipid profile.

Authors' Contribution

Study Conception: BAT, GF, KSF; Study Design: GF, KSF, TU; Supervision: GF, ÖK; Funding: GF; Materials: BAT, NPT, KSF; Data Collection and/or Processing: BAT, NPT; Statistical Analysis and/or Data Interpretation: BAT, TU, NPT; Literature Review: ÖK, BAT, GF; Manuscript Preparation: BAT, GF, TU and Critical Review: ÖK, GF.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. Katsuki A, Sumida Y, Gabazza EC, et al. Homeostasis model assessment is a reliable indicator of insulin resistance during follow-up of patients with type 2 diabetes. Diabetes Care. 2001;24(2):362-365. doi: 10.2337/diacare.24.2.362.
2. Krishnamurthy H, Siriwardhane T, Krishna K, et al. Insulin resistance in thyroid disorders: association between anti-TPO and HOMA-IR. medRxiv 2023. doi: 10.1101/2023.06.06.23291013.
3. Roberts CK, Hevener AL, Barnard RJ. Metabolic syndrome and insulin resistance: underlying causes and modification by exercise training. Compr Physiol. 2013;3(1):1-58. doi: 10.1002/cphy.c110062.
4. Silveira Rossi JL, Barbalho SM, Reverete de Araujo R, Bechara MD, Sloan KP, Sloan LA. Metabolic syndrome and cardiovascular diseases: going beyond traditional risk factors. Diabetes Metab Res Rev. 2022;38(3):e3502. doi: 10.1002/dmrr.3502.
5. Novo G, Pugliesi M, Visconti C, et al. Early subclinical ventricular dysfunction in patients with insulin resistance. J Cardiovasc Med (Hagerstown). 2014;15(2):110-114. doi: 10.2459/JCM.0b013e3283638164.
6. Hwang YC, Jee JH, Kang M, Rhee EJ, Sung J, Lee MK. Metabolic syndrome and insulin resistance are associated with abnormal left ventricular diastolic function and structure independent of blood pressure and fasting plasma glucose level. Int J Cardiol. 2012;159(2):107-111. doi: 10.1016/j.ijcard.2011.02.039.
7. Safar ME, Balkau B, Lange C, et al. Hypertension and vascular dynamics in men and women with metabolic syndrome. J Am Coll Cardiol. 2013;61(1):12-9. doi: 10.1016/j.jacc.2012.01.088.
8. Cadeddu C, Nocco S, Piano D, et al. Early impairment of contractility reserve in patients with insulin resistance in comparison with healthy subjects. Cardiovasc Diabetol. 2013;12:66. doi: 10.1186/1475-2840-12-66.
9. Onuchina EL, Solov'ev OV, Mochalova OV, Kononov SK, Onuchin SG. [Metabolic syndrome and chronic persistent atrial fibrillation]. Klin Med (Mosk). 2011;89(1):26-31. [Article in Russian]
10. Hung CL, Chao TF, Lai YH, et al. The relationship among atrium electromechanical interval, insulin resistance, and metabolic syndrome. Can J Cardiol. 2013;29(10):1263-1268. doi: 10.1016/j.cjca.2013.01.001.
11. Lang RM, Badano LP, Mor-Avi V, et al. Recommendations for cardiac chamber quantification by echocardiography in adults: an update from the American Society of Echocardiography and the European Association of Cardiovascular Imaging. J Am Soc Echocardiogr. 2015;28(1):1-39.e14. doi: 10.1016/j.echo.2014.10.003.
12. GBD 2013 Mortality and Causes of Death Collaborators. Global, regional, and national age-sex specific all-cause and cause-specific mortality for 240 causes of death, 1990-2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet. 2015;385(9963):117-171. doi: 10.1016/S0140-6736(14)61682-2.
13. Roth GA, Huffman MD, Moran AE, et al. Global and regional patterns in cardiovascular mortality from 1990 to 2013. Circulation. 2015;132(17):1667-1678. doi: 10.1161/CIRCULATIONAHA.114.008720.
14. Patel SA, Winkel M, Ali MK, Narayan KM, Mehta NK. Cardiovascular mortality associated with 5 leading risk factors: national and state preventable fractions estimated from survey data. Ann Intern Med. 2015;163(4):245-53. doi: 10.7326/M14-1753.
15. Vasan RS, Sullivan LM, Wilson PW, et al. Relative importance of borderline and elevated levels of coronary heart disease risk factors. Ann Intern Med. 2005;142(6):393-402. doi:

10.7326/0003-4819-142-6-200503150-00005.
16. Lowe LP, Greenland P, Ruth KJ, Dyer AR, Stamler R, Stamler J. Impact of major cardiovascular disease risk factors, particularly in combination, on 22-year mortality in women and men. Arch Intern Med. 1998;158(18):2007-2014. doi: 10.1001/archinte.158.18.2007.
17. Adeva-Andany MM, Martínez-Rodríguez J, González-Lucán M, Fernández-Fernández C, Castro-Quintela E. Insulin resistance is a cardiovascular risk factor in humans. Diabetes Metab Syndr. 2019;13(2):1449-1455. doi: 10.1016/j.dsx.2019.02.023.
18. Ginsberg HN. Insulin resistance and cardiovascular disease. J Clin Invest. 2000;106(4):453-458. doi: 10.1172/JCI10762.
19. Kissebah AH, Freedman DS, Peiris AN. Health risks of obesity. Med Clin North Am. 1989;73(1):111-138. doi: 10.1016/s0025-7125(16)30695-2.
20. Piché ME, Weisnagel SJ, Corneau L, Nadeau A, Bergeron J, Lemieux S. Contribution of abdominal visceral obesity and insulin resistance to the cardiovascular risk profile of postmenopausal women. Diabetes. 2005;54(3):770-777. doi: 10.2337/diabetes.54.3.770.
21. Julius U. Influence of plasma free fatty acids on lipoprotein synthesis and diabetic dyslipidemia. Exp Clin Endocrinol Diabetes. 2003;111(5):246-250. doi: 10.1055/s-2003-41284.
22. Petersen KF, Dufour S, Befroy D, Garcia R, Shulman GI. Impaired mitochondrial activity in the insulin-resistant offspring of patients with type 2 diabetes. N Engl J Med. 2004;350(7):664-671. doi: 10.1056/NEJMoa031314.
23. Nieves DJ, Cnop M, Retzlaff B, et al. The atherogenic lipoprotein profile associated with obesity and insulin resistance is largely attributable to intra-abdominal fat. Diabetes. 2003;52(1):172-179. doi: 10.2337/diabetes.52.1.172.
24. Fontes-Carvalho R, Ladeiras-Lopes R, Bettencourt P, Leite-Moreira A, Azevedo A. Diastolic dysfunction in the diabetic continuum: association with insulin resistance, metabolic syndrome and type 2 diabetes. Cardiovasc Diabetol. 2015;14:4. doi: 10.1186/s12933-014-0168-x.
25. Dinh W, Lankisch M, Nickl W, et al. Insulin resistance and glycemic abnormalities are associated with deterioration of left ventricular diastolic function: a cross-sectional study. Cardiovasc Diabetol. 2010;9:63. doi: 10.1186/1475-2840-9-63.
26. Russo C, Jin Z, Homma S, et al. Effect of obesity and overweight on left ventricular diastolic function: a community-based study in an elderly cohort. J Am Coll Cardiol. 2011;57(12):1368-1374. doi: 10.1016/j.jacc.2010.10.042.
27. Yancy CW, Jessup M, Bozkurt B, et al. 2013 ACCF/AHA guideline for the management of heart failure: executive summary: a report of the American College of Cardiology Foundation/American Heart Association Task Force on practice guidelines. Circulation. 2013;128(16):1810-1852. doi: 10.1161/CIR.0b013e31829e8807.
28. Bajraktari G, Koltai MS, Ademaj F, et al. Relationship between insulin resistance and left ventricular diastolic dysfunction in patients with impaired glucose tolerance and type 2 diabetes. Int J Cardiol. 2006;110(2):206-211. doi: 10.1016/j.ijcard.2005.08.033.
29. de las Fuentes L, Brown AL, Mathews SJ, et al. Metabolic syndrome is associated with abnormal left ventricular diastolic function independent of left ventricular mass. Eur Heart J. 2007;28(5):553-559. doi: 10.1093/eurheartj/ehl526.
30. Iacobellis G, Leonetti F. Epicardial adipose tissue and insulin resistance in obese subjects. J Clin Endocrinol Metab. 2005;90(11):6300-6302. doi: 10.1210/jc.2005-1087.
31. Ahn SG, Lim HS, Joe DY, et al. Relationship of epicardial adipose tissue by echocardiography to coronary artery disease. Heart. 2008;94(3):e7. doi: 10.1136/hrt.2007.118471.
32. Iacobellis G, Barbaro G. The double role of epicardial adipose tissue as pro- and anti-inflammatory organ. Horm Metab Res. 2008;40(7):442-445. doi: 10.1055/s-2008-1062724.
33. Mazurek T, Zhang L, Zalewski A, et al. Human epicardial adipose tissue is a source of inflammatory mediators. Circulation. 2003;108(20):2460-2466. doi: 10.1161/01.CIR.0000099542.57313.C5.
34. Picard FA, Gueret P, Laissy JP, et al. Epicardial adipose tissue thickness correlates with the presence and severity of angiographic coronary artery disease in stable patients with chest pain. PLoS One. 2014;9(10):e110005. doi: 10.1371/journal.pone.0110005.

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The relationship between heart functions and anemia in patients with end-stage renal disease receiving hemodialysis

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ABSTRACT

Objectives: We investigated the relationship between anemia and cardiac functions by conventional and speckle-tracking echocardiography (STE) in patients with end-stage renal disease (ESRD) receiving hemodialysis.

Methods: One hundred and six patients with ESRD receiving hemodialysis were included in this cross-sectional study. The conventional echocardiography and STE findings were compared between the patients with and without anemia. In addition, a comparison of the findings between the ESRD patients and healthy controls consisting of 68 participants was conducted.

Results: Compared to healthy controls, ESRD patients had lower left ventricular ejection fraction (LVEF), left ventricular global longitudinal strain (LVGLS), and left atrial reservoir strain (LASr) [53% (48-57) vs. 65% (62-68), -15.2 (-16.9- -13.6) vs. -19.7 (-16.9- -13.6), and -21.9 (-29.5- -15.3) vs. -29.9 (-35.3- -22.8), respectively, P-value <0.001 for all]. Of the ESRD patients, 70 (66%) had anemia. ESRD patients with anemia had higher interventricular septum (IVS), posterior wall (PW), and left atrial volume index (LAVi) values than patients without anemia. In addition, ESRD patients with anemia had lower LVEF, LVGLS, and LASr than patients without anemia [median (IQR), 13 (12-15) vs. 12 (11-14), P=0.004, 13 (12-15) vs. 12 (11-13.5), P<0.005, 43 (35-55) vs. 34.7 (28-50), P=0.013, 52 (48-55) vs. 56 (47.5-60), P=0.016, -14.6 (-16.4- -13.5) vs. -16 (-18.6- -14.7), P=0.003, and -21.6 (-30.5- -16.3) vs. -30.5 (-33.6- -23.3), P=0.006, respectively]. In multivariable logistic regression analysis, diabetes, PW, LASr, and LVGLS were independently associated with the presence of anemia in ESRD patients.

Conclusion: Our study confirmed impaired cardiac mechanics in ESRD hemodialysis patients and showed that anemia was associated with further worsening cardiac mechanics in this population.

Keywords: Echocardiography, end-stage renal disease, hemodialysis, anemia

End-stage renal disease (ESRD) is one of the leading causes of mortality and morbidity worldwide [1]. Cardiovascular death accounts for more than half of mortality in patients with ESRD [2-4]. On the other hand, anemia is the most common hematologic complication in ESRD, resulting in

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poorer quality of life, and is reportedly a major risk factor for cardiovascular disease in this population [5, 6].

Many studies examined the cardiac characteristics by echocardiographic examination in ESRD patients [7-9]. However, data showing the association between anemia and cardiovascular mechanics in adult patients with ESRD is rare. The purpose of the present study was to address this information gap. In other words, we aimed to evaluate the relationship between anemia and cardiac mechanics using conventional two-dimensional transthoracic echocardiography (TTE) and speckle-tracking echocardiography (STE) in ESRD patients receiving hemodialysis treatment.

METHODS

Study Population

Between February 5, 2019, and April 30, 2019, adult patients with ESRD receiving hemodialysis were included in this cross-sectional study. Patients with congenital heart disease, severe valvular heart disease, and atrial fibrillation were excluded. Intensive-care patients were also excluded from the study. ESRD (a glomerular filtration rate of less than 15 mL/min) was defined per Kidney Disease Improving Global Outcomes (KDIGO) Chronic Kidney Disease (CKD) evaluation and management guideline [10]. Anemia is defined as hemoglobin concentration <13.0 g/dL in males and <12.0 g/dL in females, based on KDIGO anemia in CKD guidelines [11]. The patients were divided into two groups according to the presence of anemia. Findings were compared between the groups. In addition, a comparison of echocardiographic findings between the patients and age and sex-matched healthy controls of 68 participants was conducted.

The study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Kafkas University (Date: January 30, 2019/ No 80576354-050-99/30). Written informed consent was obtained from all patients or their legal representatives.

Blood Sampling

Venous blood samples were taken on the day of hemodialysis before the TTE examination. Complete blood count, renal function test (blood urea and serum creatinine), lipid profile, serum iron, serum ferritin, and transferrin saturation were done as a routine examination for CKD.

2-D Transthoracic Echocardiography Examination

TTE recordings were obtained using Philips Epiq7 (Philips Ultrasound, WA, USA), according to the American Society of Echocardiography and the European Association of Cardiovascular Imaging guidelines [12]. Diameters of the left ventricle (LV) (end-systolic and end-diastolic), interventricular septum (IVS), LV posterior wall (PW), left atrial (LA) volume, right ventricle (basal), and right atrium were measured. LA volume index (LAVi) was calculated by dividing the left atrial volume by the body surface area. Assessment of mitral inflow included the peak early (E-wave) and late (A-wave) diastolic filling velocities and calculation of the E/A ratio. The peak velocity of early diastolic mitral annular motion (e') was measured by pulse wave Doppler from the apical four-chamber view. Then, the average value was calculated from septal e' and lateral e' values. The modified Simpson's method described in the European Association of Cardiovascular Imaging (EACVI) was used to calculate the left ventricular ejection fraction (LVEF) [12].

2-D speckle-tracking Echocardiography Examination

Speckle-tracking analysis was performed according to the consensus document of the European Association of Cardiovascular Imaging Task Force [13]. Strain analyses were performed by an experienced cardiologist (T.O.) using QLAB Advanced Quantification Software. The end-diastole was regarded as the peak R wave of the electrogram, and the end-systole was estimated as aortic valve closure. LV myocardial deformation analysis was achieved from 2-dimensional gray-scale loops by automatically tracking myocardial speckles after manually selecting landmark points using apical views of the LV. Manually corrected when needed. The region of interest was the endo-myocardium (from the endocardial border to the myocardial mid-line). The left ventricular global longitudinal strain (LVGLS) calculation was obtained by averaging the negative peak of longitudinal strain from 17 ventricular segments from the apical 4-chamber, 3-cham-

ber, and 2-chamber views. The change in the percentage (%) was regarded as LVGLS. Higher negative values represent the ability of myocardial contractility (The less negative value, the worse LVGLS performance). The LA strain was measured in the reservoir phase (LASr) from the apical 4- and 2-chamber views using the QRS complex as a reference point. Then, the mean value was calculated.

Statistical Analysis

Statistical analysis was performed using SPSS software version 20.0 (SPSS, Inc., Chicago, IL, USA). The continuous variables were presented as mean values and standard deviation (SD). Categorical variables were expressed as frequencies and percentages. Kolmogorov–Smirnov test was used to investigate the distribution of the data. While the independent t-test was

Table 1. Demographic, clinical, and laboratory characteristics of the patients

	Overall (n=106)	Anemia (+) (n=70)	Anemia (-) (n=36)	P value
Male sex, n (%)	66 (62.3)	44 (62.9)	22 (61.1)	0.861
Age (years), median [IQR]	71 [60-77]	68 [59-78]	72 [66-77]	0.285
BMI (kg/m^2) mean±SD	25.5±4.2	24.9±3.9	26.8±4.5	**0.027**
Laboratory				
Hemoglobin (g/dL), median [IQR]	10.7 [9.8-12.5]	10.3 [9.3-10.6]	13.1 [12.3-13.7]	**<0.001**
Hematocrit (%)	32.9 [30.4-38]	31.9 [28.7-32.9]	39 [38-41]	**<0.001**
MCV, mean±SD	89.7±6.5	90.4±5.3	89.3± 7	0.402
RDW (fL)	14.9 [14-16.2]	14.6 [13.8-16]	15.5 [14.5-16.5]	0.053
WBC (× 10^3/µL), median [IQR]	6.73 [5.49-9.22]	6.55 [5.3-9.05]	7.06 [5.49-9.22]	0.395
Lymphocyte (× 10^3/µL), median [IQR]	1.4 [1-1.81]	1.27 [1-1.63]	1.65 [1.05-1.96]	**0.039**
Neutrophil (× 10^3/µL), median [IQR]	4.34 [3.48-6.13]	4.3 [3.31-6.13]	4.83 [3.88-5.94]	0.431
Platelet (× 10^3/µL), median [IQR]	198 [156-251]	208 [158-256]	190 [154-223]	0.327
MPV (fL)	9.4 [8.8-10.5]	9.4 [8.8-10.3]	9.3 [8.6-10.8]	0.805
Total cholesterol, (mg/dL) median [IQR]	139 [120-170]	139 [113-166]	139 [128-177]	0.314
Triglyceride, (mg/dL) median [IQR]	113 [85-161]	112 [86-161]	114 [78-167]	0.878
LDL, (mg/dL) median [IQR]	75 [57-100]	75 [53-1008]	76 [60-100]	0.568
HDL, (mg/dL) median [IQR]	36 [26-42]	35 [25-40]	37 [28-44]	0.315
Creatinine (mg/dL), mean±SD	7.24±3.27	7.42±2.73	6.90±4.14	0.442
CRP (mg/L), median [IQR]	9.87 [3.92-32.3]	10 [4.46-30]	7.2 [3.8-31.3]	0.422
Albumin (g/dL), median [IQR]	37.9 [33.2-41.2]	37.6 [33-40.5]	38.1 [35.9-42]	0.454
Hs-TnT (ng/L), median [IQR]	72.9 [29.8-131.2]	66.7 [31-131.3]	91.3 [15.7-123]	0.915
Comorbidities, n (%)				
Hypertension	79 (74.5)	53 (75.7)	26 (2.2)	0.696
Diabetes	47 (44.3)	36 (51.4)	11 (30.6)	**0.040**
Smoking	20 (19.2)	11 (15.9)	9 (25.7)	0.232
Hyperlipidemia	35 (33)	25 (35.)	10 (27.8)	0.411
Coronary artery disease	25 (23.6)	17 (24.3)	8 (22.2)	0.813

BMI=body mass index, CRP=C-reactive protein, fL=femtoliters, HDL=high-density lipoprotein, Hs-TnT=high-sensitive troponin T, IQR=interquartile range, MCV=mean cell volume, MPV=mean platelet volume, LDL=low-density lipoprotein, RDW=red cell distribution width, SD=standard deviation, WBC=white blood count

used to analyze normally distributed continuous data, the Mann-Whitney U test was used to analyze non-normally distributed variables. Categorical variables were compared with the Chi-squared or Fisher exact test. Univariate regression analysis was carried out to find the variables related to the presence of anemia. Moreover, a multivariate logistic regression analysis with a backward conditional method, including body mass index (BMI), diabetes, IVS, PW, LAVi, E/A, TAPSE, LASr, and LVGLS, was used to describe the independently associated variables with the presence of anemia. Data are displayed as odds ratios (95% confidence intervals). Also, spearman correlation analysis was conducted between hemoglobin value and echocardiographic parameters, including LVEF, LVGLS, LASr, and LAVi. The statistical significance level was accepted as two-tailed P values <0.05.

RESULTS

A total of 106 patients were included in the study. The demographical and laboratory findings of the patients are presented in Table 1. The median age was 71 (IQR, 60-77) years. Of the patients, 70 (66%) had anemia. The hemoglobin value in the global patient population was 10.7 gr/dL (IQR, 9.8-12.5). It was 10.3 g/dL (IQR, 9.3-10.6) in the patients with anemia and 13.1 g/dL (IQR, 12.3-13.7) in the patients without anemia (P<0.001). The BMI of the patients with anemia was

Table 2. Comparison of the echocardiographic findings based on anemia in ESRD patients receiving hemodialysis

	Overall (n=106)	Anemia (+) (n=70)	Anemia (-) (n=36)	P value
LVDD (mm), median [IQR]	48 [44-50]	48 [45-50]	47.5 [44-50]	0.469
LVSD (mm), mean±SD	33.4±6.5	34.3±6.8	31.8±5.5	0.068
IVS (mm), median [IQR]	13 [12-15]	13 [12-15]	12 [11-14]	0.004
PW (mm), median [IQR]	13 [12-14]	13 [12-15]	12 [11-13.5]	<0.001
LA volume index, median [IQR]	39 [29.5-51]	43 [35-55]	34.7 [28-50]	0.013
Right ventricle(mm), median [IQR]	42 [36-46]	42 [36-46]	40 [35-46]	0.194
Right atrium (mm), median [IQR]	44 [36-49]	45 [37-49]	42 [35-47]	0.057
LVEF (%), median [IQR]	53 [48-57]	52 [48-55]	56 [47.5-60]	0.016
E, median [IQR]	84 [66.7-95]	83.5 [65-100]	85.5 [72-93]	0.947
A, median [IQR]	89.5 [72.8-104.8]	91 [75-115]	85.5 [68-90.5]	0.010
e', median [IQR]	14.65 [11-17.9]	14.5 [10.4-17.2]	15.7 [12.7-20]	0.0450
E/A, median [IQR]	0.88 [0.73-1.18]	0.85 [0.69-1.03]	1.01 [0.85-1.22]	0.027
E/e', median [IQR]	5.86 [4.10-7.56]	6.40 [4.10-7.72]	4.72 [4.35-6.47]	0.106
TAPSE, median [IQR]	16.2 [13.8-18.4]	15.2 [13.2-18.2]	18 [15.2-19]	0.004
S', median [IQR]	11.5 [10-12.9]	11 [9.9-12]	12 [11-13]	0.047
PASB, mean±SD	24.8±2.1	26.7±2.7	23.9±4.4	0.001
LASr, median [IQR]	-24 [-33- -16.8]	-21.6 [-30.5- -16.3]	-30.5 [-33.6- -23.3]	0.006
LVGLS, median [IQR]	-15.2 [-16.9- -13.6]	-14.6 [-16.4- -13.5]	-16 [-18.6- -14.7]	0.003

A=late diastolic filling mitral velocity, E=early diastolic filling mitral velocity, E/A=E to A raito, IVS=interventricular septum thickness, IQR=interquartile range, LA=left atrium, LASr=left atrial reservoir strain, LVDD=left ventricle end-diastolic diameter, LVEF=left ventricular ejection fraction, LVGLS=left ventricle global longitudinal strain, LVSD=left ventricle end-systolic diameter, RA=right atrium diameter, RV=right ventricle diameter, PASP=pulmonary arterial systolic pressure, SD=standard deviation, TAPSE=tricuspid annular plane systolic excursion, PW=left ventricular posterior wall thickness, e'=the peak early diastolic velocity of the mitral annulus by tissue Doppler, S'=tissue Doppler velocity of the basal free lateral wall of the right ventricle

significantly lower than those without anemia (mean±SD, 24.9±3.9 vs. 26.8±4.5, P=0.027). Lymphocyte was also significantly lower in patients with anemia [median (IQR), 1.27 (1-1.63) vs. 1.65 (1.05-1.96), P=0.039]. The remaining laboratory findings were similar between the two groups. Regarding comorbidities, the proportion of diabetes was significantly higher in patients with anemia than those without anemia (51% vs. 30.6%, P=0.040).

The comparison of echocardiographic findings in the patients based on the presence of anemia is presented in Table 2. Values of IVS, PW, and LAVi were significantly higher in patients with anemia than those without, while the LVEF was significantly lower. [median (IQR), 13 (12-15) vs. 12 (11-14), P=0.004, 13 (12-15) vs. 12 (11-13.5), P<0.005, 43 (35-55) vs. 34.7 (28-50), P=0.013, and 52 (48-55) vs. 56 (47.5-60), P=0.016, respectively]. Also, the A-wave was significantly higher in patients with anemia, and the E-wave was significantly lower [median (IQR), 91 (75-115) vs. 85.5 (68-90.5), P=0.010 and 6 (4.6-8.1) vs. 6.8 (5.2-9.9), P=0.024, respectively]. Respecting STE analysis, both LVGLS and LASr were significantly lower (less negative, which indicates worse function) the patients with anemia than those without anemia [median (IQR), -14.6 (-16.4- -13.5) vs. -16 (-18.6- -14.7, P=0.003 and -21.6 (-30.5- -16.3) vs. -30.5 (-33.6- -23.3), P=0.006, respectively]. Considering the right ventricular function, both TAPSE and S' were significantly lower in patients with anemia than in patients without anemia [median (IQR), 15.2 (13.2-18.2) vs. 18 (15.2-19), P=0.004 and 11 (9.9-12) vs. 12 (11-13), P=0.047, respectively]. The PASB was significantly higher in patients with anemia (mean ±SD 26.7±2.7 vs. 23.9±4.4, P=0.001). According to correlation analysis, hemoglobin level was significantly correlated with LVEF, LVGLS, LASr, and LAVi (r=0.019, P=0.041, r=0.139, P=0.001, r=0.121, and r=116, P<0.001, respectively) (Fig. 1).

In univariable logistic regression analysis, the fol-

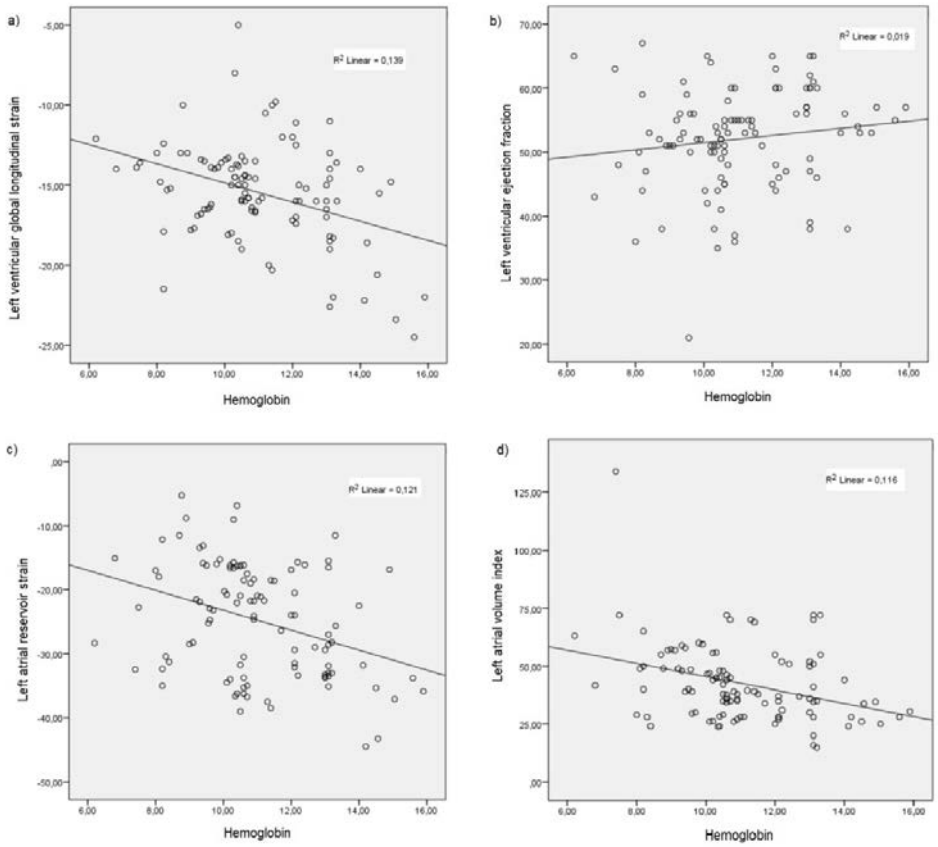

Fig. 1. The correlation between hemoglobin value and (a) LVGLS, (b) LVEF, (c) LASr, and (d) LAVi.LVEF=left ventricular ejection fraction, LVGLS=left ventricular global longitudinal strain, LASr=left atrial reservoir strain,LAVi=left atrial volume index

lowing parameters demonstrated a significant relationship with the presence of anemia: BMI, diabetes, IVS, PW, LAVi, E/A, TAPSE, LVEF, LVGLS, and LASr. In multivariable logistic regression analysis, diabetes, PW, LASr, and LVGLS were independently associated with the presence of anemia in ESRD patients receiving hemodialysis (Table 3).

When the findings were compared between hemodialysis patients and the healthy controls (Table 4), the hemoglobin value was significantly lower in the patients than in the healthy controls [median (IQR), 10.7 (9.8-12.4) vs. 14.7 (13.8-15.9), P<0.001]. Echocardiographic findings, including IVS, PW, and LAVi, were significantly higher in the patients than the control group [median (IQR), 13 (12-15 vs. 11 (11-11.75), 13 (12-14 vs. 11 (11-12), and 39 (29.6-51) vs. 34 (30-44.2), respectively, the P-value for all <0.001]. The medians of LVEF, LVGLS, LASr, TAPSE, and S' were significantly lower in the hemodialysis patients [median (IQR), 53 (48-57) vs. 65 (62-68), -15.2 (-16.9- -13.6) vs. -19.7 (-16.9- -13.6), -21.9 (-29.5- -15.3) vs. -29.9 (-35.3- -22.8), 16.2 (14.-18.2) vs. 21 (20-22) and 11.5 (10-12.8) vs. 12.9 (11.9-14.5), respectively, the P-value for all <0.001]. In the patients, the diameters of cardiac chambers were also significantly higher.

DISCUSSION

This study investigated the relationship between anemia and cardiac mechanics, including systolic and diastolic LV functions and LA and RV functions, using standard TTE and STE analysis in patients with ESRD receiving hemodialysis. First, our study confirmed the previous literature findings that cardiac mechanics in patients with ESRD were impaired compared to healthy controls. Of note, for the first time, in patients with ESRD receiving hemodialysis, we found that LAVi, IVS, and PW thickness were higher in patients with anemia than those without anemia. Moreover, using both TTE and STE, we observed that left atrial, left ventricular, and right ventricular functions were further impaired in patients with anemia. Finally, LV PW thickness, LASr, and LVGLS were independently associated with the presence of anemia in ESRD patients receiving hemodialysis. Our results provided robust evidence regarding the relationship between anemia and cardiac functions, specifically in patients with ESRD receiving hemodialysis.

LV hypertrophy and increased LV mass are well-established identifiable risk factors for cardiovascular events in patients with ESRD [9]. In early reports, cardiac hypertrophy has been reported in about 2/3 of pa-

Table 3. Univariable and multivariable analysis of the variables related to ESRD patients

	Univariate analysis		Multivariate analysis	
	OR (95% CI)	P value	OR (95% CI)	P value
BMI	0.89 (0.80-0.99)	**0.031**		
Diabetes	2.40 (1.03-5.63)	**0.043**	3.6 (1.3-10.3)	**0.017**
IVS	1.37 (1.08-1.74)	**0.010**		
PW	1.57 (1.2-2.08)	**0.001**	1.4 (1.03-1.9)	**0.031**
LAVi	1.04 (1.002-1.07)	**0.032**		
E/A	0.29 (0.09-0.97)	**0.044**		
TAPSE	0.90 (0.81-0.99)	**0.047**		
LVEF	0.95 (0.89-0.99)	**0.048**		
LASr	1.08 (1.03-1.14)	**0.003**	1.09 (1.03-1.16)	**0.005**
LVGLS	1.27 (1.09-1.48)	**0.002**	1.27 (1.06-1.51)	**0.008**

A=late diastolic filling mitral velocity, BMI=body mass index,E=early diastolic filling mitral velocity, E/A=E to A raito, IVS=interventricular septum thickness, LAVi=left atrial volume index, LASr=left atrial reservoir strain, LVGLS=left ventricle global longitudinal strain, LVEF=left ventricular ejection fraction, PW=left ventricular posterior wall thickness, TAPSE=tricuspid annular plane systolic excursion

Table 4. Comparison of the echocardiographic characteristics between hemodialysis patients and healthy controls

	Overall (n=174)	Hemodialysis (n=106)	Control (n=68)	P value
Age (years), median [IQR]	70.5 [62-77]	71 [62-77]	70 [62.5-76]	0.806
Male, sex, n (%)	105 (60.3)	66 (62.3)	39 (57.4)	0.518
BMI (kg/m^2) mean±SD	26.1+4.1	25.5+4.2]	27.1+3.9]	**0.012**
Hemoglobin (g/dL), median [IQR]	12.5 [10.5-14.4]	10.7 [9.8-12.4]	14.7 [13.8-15.9]	**<0.001**
Echocardiographic findings				
LVDD (mm), median [IQR]	47 [43-49]	48 [44-50]	44 [41-48]	**<0.001**
LVSD (mm), mean±SD	32 [29-35]	33 [30-37]	30 [28-35]	**0.001**
IVS (mm), median [IQR]	12 [11-14]	13 [12-15]	11 [11-11.75]	**<0.001**
PW (mm), median [IQR]	12 [11-13]	13 [12-14]	11 [11-12]	**<0.001**
LA volume index, median [IQR]	34 [30-44.2]	39 [29.6-51]	34 [30-44.2]	**<0.001**
RV (mm), median [IQR]	36 [33-43.3]	42 [36-46]	32 [29.5-34]	**<0.001**
RA (mm), median [IQR]	36 [34-46]	44 [36-49]	33 [31-36]	**<0.001**
LVEF (%), median [IQR]	59 [51-64.3]	53 [48-57]	65 [62-68]	**<0.001**
E/A, median [IQR]	1.1 [0.83-1.33]	0.88 [0.74-1.17]	1.3 [1.1-1.5]	**<0.001**
E/e', median [IQR]	6.2 [4.6-7.7]	5.9 [4.1-7.5]	6.4 [5.5-8]	**0.007**
TAPSE, median [IQR]	19 [15.2-21.4]	16.2 [14-18.2]	21 [20-22]	**<0.001**
S', median [IQR]	12 [11-13.7]	11.5 [10-12.8]	12.9 [11.9-14.5]	**<0.001**
LASr, median [IQR]	-24.9 [32.3- -17.7]	-21.9 [-29.5- -15.3]	-29.9 [-35.3- -22.8]	**<0.001**
LVGLS, median [IQR]	-17 [-19.8- -14.5]	-15.2 [-16.9- -13.6]	-19.7[-16.9- -13.6]	**<0.001**

A=late diastolic filling mitral velocity, BMI=body mass index, E=early diastolic filling mitral velocity, E/A=E to A raito, IVS=interventricular septum thickness, IQR=interquartile range, LA=left atrium, LASr=left atrial reservoir strain, LVDD=left ventricle end-diastolic diameter, LVEF=left ventricular ejection fraction, LVGLS=left ventricle global longitudinal strain, LVSD=left ventricle end-systolic diameter, RA=right atrium diameter, RV=right ventricle diameter, SD=standard deviation, TAPSE=tricuspid annular plane systolic excursion, PW=left ventricular posterior wall thickness,e'=the peak early diastolic velocity of the mitral annulus by tissue Doppler, S'=tissue Doppler velocity of the basal free lateral wall of the right ventricle

tients receiving renal replacement therapy [4, 14, 15]. In line with these findings, our results showed that hemodialysis patients had increased LV wall thicknesses compared to healthy controls. However, the association between anemia and hypertrophy has remained unknown in these patients. Our study observed a notable increase in LV posterior and septum thicknesses in hemodialysis patients with anemia than those without anemia. There is evidence that anemia, the most common complication of ESRD, increases the risk of cardiovascular diseases [9, 16]. Since anemia reduces the oxygen-carrying capacity of the blood, a greater cardiac output is needed to maintain a sufficient supply of oxygen. As anemia becomes severe, it leads to a hyperdynamic circulatory system and eventually may cause cardiac enlargement, hypertrophy, and dysfunction [9, 17]. These facts may explain the observed further advanced LV hypertrophy in anemic hemodialysis patients in our study.

Several studies have demonstrated that cardiac mechanics, including the left ventricular, right ventricular, and left atrial structural and functional characteristics, are altered in dialysis patients [18-20]. Some factors such as fluid retention, volume overload, anemia, and calcium phosphate metabolism abnormalities may provoke cardiac dysfunction and may lead to my-

ocardial changes in this population [21]. Studies using STE, which better detects subtle cardiac dysfunction, have also revealed LV systolic dysfunction in hemodialysis patients. According to previous reports, LVGLS was decreased in hemodialysis patients compared to healthy controls, although LVEF was similar [22, 23]. In parallel, our standard and STE echocardiography results showed that cardiac functions in hemodialysis patients were more impaired than in the healthy controls. Of note, previous works have not investigated the relationship between anemia and cardiac functions in the hemodialysis population. Our study addressed this important knowledge gap. Our study revealed that hemodialysis patients with anemia had further deteriorated cardiac functions than those without anemia. Notably, our study identified LASr, and LVGLS as independent markers for association with the presence of anemia in hemodialysis patients but not LVEF. This may be because strain measurement better reflects cardiac mechanics by directly assessing myocardial motion [24]. LVEF is more volume and cardiac-filling dependent. Thus, strain measurement may be a more accurate parameter for hemodialysis patients to follow-up cardiac mechanics, particularly in anemic patients. To our knowledge, only Bhagat *et al*.[25] investigated the association between anemia and cardiac mechanics in children with different stages of chronic kidney disease. They found a significant adverse change in standard echocardiographic findings, including chamber diameters, left ventricular systolic and diastolic functions, and right ventricular function in the anemic group. Unlike ours, their study performed cardiac function analysis using only conventional echocardiographic parameters such as LVEF and did not include the STE technique.

In patients receiving hemodialysis, due to pressure and volume overload, the left atrium is repeatedly subjected to abnormal filling pressure [23]. Dialysis may not be enough to prevent morphological and functional changes in atriums alongside ventricles. It was shown that LA characteristics, including maximum LA volume and LA active and passive emptying volumes, were higher in dialysis patients than in healthy subjects [26, 27]. Some studies showed subclinical LA dysfunction by STE in individuals with ESRF [28]. Our study found that conventional LV diastolic function parameters, including E/A and A wave and LA strain parameters, were more impaired in ESRD patients with anemia.

Our findings align with the literature regarding impaired cardiac mechanics in patients. Furthermore, our results by conventional and myocardial motion tracking techniques showed that cardiac mechanics were even further impaired in hemodialysis patients with anemia. Therefore, anemia may have a further pathophysiological adverse impact on the cardiac structures and functions in ESRD adult patients receiving hemodialysis.

Limitations

Our study had some limitations. Our study had a relatively small sample size. Because our research was a cross-sectional study, we cannot draw cause-and-effect connections from our results. Multicenter prospective analyses should verify the generalizability of these results. Other speckle tracking parameters to evaluate LV systolic function, such as global circumferential and radial strain, were not obtained.

CONCLUSION

Our study confirmed impaired cardiac mechanics in hemodialysis patients and showed that anemia was associated with further worsening cardiac mechanics in this population. These pathophysiological findings indicate the role of anemia in the alteration of cardiac mechanics in patients with ESRD receiving hemodialysis.

Authors' Contribution

Study Conception: TO, MA; Study Design: TO; Supervision: İR, MÇ; Funding: TO; Materials: MK; Data Collection and/or Processing, İA; Statistical Analysis and/or Data Interpretation: YK; Literature Review: MY, TO; Manuscript Preparation: TO and Critical Review: Dİ, AA.

Conflict of interest

The authors disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

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REFERENCES

1. Thurlow JS, Joshi M, Yan G, et al. Global epidemiology of end-stage kidney disease and disparities in kidney replacement therapy. Am J Nephrol. 2021;52(2):98-107. doi: 10.1159/000514550.
2. Levey AS, Beto JA, Coronado BE, et al. Controlling the epidemic of cardiovascular disease in chronic renal disease: what do we know? What do we need to learn? Where do we go from here? National Kidney Foundation Task Force on Cardiovascular Disease. Am J Kidney Dis. 1998;32(5):853-906. doi: 10.1016/s0272-6386(98)70145-3.
3. Foley RN, Parfrey PS, Sarnak MJ. Clinical epidemiology of cardiovascular disease in chronic renal disease. Am J Kidney Dis. 1998;32(5 Suppl 3): S112-9. doi: 10.1053/ajkd.1998.v32.pm9820470.
4. Jankowski J, Floege J, Fliser D, Böhm M, Marx N. Cardiovascular disease in chronic kidney disease: pathophysiological insights and therapeutic options. Circulation. 2021;143(11):1157-1172. doi: 10.1161/CIRCULATION AHA.120.050686.
5. Georgatzakou HT, Antonelou MH, Papassideri IS, Kriebardis AG. Red blood cell abnormalities and the pathogenesis of anemia in end-stage renal disease. Proteomics Clin Appl. 2016;10(8):778-790. doi: 10.1002/prca.201500127.
6. Sofue T, Nakagawa N, Kanda E, et al. Prevalence of anemia in patients with chronic kidney disease in Japan: a nationwide, cross-sectional cohort study using data from the Japan Chronic Kidney Disease Database (J-CKD-DB). PLoS One. 2020;15(7):e0236132. doi: 10.1371/journal.pone.0236132.
7. Omrani H, Golshani S, Sharifi V, Almasi A, Sadeghi M. The relationship between hemodialysis and the echocardiographic findings in patients with chronic kidney disease. Med Arch. 2016;70(5):328-331. doi: 10.5455/medarh.2016.70.328-331.
8. Parfrey PS, Harnett JD, Barre PE. The natural history of myocardial disease in dialysis patients. J Am Soc Nephrol. 1991;2(1):2-12. doi: 10.1681/ASN.V212.
9. Li S, Foley RN, Collins AJ. Anemia and cardiovascular disease, hospitalization, end stage renal disease, and death in older patients with chronic kidney disease. Int Urol Nephrol. 2005;37(2):395-402. doi: 10.1007/s11255-004-3068-2.
10. Eknoyan G, Lameire N, Eckardt K, et al. KDIGO 2012 Clinical Practice Guideline for the Evaluation and Management of Chronic Kidney Disease. Kidney Int Suppl. 2013;3(1):5-14. doi: 10.1038/kisup.2012.76.
11. McMurray JJV, Parfrey PS, Adamson JW, et al. Kidney disease: Improving global outcomes (KDIGO) anemia work group. KDIGO clinical practice guideline for anemia in chronic kidney disease. Kidney Int Suppl. 2012;2(4):279-335. doi: 10.1038/kisup.2012.37.
12. Recommendations for Cardiac Chamber Quantification by Echocardiography in Adults: An Update from the American Society of Echocardiography and the European Association of, Cardiovascular Imaging. Eur Heart J Cardiovasc Imaging. 2016;17(4):412. doi: 10.1093/ehjci/jew041.
13. Voigt JU, Pedrizzetti G, Lysyansky P, et al. Definitions for a common standard for 2D speckle tracking echocardiography: consensus document of the EACVI/ASE/Industry Task Force to standardize deformation imaging. J Am Soc Echocardiogr. 2015;28(2):183-193. doi: 10.1016/j.echo.2014.11.003.
14. Foley RN, Parfrey PS, Harnett JD, et al. Clinical and echocardiographic disease in patients starting end-stage renal disease therapy. Kidney Int. 1995;47(1):186-192. doi: 10.1038/ki.1995.22.
15. Hickson LJ, Negrotto SM, Onuigbo M, et al. Echocardiography Criteria for Structural Heart Disease in Patients With End-Stage Renal Disease Initiating Hemodialysis. J Am Coll Cardiol. 2016;67(10):1173-1182. doi: 10.1016/j.jacc.2015.12.052.
16. Sikole A, Polenakovic M, Spirovska V, Polenakovic B, Masin G. Analysis of heart morphology and function following erythropoietin treatment of anemic dialysis patients. Artif Organs. 1993;17(12):977-984. doi: 10.1111/j.1525-1594.1993.tb03179.x.
17. Levin A, Thompson CR, Ethier J, et al. Left ventricular mass index increase in early renal disease: impact of decline in hemoglobin. Am J Kidney Dis. 1999;34(1):125-134. doi: 10.1016/s0272-6386(99)70118-6.
18. Bansal N, Keane M, Delafontaine P, et al; CRIC Study Investigators. A longitudinal study of left ventricular function and structure from CKD to ESRD: the CRIC study. Clin J Am Soc Nephrol. 2013;8(3):355-362. doi: 10.2215/CJN.06020612.
19. Gulel O, Soylu K, Yuksel S, et al. Evidence of left ventricular systolic and diastolic dysfunction by color tissue Doppler imaging despite normal ejection fraction in patients on chronic hemodialysis program. Echocardiography. 2008;25(6):569-574. doi: 10.1111/j.1540-8175.2008.00657.x.
20. De Lima JJG, Macedo TA, Gowdak LHW, David-Neto E, Bortolotto LA. Diastolic and systolic left ventricular dysfunction and mortality in chronic kidney disease patients on haemodialysis. Nephrology (Carlton). 2022;27(1):66-73. doi: 10.1111/nep.13960.
21. Schärer K, Schmidt KG, Soergel M. Cardiac function and structure in patients with chronic renal failure. Pediatr Nephrol. 1999;13(9):951-965. doi: 10.1007/s004670050737.
22. Liu YW, Su CT, Huang YY, et al. Left ventricular systolic strain in chronic kidney disease and hemodialysis patients. Am J Nephrol. 2011;33(1):84-90. doi: 10.1159/000322709.
23. Chen R, Wu X, Shen LJ, et alLeftventricularmyocardialfunction in hemodialysis and non-dialysis uremia patients: a three-dimensional speckle-tracking echocardiography study. PLoSOne. 2014;9(6):e100265. doi: 10.1371/journal.pone.0100265.
24. Cameli M, Mandoli GE, Sciaccaluga C, Mondillo S. Morethan 10 years of speckle tracking echocardiography: Still a novel technique or a definite tool for clinical practice? Echocardiography. 2019;36(5):958-970. doi: 10.1111/echo.14339.
25. Bhagat N, Dawman L, Naganur S, et al. Impact of anemia on the cardiovascular status in children with chronic kidney disease: a pilot study. Clin Nutr ESPEN. 2022;47:283-287. doi: 10.1016/j.clnesp.2021.11.031.
26. Sulemane S, Panoulas VF, Nihoyannopoulos P. Echocardiographic assessment in patients with chronic kidney disease: current update. Echocardiography. 2017;34(4):594-602. doi: 10.1111/echo.13495.
27. Demirtas L, Turkmen K, Buyuklu M, Kocyigit I, Orscelik O. Atrial electromechanical delay and left atrial mechanical functions in hemodialysis and peritoneal dialysis patients. Int Urol Nephrol. 2016;48(5):781-789. doi: 10.1007/s11255-016-1238-7.
28. Calleja AM, Rakowski H, Williams LK, Jamorski M, Chan CT, Carasso S. Left atrial and ventricular systolic and diastolic myocardial mechanics in patients with end-stage renal disease. Echocardiography. 2016;33(10):1495-1503. doi: 10.1111/echo.13284.

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The use of mobile health applications in empowering self-management of type 2 diabetes: a literature review

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ABSTRACT

The use of mobile health applications is vital for achieving glycemic control, promoting lifestyle changes, and empowering self-management in individuals with type 2 diabetes. This literature review aims to assess the effectiveness of mobile health applications in empowering self-management among type 2 diabetes patients. A thorough search was conducted in databases like PubMed, CINAHL, Web of Science, Cochrane Library, Scopus, ULAKBIM National Database, and Medline between January 2020 and March 2023, using keywords such as "mobile health, mobile applications, type 2 diabetes, diabetes self-management, nursing." The search yielded 525 articles, out of which 34 studies in Turkish or English that evaluated the effectiveness of mobile health applications in individuals aged 18 years and above with type 2 diabetes were included in the review. Recent studies demonstrate a growing utilization of mobile health applications for the management of treatment and care in individuals with type 2 diabetes. These applications have been shown to empower self-management by promoting dietary adherence, regular blood sugar monitoring, regular physical activity, reduced medication requirements, and decreased HbA1c levels. Additionally, mobile health applications have been found to reduce face-to-face counseling time and healthcare costs. In conclusion, mobile health applications offer promising solutions for improving self-management and healthcare outcomes for individuals with type 2 diabetes. Further research and continued integration of these applications into clinical practice are essential to optimize their benefits and address the challenges faced by diabetes patients worldwide.

Keywords: Diabetes self-management, nursing, mobile applications, mobile health, type 2 diabetes

Diabetes Mellitus is one of the most prevalent chronic diseases today, and its prevalence continues to increase. Type 2 diabetes constitutes approximately 90% of the entire diabetic population. When type 2 diabetes is poorly managed, complications such as coronary heart disease, stroke, kidney failure, retinopathy, and foot ulcers can occur. These complications progressively lead to significant morbidity and mortality rates over time [1]. Unhealthy lifestyle factors play a significant role in the pathogenesis of type 2 diabetes. Therefore, one of the key elements in preventing and treating diabetes is promoting and adopting healthy lifestyle changes in individuals [2].

Due to the lack of a definitive cure for type 2 diabetes, the concept of self-management plays a vital role throughout the lives of individuals with diabetes.

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Self-management is a term used to define individuals' responsibilities in maintaining and sustaining their health. It involves enabling individuals to control their blood glucose levels, which is considered the most crucial factor in preventing diabetes complications [3]. Self-management also promotes individuals' autonomy, facilitates disease monitoring outside clinical settings, and has the potential to reduce the burden on the healthcare system [4]. Self-management strategies for type 2 diabetes encompass multidimensional components such as regular blood sugar monitoring and recording, healthy lifestyle changes including proper diet, regular physical activity, smoking cessation, weight management, stress coping mechanisms, adherence to pharmacological treatment, and education. Studies have shown that medical nutrition therapy and regular physical activity contribute to achieving glycemic control and improving the quality of life in individuals with type 2 diabetes [5-7]. Despite the significant role of self-management in the treatment and care of individuals with type 2 diabetes, it has been reported that individuals generally have low levels of self-management. This low level of self-management results in fluctuations in blood sugar levels, increasing the risk of complications and reducing individuals' quality of life [8].

The increasing prevalence of type 2 diabetes worldwide and the growing number of affected patients necessitate the development of new strategies for diabetes treatment, adherence to treatment, lifestyle changes, and care. The rising prevalence of type 2 diabetes puts pressure on healthcare systems to enable individuals to manage their conditions effectively [9]. Consequently, numerous mobile health (mHealth) applications have been designed to enhance self-management of type 2 diabetes as a new strategy [10].

The World Health Organization (WHO) recognizes mHealth as a component of electronic health. According to WHO, mHealth is defined as "the use of mobile communication devices for the delivery of health services" [11]. mHealth involves the transmission and structuring of health information through mobile communication and multimedia technologies, such as mobile phones, computers, and wireless communication infrastructure [11]. A report published by the Health Information Institute in 2015 stated that the number of mHealth applications available to consumers has exceeded 165,000 [12]. The majority of existing mHealth applications focus on promoting healthy lifestyles, diet, and exercise. The fact that approximately one-fourth of existing mHealth applications are intended for the management of chronic diseases indicates the increasing interest in their use [13]. A study investigating the use of mHealth applications for health search behavior reported that 36% of individuals with smartphones or tablets use mHealth applications. It further revealed that 60% of individuals who use these applications find them helpful in achieving health-related goals, 35% consider them useful for making decisions about medical care, and 38% find them valuable for asking questions or seeking advice from healthcare professionals [14].

Currently, there are many mHealth applications available for enhancing self-management in individuals with diabetes. These applications enable individuals to be closely monitored, receive feedback, and overcome geographic barriers. The use of mHealth applications facilitates the delivery of healthcare services outside clinical settings [13]. mHealth applications have been integrated into various aspects of daily life and are increasingly being utilized for disease management. Literature indicates the existence of over 2,000 applications that can be used for diabetes self-management [14, 15]. Compared to web-based applications, mHealth applications are considered more accessible, cost-effective, and convenient in terms of accessing information due to their enhanced technological features such as portability, increased interaction with healthcare professionals, compatibility with other devices, and ease of data collection [16]. A systematic review and meta-analysis published in the literature demonstrated the effectiveness of mHealth applications in empowering self-management across a wide range of disease management and lifestyle change areas [2]. The American Diabetes Association's guidelines also suggest that mHealth applications can be a beneficial component in achieving effective lifestyle changes for diabetes prevention [1].

The empowerment of self-management in individuals with type 2 diabetes is important from the perspective of healthcare professionals. However, due to the limited number of medical appointments that can be provided, healthcare professionals are often only able to assess and encourage diabetes self-manage-

ment a few times a year. Moreover, the increasing number of individuals with type 2 diabetes and the inadequate number of healthcare professionals in some countries necessitate innovative and cost-effective solutions, such as mHealth applications, to promote self-management [17]. The use of mHealth applications allows continuous contact with nurses, and nurses play educational and organizational roles in this field. Additionally, mHealth applications contribute to individuals' autonomous living and minimize their need for prolonged hospital stays [9].

To enhance self-management in individuals with type 2 diabetes, continuous and regular education is necessary. It has been observed that routine follow-ups and support for self-management are insufficient due to the heavy workload in hospital settings. The need for support in home environments has become even more apparent due to the recent pandemic, both globally and in our country. This increased need further emphasizes the importance of conducting studies in this field. The aim of this literature review is to determine the effectiveness of mHealth applications used in empowering self-management of type 2 diabetes.

METHODS

This literature review conducted a search in the PubMed, CINAHL, Web of Science, Cochrane Library, Scopus, ULAKBİM National Database, and Medline databases between March 2020 and May 2023 without any year limitations. The search was performed using Turkish and English keywords such as "mobile health, mobile applications, type 2 diabetes, diabetes self-management, nursing." As a result of the literature search, a total of 525 articles were identified using the specified keywords. The titles and abstracts of the identified articles were individually examined, and their subject content, which evaluated the effectiveness of mHealth applications in enhancing self-management of individuals with type 2 diabetes, were independently evaluated by the researchers. Based on this evaluation, a total of 34 studies including systematic reviews, meta-analyses, and randomized controlled trials, which were published between 2020 and 2023, were included in the literature review.

RESULTS

In recent years, mobile health (mHealth) applications have been increasingly used in the care of individuals with Type 2 diabetes. Studies in the literature have shown that mHealth applications used in Type 2 diabetes self-management have a positive impact on individuals' health outcomes [8-23]. The findings obtained from the literature review were grouped and analyzed based on the effects of mHealth applications on patient outcomes, their impact on self-management behaviors, and the factors influencing the use of mHealth applications.

Impact of Mobile Health Applications On Patient Outcomes

Studies have shown that mHealth applications have positive effects on patient outcomes such as HbA1c levels, blood pressure, lipid profile, self-efficacy, quality of life, utilization of health services, and patient satisfaction. Research studies in the literature have demonstrated that mHealth applications used for diabetes self-management significantly reduce HbA1c levels in individuals [24-26]. In a meta-analysis conducted by Cui *et al*. [2], it was observed that individuals using mHealth applications had a significant 40% reduction in HbA1c levels compared to standard diabetes care ($P<0.05$). No statistically significant difference was found in blood pressure, lipid profile, or weight loss [2]. Another meta-analysis by Wu *et al*. [4] reported that mHealth applications assist in strengthening self-management in individuals with Type 2 diabetes and result in a clinically significant reduction in HbA1c levels ($P<0.05$) [4]. A randomized controlled study involving 163 patients with Type 2 diabetes found that a comprehensive mHealth application reduced HbA1c levels by 1.9% ($P<0.05$) in the intervention group [27]. Koot *et al*. [23] conducted a study examining the effect of mHealth applications on HbA1c levels and found that 59% of application users experienced a decrease of ≥1 in their HbA1c levels ($P<0.001$). Another meta-analysis indicated that the use of mHealth applications for diabetes self-management resulted in an average reduction of 0.5% in HbA1c levels over a 6-month follow-up period ($P<0.001$) [24]. A 1% decrease in HbA1c levels leads

to a 21% reduction in mortality rate, a 14% decrease in myocardial infarction, and a 37% reduction in the risk of developing microvascular complications [24]. Other studies have also identified a statistically significant difference in HbA1c levels between intervention and control groups, indicating the effectiveness of mHealth applications [28, 29]. The reduction in HbA1c levels among individuals with diabetes is attributed to the interaction and feedback between individuals and healthcare professionals, the intensity of self-management interventions in applications, and the effective use of techniques to promote healthy lifestyle changes [2, 16].

However, alongside studies demonstrating the significant reduction in HbA1c levels achieved by mHealth applications for diabetes self-management, there are also studies indicating no statistically significant difference. Agarwal *et al*. [10] found no statistically significant difference in HbA1c levels between the intervention and control groups (P>0.05). Additionally, no significant impact of mHealth applications was observed on secondary outcomes such as self-efficacy, quality of life, and utilization of health services. This may be attributed to inadequate utilization of the application, as nearly half of the participants in the intervention group had minimal interaction with the application. It was noted that many features of the applications, including diet and exercise tracking, which have been shown to play a significant role in Type 2 diabetes self-management, were underutilized [10].

In addition to studies demonstrating the benefits of mHealth applications, there are also studies evaluating the interest and satisfaction of individuals with Type 2 diabetes regarding these applications [30, 31]. It has been found that the recommendation of health applications by healthcare professionals and interaction with them are factors that increase individuals' satisfaction. Individuals also express their belief that the use of mHealth applications enhances interaction between themselves and healthcare professionals. One participant in the study conducted by Lie *et al*. [32] expressed their interaction with healthcare professionals as follows: "Previously, after routine tests during standard care, I used to leave without having time to express my emotions and thoughts. However, with these applications, I can finally communicate with healthcare professionals on this matter." A study by Veazie *et al*. [33] suggested that when mHealth applications are associated with healthcare professional support, they can have a greater impact on diabetes-related outcomes, especially HbA1c levels. A study examining the feedback systems in applications indicated differences between automated feedback and personalized feedback provided by healthcare professionals. While automated feedback offers the advantage of being interactive and dynamic, it is believed to have limitations in predefined pathways. Personalized feedback provided by healthcare professionals is considered more individualized and valuable, particularly in emergency situations [26].

In a study comparing feedback-receiving and non-feedback-receiving groups among individuals using mHealth applications, it was found that individuals receiving feedback through the application engaged in more physical activity after a 3-month follow-up period, with an average daily difference of 10.59 minutes compared to the non-feedback group ($p < 0.001$) [34]. Georgsson and Staggers [35] reported that individuals who used the mHealth application perceived the benefits of receiving feedback and indicated positive lifestyle changes after using the application. The majority of participants in the study stated that mHealth applications were useful for healthcare services and mentioned personal advantages associated with their use. They also emphasized that mHealth applications facilitated disease monitoring and management and supported interaction with healthcare professionals. Participants stated that the use of mHealth applications reduced their need for medical appointments and recommended the applications to others [35]. In another study, individuals reported that receiving weekly SMS reminders related to their self-management would enhance their effective use of the application [22]. Koot *et al*. [23] found that individuals using the application were generally satisfied with it and would recommend it to others for diabetes self-management.

Impact of Mobile Health Applications On Self-Management Behaviors

Studies have indicated that mHealth applications have positive contributions to self-management behaviors such as healthy lifestyle changes, physical activity, and medication adherence. It has been emphasized that healthy lifestyle changes, including the implementation of a healthy diet, increased physical activity, and regular blood sugar monitoring, which are essential

for diabetes self-management, significantly improve with the use of mHealth applications. Regular blood sugar monitoring is particularly beneficial for individuals with high blood sugar levels who struggle to control them. Additionally, mHealth applications are reported to support and guide individuals in achieving healthy lifestyle changes, including individuals at risk of developing prediabetes [36]. Koot *et al*. [23] found that the use of mHealth applications significantly increased the rate of regular blood sugar monitoring among individuals with Type 2 diabetes (P<0.001). Furthermore, 68 out of 80 participants (85%) reported positive changes in their diets after using the mHealth application (P<0.001). It was determined that individuals using the application consumed the recommended amounts of vegetables and fruits, avoided fatty foods (P<0.001), lost an average of 2.3 kilograms, and 20% of individuals achieved a weight loss of ≥5% of their initial body weight (P<0.001) [23]. A meta-analysis by Cui *et al*. [2] concluded that mHealth applications have a moderate effect on adopting healthy lifestyle changes, including daily physical activity and medication requirements.

The use of mHealth applications for strengthening Type 2 diabetes self-management has been shown to increase the level of physical activity among individuals. In a study by van der Weegen *et al*. [34], individuals using a self-management-based mHealth application engaged in more physical activity compared to the standard care group (P<0.001). In the study conducted by Koot *et al*. [23], although 30 out of 80 participants (38%) reported an increase in their weekly physical activity levels due to the use of the mHealth application, there was no statistically significant difference in the number of days with at least 30 minutes of physical activity compared to the control group (P>0.05).

mHealth applications used for strengthening diabetes self-management include various reminders for blood sugar, insulin treatment, nutrition, and physical activity measurement and recording. It is stated that all types of reminders included in these applications are effective in improving medication adherence among individuals with Type 2 diabetes. Studies have shown that receiving reminder messages through SMS and being monitored encourage medication adherence [37, 38]. In a study by Huang *et al*. [39], it was found that more than half of the included studies had medication reminder features, 16.8% had medication adherence monitoring features, 5.6% provided medication information, and 4.2% sent motivational messages to encourage medication intake. Similarly, Martinez *et al*. [40] found that fewer than half of the mHealth applications included features for medication adherence monitoring. Although many applications have comprehensive feature lists, including calorie tracking and cloud backup, it was determined that medication reminders were not present in mHealth applications despite forgetfulness being a significant non-adherence factor. Therefore, it is emphasized that more importance should be given to the design of medication management features in mHealth applications, and further evidence-based research should be conducted to improve medication adherence among individuals with diabetes [39].

Existing studies demonstrate that mHealth applications are feasible tools for improving individuals' self-management [26, 36]. The use of mHealth applications has been shown to result in positive self-management behaviors such as adherence to TBT, increased physical activity, and regular blood sugar monitoring [36, 41]. A study by Adu *et al*. [42] found significant improvements in TBT adherence, blood glucose levels, and self-management skills among individuals using the mHealth application (P<0.05). Moreover, after the intervention, the skills and self-efficacy of individuals in the intervention group increased significantly (P<0.05). Participants stated that the application provided motivation to enhance their self-management and encouraged their engagement in various aspects of self-management, such as regular blood sugar monitoring, healthy eating, and physical activity [42]. In a study examining the effects of an mHealth application-based continuous care, Wang *et al*. [8] found that individuals in the intervention group had increased awareness levels and improved self-management skills compared to the control group (P<0.05). Furthermore, when compared to the control group, the intervention group had significantly reduced readmission frequencies and lower hospital readmission rates within 6 months after discharge (P<0.05) [8]. Puzozerov *et al*. [43] determined that mHealth applications encouraged individuals to engage in regular blood sugar monitoring and TBT adherence, gradually strengthening their self-management skills. Similarly, Hoppe *et al*. [44]

reported that nursing care-based mHealth applications significantly improved individuals' self-management skills. In a study by Torbjørnsen *et al*. [45], a positive relationship was found between high self-management ability and mHealth applications, indicating that the applications were beneficial for treatment adherence.

In a cross-sectional study on the current use of mHealth applications in Australia, it was found that the use of multiple functions in mHealth applications, such as monitoring blood glucose levels, setting reminders, and accessing information on nutrition and exercise, was associated with increased self-management behaviors. Furthermore, it was observed that individuals who received recommendations from healthcare professionals were more likely to use mHealth applications for diabetes self-management [46]. The study conducted by Carroll and Richardson [47] revealed that individuals who used mHealth applications to manage their diabetes reported improved self-management behaviors, including increased physical activity, healthy eating, blood glucose monitoring, and medication adherence.

Factors Influencing The Use of Mobile Health Applications

The utilization of mHealth applications for Type 2 diabetes self-management is influenced by various factors, including individual characteristics, technological factors, social factors, and healthcare system-related factors. Individual characteristics, such as age, gender, education level, digital literacy, and motivation, play a significant role in the adoption and use of mHealth applications. It has been found that younger individuals, females, and those with higher education levels are more likely to use mHealth applications for self-management [10, 11, 16, 48]. Studies have also shown that individuals with higher levels of digital literacy and motivation have a higher likelihood of using mHealth applications [48]. In a study by Lie *et al*. [32], it was determined that older individuals had less confidence in using mHealth applications and felt that these applications were time-consuming. Similarly, Agarwal *et al*. [10] found that older individuals had difficulty using mHealth applications due to factors such as unfamiliarity with smartphones, visual impairment, and difficulty in understanding the application's functions. Therefore, it is important to consider individual characteristics when designing and implementing mHealth applications to ensure inclusiveness and accessibility for all user groups.

Technological factors, including the usability, design, and functionality of mHealth applications, influence individuals' adoption and continued use of these applications. Studies have highlighted the importance of user-friendly interfaces, intuitive navigation, clear instructions, and compatibility with different devices and operating systems [10, 16, 31, 48]. It has been noted that mHealth applications should provide personalized and tailored features to meet individual needs and preferences [10, 16]. Furthermore, the integration of mHealth applications with other technologies, such as wearable devices and sensors, can enhance their effectiveness in diabetes self-management [16].

Social factors also impact the use of mHealth applications for Type 2 diabetes self-management. Peer support and social interactions facilitated through mHealth applications have been found to positively influence individuals' engagement and adherence to self-management behaviors [16, 49]. In a study by Schnall *et al*. [49], it was observed that individuals who used a social networking-based mHealth application for diabetes self-management reported increased motivation and support from peers. Similarly, Osborn *et al*. [16] found that social support and encouragement from family and friends influenced individuals' engagement with mHealth applications and their self-management behaviors.

Healthcare system-related factors, including healthcare professionals' recommendations, support, and integration of mHealth applications into clinical practice, play a crucial role in the adoption and sustained use of these applications. Studies have shown that healthcare professionals' endorsement and encouragement of mHealth applications can increase individuals' trust and confidence in using them [10, 31, 32]. Moreover, healthcare professionals' involvement in the monitoring and feedback process through mHealth applications can enhance individuals' adherence to self-management behaviors [26, 34]. However, challenges related to the integration of mHealth applications into clinical workflows, data privacy and security concerns, and reimbursement issues need to be addressed for successful implementation in healthcare settings [31, 50].

CONCLUSION

The use of mHealth applications for Type 2 diabetes self-management has shown promising results in improving patient outcomes, self-management behaviors, and patient satisfaction. These applications have been found to significantly reduce HbA1c levels, improve medication adherence, facilitate healthy lifestyle changes, and support regular blood sugar monitoring. Factors such as individual characteristics, technological factors, social factors, and healthcare system-related factors influence the adoption and continued use of mHealth applications. To maximize the effectiveness of mHealth applications, it is crucial to consider user preferences, ensure usability and compatibility, promote social support and interaction, and integrate these applications into clinical practice with the support and guidance of healthcare professionals. Further research is needed to explore the long-term effects, cost-effectiveness, and scalability of mHealth applications for type 2 diabetes self-management.

Authors' Contribution

Study Conception: PÖ; Study Design: PÖ; Supervision: PÖ; Funding: N/A; Materials: N/A; Data Collection and/or Processing: PÖ; Statistical Analysis and/or Data Interpretation: PÖ; Literature Review: PÖ; Manuscript Preparation: PÖ and Critical Review: PÖ.

Conflict of interest

The author disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. American Diabetes Association. Standards of medical care in diabetes-2020. Diabetes Care. 2020;43:S14-31.
2. Cui M, Wu X, Mao J, Wang X, Nie M. T2DM Self-Management via Smartphone Applications: A Systematic Review and Meta-Analysis. PLoS One. 2016;11(11):e0166718. doi: 10.1371/journal.pone.0166718.
3. Blondon K, Klasnja P, Coleman K, Pratt W. An exploration of attitudes toward the use of patient incentives to support diabetes self-management. Psychol Health. 2014;29(5):552-563. doi: 10.1080/08870446.2013.867346.
4. Wu IXY, Kee JCY, Threapleton DE, et al. Effectiveness of smartphone technologies on glycaemic control in patients with type 2 diabetes: systematic review with meta-analysis of 17 trials. Obes Rev. 2018;19(6):825-838. doi: 10.1111/obr.12669.
5. Moncrieft AE, Llabre MM, McCalla JR, et al. Effects of a Multicomponent Life-Style Intervention on Weight, Glycemic Control, Depressive Symptoms, and Renal Function in Low-Income, Minority Patients With Type 2 Diabetes: Results of the Community Approach to Lifestyle Modification for Diabetes Randomized Controlled Trial. Psychosom Med. 2016;78(7):851-860. doi: 10.1097/PSY.0000000000000348.
6. Whitehead LC, Crowe MT, Carter JD, et al. A nurse-led interdisciplinary approach to promote self-management of type 2 diabetes: a process evaluation of post-intervention experiences. J Eval Clin Pract. 2017;23(2):264-271. doi: 10.1111/jep.12594.
7. Wu Y, Yao X, Vespasiani G, et al. Mobile App-Based Interventions to Support Diabetes Self-Management: A Systematic Review of Randomized Controlled Trials to Identify Functions Associated with Glycemic Efficacy. JMIR Mhealth Uhealth. 2017;5(3):e35. doi: 10.2196/mhealth.6522.
8. Wang Y, Li M, Zhao X, et al. Effects of continuous care for patients with type 2 diabetes using mobile health application: A randomised controlled trial. Int J Health Plann Manage. 2019;34(3):1025-1035. doi: 10.1002/hpm.2872.
9. Klösch M, Klösch C, Kundt FS, van der Zee-Neuen A, Dieplinger AM. eHealth systems for the optimised care of patients with type 2 diabetes. Br J Nurs. 2020;29(5):274-278. doi: 10.12968/bjon.2020.29.5.274.
10. Agarwal P, Mukerji G, Desveaux L, et al. Mobile App for Improved Self-Management of Type 2 Diabetes: Multicenter Pragmatic Randomized Controlled Trial. JMIR Mhealth Uhealth. 2019;7(1):e10321. doi: 10.2196/10321.
11. World Health Organization. mHealth: New Horizons for Health Through Mobile Technologies: Second Global Survey on eHealth. Global Observatory for eHealth series. Volume 3, 2011.
12. Aitken M, Lyle J. Patient adoption of mHealth: Use, Evidence and Remaining Barriers to Mainstream Acceptance. Parsippany, New York: IMS Institute for Healthcare Informatics; 2015.
13. Brzan PP, Rotman E, Pajnkihar M, Klanjsek P. Mobile Applications for Control and Self Management of Diabetes: A Systematic Review. J Med Syst. 2016;40(9):210. doi: 10.1007/s10916-016-0564-8.
14. Bhuyan SS, Lu N, Chandak A, et al. Use of Mobile Health Applications for Health-Seeking Behavior Among US Adults. J Med Syst. 2016;40(6):153. doi: 10.1007/s10916-016-0492-7.
15. Huang Z, Lum E, Car J. Medication Management Apps for Diabetes: Systematic Assessment of the Transparency and Reliability of Health Information Dissemination. JMIR Mhealth Uhealth. 2020;8(2):e15364. doi: 10.2196/15364.
16. Osborn J, Ajakaiye A, Cooksley T, Subbe CP. Do mHealth applications improve clinical outcomes of patients with cancer? A critical appraisal of the peer-reviewed literature. Support Care Cancer. 2020;28(3):1469-1479. doi: 10.1007/s00520-019-04945-4.
17. Boels AM, Rutten G, Zuithoff N, de Wit A, Vos R. Effectiveness of diabetes self-management education via a smartphone ap-

plication in insulin treated type 2 diabetes patients - design of a randomised controlled trial ('TRIGGER study'). BMC Endocr Disord. 2018;18(1):74. doi: 10.1186/s12902-018-0304-9.
18. Holmen H, Torbjørnsen A, Wahl AK, et al. A Mobile Health Intervention for Self-Management and Lifestyle Change for Persons With Type 2 Diabetes, Part 2: One-Year Results From the Norwegian Randomized Controlled Trial RENEWING HEALTH. JMIR Mhealth Uhealth. 2014;2(4):e57. doi: 10.2196/mhealth.3882.
19. Whitehead L, Seaton P. The Effectiveness of Self-Management Mobile Phone and Tablet Apps in Long-term Condition Management: A Systematic Review. J Med Internet Res. 2016;18(5):e97. doi: 10.2196/jmir.4883.
20. Munster-Segev M, Fuerst O, Kaplan SA, Cahn A. Incorporation of a Stress Reducing Mobile App in the Care of Patients With Type 2 Diabetes: A Prospective Study. JMIR Mhealth Uhealth. 2017;5(5):e75. doi: 10.2196/mhealth.7408.
21. Kumar S, Moseson H, Uppal J, Juusola JL. A Diabetes Mobile App With In-App Coaching From a Certified Diabetes Educator Reduces A1C for Individuals With Type 2 Diabetes. Diabetes Educ. 2018;44(3):226-236. doi: 10.1177/0145721718765650.
22. Jeffrey B, Bagala M, Creighton A, et al. Mobile phone applications and their use in the self-management of Type 2 Diabetes Mellitus: a qualitative study among app users and non-app users. Diabetol Metab Syndr. 2019;11:84. doi: 10.1186/s13098-019-0480-4.
23.Koot D, Goh PSC, Lim RSM, et al. A Mobile Lifestyle Management Program (GlycoLeap) for People With Type 2 Diabetes: Single-Arm Feasibility Study. JMIR Mhealth Uhealth. 2019;7(5):e12965. doi: 10.2196/12965.
24. Liang X, Wang Q, Yang X, et al. Effect of mobile phone intervention for diabetes on glycaemic control: a meta-analysis. Diabet Med. 2011;28(4):455-463. doi: 10.1111/j.1464-5491.2010.03180.x.
25. Desveaux L, Agarwal P, Shaw J, et al. A randomized wait-list control trial to evaluate the impact of a mobile application to improve self-management of individuals with type 2 diabetes: a study protocol. BMC Med Inform Decis Mak. 2016;16(1):144. doi: 10.1186/s12911-016-0381-5.
26. Hou C, Carter B, Hewitt J, Francisa T, Mayor S. Do Mobile Phone Applications Improve Glycemic Control (HbA1c) in the Self-management of Diabetes? A Systematic Review, Meta-analysis, and GRADE of 14 Randomized Trials. Diabetes Care. 2016;39(11):2089-2095. doi: 10.2337/dc16-0346.
27. Quinn CC, Shardell MD, Terrin ML, Barr EA, Ballew SH, Gruber-Baldini AL. Cluster-randomized trial of a mobile phone personalized behavioral intervention for blood glucose control. Diabetes Care. 2011;34(9):1934-42. doi: 10.2337/dc11-0366.
28. Waki K, Fujita H, Uchimura Y, et al. DialBetics: A Novel Smartphone-based Self-management Support System for Type 2 Diabetes Patients. J Diabetes Sci Technol. 2014;8(2):209-215. doi: 10.1177/1932296814526495.
29. Zhou W, Chen M, Yuan J, Sun Y. Welltang - A smart phone-based diabetes management application - Improves blood glucose control in Chinese people with diabetes. Diabetes Res Clin Pract. 2016;116:105-110. doi: 10.1016/j.diabres.2016.03.018.
30. Humble JR, Tolley EA, Krukowski RA, Womack CR, Motley TS, Bailey JE. Use of and interest in mobile health for diabetes self-care in vulnerable populations. J Telemed Telecare. 2016;22(1):32-38. doi: 10.1177/1357633X15586641.
31. Pludwinski S, Ahmad F, Wayne N, Ritvo P. Participant experiences in a smartphone-based health coaching intervention for type 2 diabetes: A qualitative inquiry. J Telemed Telecare. 2016 Apr;22(3):172-178. doi: 10.1177/1357633X15595178.
32. Lie SS, Karlsen B, Oord ER, Graue M, Oftedal B. Dropout From an eHealth Intervention for Adults With Type 2 Diabetes: A Qualitative Study. J Med Internet Res. 2017;19(5):e187. doi: 10.2196/jmir.7479.
33. Veazie S, Winchell K, Gilbert J, et al. Rapid Evidence Review of Mobile Applications for Self-management of Diabetes. J Gen Intern Med. 2018;33(7):1167-1176. doi: 10.1007/s11606-018-4410-1.
34. van der Weegen S, Verwey R, Spreeuwenberg M, Tange H, van der Weijden T, de Witte L. It's LiFe! Mobile and Web-Based Monitoring and Feedback Tool Embedded in Primary Care Increases Physical Activity: A Cluster Randomized Controlled Trial. J Med Internet Res. 2015;17(7):e184. doi: 10.2196/jmir.4579.
35. Georgsson M, Staggers N. Patients' Perceptions and Experiences of a mHealth Diabetes Self-management System. Comput Inform Nurs. 2017;35(3):122-130. doi: 10.1097/CIN.0000000000000296.
36. El-Gayar O, Timsina P, Nawar N, Eid W. Mobile applications for diabetes self-management: status and potential. J Diabetes Sci Technol. 2013;7(1):247-262. doi: 10.1177/193229681300700130.
37. Vervloet M, Linn AJ, van Weert JC, de Bakker DH, Bouvy ML, van Dijk L. The effectiveness of interventions using electronic reminders to improve adherence to chronic medication: a systematic review of the literature. J Am Med Inform Assoc. 2012;19(5):696-704. doi: 10.1136/amiajnl-2011-000748.
38. Dayer L, Heldenbrand S, Anderson P, Gubbins PO, Martin BC. Smartphone medication adherence apps: potential benefits to patients and providers. J Am Pharm Assoc (2003). 2013;53(2):172-181. doi: 10.1331/JAPhA.2013.12202.
39. Huang Z, Lum E, Jimenez G, Semwal M, Sloot P, Car J. Medication management support in diabetes: a systematic assessment of diabetes self-management apps. BMC Med. 2019;17(1):127. doi: 10.1186/s12916-019-1362-1.
40. Martinez M, Park SB, Maison I, Mody V, Soh LS, Parihar HS. iOS Appstore-Based Phone Apps for Diabetes Management: Potential for Use in Medication Adherence. JMIR Diabetes. 2017;2(2):e12. doi: 10.2196/diabetes.6468.
41. Kebede MM, Pischke CR. Popular Diabetes Apps and the Impact of Diabetes App Use on Self-Care Behaviour: A Survey Among the Digital Community of Persons With Diabetes on Social Media. Front Endocrinol (Lausanne). 2019;10:135. doi: 10.3389/fendo.2019.00135.
42. Adu MD, Malabu UH, Malau-Aduli AEO, Drovandi A, Malau-Aduli BS. Efficacy and Acceptability of My Care Hub Mobile App to Support Self-Management in Australians with Type 1 or Type 2 Diabetes. Int J Environ Res Public Health. 2020;17(7):2573. doi: 10.3390/ijerph17072573.
43. Pustozerov E, Popova P, Tkachuk A, Bolotko Y, Yuldashev Z, Grineva E. Development and Evaluation of a Mobile Personalized Blood Glucose Prediction System for Patients With Gestational Diabetes Mellitus. JMIR Mhealth Uhealth. 2018;6(1):e6. doi: 10.2196/mhealth.9236.

44. Hoppe CD, Cade JE, Carter M. An evaluation of diabetes targeted apps for Android smartphone in relation to behaviour change techniques. J Hum Nutr Diet. 2017;30(3):326-338. doi: 10.1111/jhn.12424.
45. Torbjørnsen A, Småstuen MC, Jenum AK, Årsand E, Ribu L. Acceptability of an mHealth App Intervention for Persons With Type 2 Diabetes and its Associations With Initial Self-Management: Randomized Controlled Trial. JMIR Mhealth Uhealth. 2018;6(5):e125. doi: 10.2196/mhealth.8824.
46. Trawley S, Baptista S, Browne JL, Pouwer F, Speight J. The Use of Mobile Applications Among Adults with Type 1 and Type 2 Diabetes: Results from the Second MILES-Australia (MILES-2) Study. Diabetes Technol Ther. 2017;19(12):730-738. doi: 10.1089/dia.2017.0235.
47. Carroll N, Richardson I. Software-as-a-medical device: demystifying connected health regulations. J Syst Inform Tech. 2016;18(2):186-215. doi: 10.1108/JSIT-07-2015-0061.
48. Png ME, Yoong J, Phan TP, Wee HL. Current and future economic burden of diabetes among working-age adults in Asia: conservative estimates for Singapore from 2010-2050. BMC Public Health. 2016;16:153. doi: 10.1186/s12889-016-2827-1.
49. Schnall R, Rojas M, Bakken S, et al, Mosley JP, Travers J. A user-centered model for designing consumer mobile health (mHealth) applications (apps). J Biomed Inform. 2016;60:243-251. doi: 10.1016/j.jbi.2016.02.002.
50. Adu MD, Malabu UH, Malau-Aduli AEO, Malau-Aduli BS. Users' preferences and design recommendations to promote engagements with mobile apps for diabetes self-management: Multi-national perspectives. PLoS One. 2018;13(12):e0208942. doi: 10.1371/journal.pone.0208942.

The European Research Journal 2024;10(1):136-143 Review

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Managing *Helicobacter pylori* infection: transitioning from conventional to alternative treatment approaches

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ABSTRACT

Helicobacter pylori, an essential constituent of the gastric microbiome in those infected, is commonly associated with medical conditions such as chronic gastritis, peptic ulcer disease, and gastric cancer. In recent years, the growing resistance to antibiotics worldwide has emerged as a substantial hurdle in the effective treatment of *H. pylori* infection. Consequently, it has necessitated the exploration of innovative treatment strategies aimed at bolstering the potency of existing antibiotic-based eradication therapies. Such avant-garde strategies include the incorporation of probiotics and prebiotics as complementary measures to *H. pylori* treatment, the use of antimicrobial peptides as potential replacements for traditional antibiotics, and the application of photodynamic therapy via ingestible devices. Other advanced methodologies entail deploying drug delivery systems that utilize microparticles and nanoparticles, the invention of vaccines, the exploration of natural products, and the potential use of phage therapy. This review offers a contemporary synopsis of these burgeoning strategies designed to suppress *H. pylori*, delving into their strengths, hurdles, and aspects to consider during their development. A significant achievement would be the creation of an efficient human vaccine; however, previous attempts at developing such vaccines have met with obstacles or even cessation. Numerous natural products have displayed anti-*H. pylori* properties, predominantly in laboratory environments. Nonetheless, a requirement remains for more extensive clinical studies to fully comprehend their role in exterminating *H. pylori*. Finally, phage therapy, while demonstrating potential as a suitable alternative, grapples with considerable challenges, chiefly the isolation of highly virulent bacteriophages that specifically target *H. pylori*.

Keywords: *Helicobacter pylori*, antibiotic resistance, innovative treatment strategies

Helicobacter pylori (H. pylori), a Gram-negative, helical bacterium, exhibits high global prevalence, infecting over half of the worldwide population [1, 2]. It primarily colonizes the gastric mucosa, constituting the majority of the gastric microbiota in those infected [3-7]. Infections with *H. pylori*, which typically initiates in childhood and can persist lifelong if left untreated, are linked to an array of gastric and extragastric conditions. These include chronic gastritis, peptic ulcer disease, gastric carcinoma, and gastric mucosa-associated lymphoid tissue (MALT) lymphoma [8]. The pathogenic potential of individual *H. pylori* strains significantly influences these clinical outcomes, with certain virulence factors associated with an elevated risk of disease development [9-11].

H. pylori infection also impinges upon the gastric and gut microbiota, potentially playing a contributory

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role in both gastric and extragastric manifestations [12]. Nevertheless, the attempt to eradicate *H. pylori* via antibiotic therapy elicits concerns regarding the emergence of antibiotic-resistant strains and disruptions in the composition of the intestinal microbiota [13-17].

Owing to the high prevalence of *H. pylori* infection and its concomitant diseases, efficient eradication therapy is vital for clinical management. Contemporary treatment guidelines take into account previous antibiotic exposure and regional resistance rates to select the apt regimen. Triple therapy involving a proton-pump inhibitor (PPI), amoxicillin, and clarithromycin for a 14-day period is recommended as the first-line therapy in regions with low clarithromycin resistance [18]. An alternative option is Bismuth-containing quadruple therapy (BQT). In cases of high clarithromycin resistance, quadruple concomitant therapy or BQT is suggested [19]. Levofloxacin-based therapy is discouraged as a first-line treatment due to the escalating fluoroquinolone resistance [20]. Other recommended options include sequential therapy and fluoroquinolone-based LOAD therapy [21, 22]. The selection of the PPI molecule is also of importance, with second-generation PPIs demonstrating higher efficacy than their first-generation counterparts [23]. BQT is considered the most effective first-line therapy, while dual therapy employing amoxicillin and vonoprazan exhibits potential in East Asia [18, 24, 25].

Antibiotic resistance poses a major obstacle in *H. pylori* treatment, with eradication rates dwindling and resistance rates surging globally [25-29]. The improvement of diagnostic tools, such as non-invasive testing methods, is instrumental in combating antibiotic resistance [18, 30]. Additionally, factors related to patient compliance, side effects, and host genetic variants necessitate the exploration of alternative therapeutic approaches for *H. pylori* treatment.

Alternative Approaches for *H. pylori* Treatment

Innovative therapies for *H. pylori* infections strive to meet WHO standards for eradication rates, patient compliance, and prevention of antimicrobial resistance. These include strategies like the use of probiotics and prebiotics, antimicrobial peptides, photodynamic therapy, natural products, vaccines, micro- and nanoparticles, and phage therapy [31-34].

Probiotics are live microorganisms that confer health benefits. They modulate the immune response, generate antimicrobial substances, and compete with *H. pylori* for adhesion sites. Lactobacillus and Bifidobacterium are the commonly used probiotic genera [31, 35, 36]. Prebiotics are non-digestible nutrients metabolized by bacteria. They stimulate the growth of specific intestinal bacteria and are utilized as supplements to bolster *H. pylori* treatment [32].

S upplementation with probiotics and prebiotics in *H. pylori* therapy has demonstrated increased eradication rates and reduced side effects in patients [33, 34]. Mechanisms of probiotic action include immunological modulation, production of antimicrobial substances, inhibition of adhesion, and enhancement of mucin secretion [35, 36].

Antimicrobial peptides (AMPs), naturally produced by organisms, play a pivotal role in innate immunity. They interact with microbial cell membranes, leading to cell lysis. Synthetic AMP analogs have exhibited anti-*H. pylori* activity [37, 38]. AMPs produced by gastric epithelial cells regulate the bacterial population in the stomach, but *H. pylori* has evolved resistance mechanisms [39-41].

Present research focuses on identifying efficacious probiotic strains and evaluating their efficiency either alone or in combination with antibiotics. Discrepant results have been reported, necessitating further studies to ascertain their impact on *H. pylori* eradication rates and gut microbiota [42-47]. In summary, while alternative approaches such as probiotics, prebiotics, and antimicrobial peptides exhibit potential in *H. pylori* treatment, additional research is required to determine their effectiveness and optimal usage [37].

Antimicrobial Peptides

Antimicrobial peptides (AMPs) are short oligopeptides possessing a positive charge, produced naturally by numerous organisms. Their role in innate immunity is essential, enabling them to defend against a variety of pathogens, including *H. pylori*. AMPs interfere with microbial cell membranes, instigating increased membrane permeability, pore formation, and leading to cell lysis. Synthetic AMP analogues, for instance, pexiganan, tilapia piscidins, and PGLa-AM1, have shown a remarkable ability to combat *H. pylori*. Bicarinalin and cathelicidins have also displayed anti-*H. pylori* activity. Despite their effectiveness, *H. pylori*

has evolved specific resistance mechanisms against host AMPs. The development of new synthetic AMP analogues and further research is actively underway [37, 38, 48-50].

Photodynamic Therapy

Photodynamic therapy (PDT) combines a photoactive molecule, termed a photosensitizer, with visible light and oxygen to generate cytotoxic reactive oxygen species (ROS). The potential of PDT as a treatment for *H. pylori* infections is currently under investigation. *H. pylori* inherently synthesizes and stores photosensitizers, making it suitable for antibacterial PDT without the requirement for external photosensitizers. In vitro research has shown significant reductions in *H. pylori* colony-forming units (CFU) following PDT using both red and blue lights. Moreover, the conjunction of PDT with antibiotics has resulted in a synergistic antibacterial effect. The use of PDT without the addition of exogenous photosensitizers has shown minimal side effects on gastric mucosa. Current research aims to refine PDT efficacy by considering tissue interactions and optical properties [51-58].

Micro- and Nanoparticles for Drug Delivery

Microparticles (MPs) and nanoparticles (NPs) have demonstrated potential in drug delivery for the treatment of microbial infections, including *H. pylori*. The small size and high surface-to-volume ratio of these particles enhance therapeutic efficacy and diminish side effects in comparison to traditional antibiotic-based treatments. Specifically, NPs can navigate physiological barriers and interact with pathogen membranes and cell walls. Drugs, such as antibiotics, can be loaded onto these particles, ensuring delivery to the infection site and protection from degradation and resistance mechanisms [57-60].

Expanding on these alternative approaches, AMPs, PDT, and micro- and nanoparticles provide potential novel treatments for *H. pylori*. Currently, research is directed towards optimizing their efficacy and suitability for clinical settings.

Microparticles and Nanoparticles for *H. pylori* Eradication

In the quest to enhance *H. pylori* eradication, microparticles and nanoparticles have come under investigation. Chitosan-based MPs and NPs have shown promise as gastric drug delivery systems, due to their biocompatibility, antimicrobial attributes, and mucoadhesiveness. Progress has been made using chitosan-based mini-tablets, nanoparticles, or hydrogels loaded with amoxicillin. Moreover, Poly (lactic-co-glycolic acid) nanoparticles possessing pH-sensitive and acid-resistant properties have been developed for targeted drug delivery to *H. pylori* infection sites. Metal nanoparticles, including gold, silver, and zinc oxide, have demonstrated good biocompatibility and anti-*H. pylori* activity. Another breakthrough was the coating of polymeric nanoparticles with *H. pylori* outer membrane proteins, which led to reduced *H. pylori* adhesion to gastric tissues. The combination of lytic bacteriophages with nanoparticles resulted in a synergistic effect, decreasing *H. pylori* colonization. Further optimization of these methods is required [61-64].

Vaccines against *H. pylori*

The development of a highly effective vaccine against *H. pylori* is perceived as a cost-effective method to prevent infection and associated diseases. Although prophylactic and therapeutic vaccines have been tested, no large-scale vaccine has been successfully produced yet. Clinical trials of different vaccine formulations have exhibited some immune responsiveness but have not consistently reduced bacterial load or stimulated protective immunity. Current research is centered on multivalent epitope-based vaccines, recombinant or fusion protein-based vaccines, and mucosal adjuvants. Oral vaccines face obstacles, but nanoparticles have shown promise in safeguarding antigens and eliciting immune responses. For instance, chitosan nanoparticles have displayed stability, enhanced immune response, and prolonged release of DNA vaccines. Immunoinformatics has also contributed to the screening of antigen targets and designing epitope-based vaccines. Vaccine development necessitates further research [65-67].

Natural Products against *H. pylori*

Natural products like plants, fruits, and spices have been explored for their anti-*H. pylori* properties. These products exhibit inhibitory effects on bacterial enzymes, possess anti-adhesive and anti-inflammatory properties, and provide bactericidal or bacteriostatic effects. Investigations have been carried out on Citrus bergamia derivatives, blueberry, grape seed extract,

mastic gum, cinnamon, ginger, curcumin, chestnut, oak honey, and propolis. However, further studies are needed to identify the specific compounds and mechanisms responsible for their activity [68-71].

Phage Therapy for *H. pylori*

The concept of phage therapy, which employs bacteriophages to target and eradicate bacterial infections, has sparked interest in treating *H. pylori*. Phages are known for their ability to selectively lyse specific bacterial strains while leaving the microbiota unharmed. Deemed safe for clinical use, phages replicate inside bacterial host cells, enabling them to target new bacterial cells. Phage therapy has proven effective in treating infections caused by pathogenic and antibiotic-resistant bacteria. Despite its potential, further research is required to translate phage therapy into a clinical application for *H. pylori* [72-76].

Interestingly, phage therapy for *H. pylori* infections has shown potential. In a study , a therapy was developed combining phage Hp ϕ with lactoferrin adsorbed on hydroxyapatite nanoparticles (NPs). This novel treatment demonstrated enhanced antimicrobial effects against *H. pylori* in human gastric cancer cells [77]. Yet, comprehensive data on phages and phage-*H. pylori* interactions in the gastric environment are lacking. The absence of sequenced phage genomes restricts our understanding of key factors like toxins, antimicrobial resistance genes, and virulence in *H. pylori* phages. Moreover, reports of endolysins present in *H. pylori* phages are nonexistent [78].

Endolysins are phage proteins responsible for breaking down bacterial cell walls and present an alternative approach in phage therapy. Endolysins show specificity to the bacterial host, and to date, bacterial resistance to endolysins hasn't been reported [79]. However, treating Gram-negative bacteria like *H. pylori* is challenging due to the presence of an outer membrane barrier. Strategies involving engineering approaches or combining lysins with weak acids have shown success in permeabilizing the outer membrane [80, 81].

CONCLUSION

The landscape for *H. pylori* eradication continues to evolve, with promising alternative approaches emerging from recent research. Antimicrobial peptides, phage therapy, nanoparticle-enhanced delivery, vaccines, and natural products represent novel strategies that are redefining the treatment modalities available for *H. pylori* infection.

Antimicrobial peptides (AMPs), both natural and synthetic, offer a new way to combat *H. pylori*. The ongoing development of synthetic AMP analogues and the further understanding of their interaction with microbial cell membranes are expected to enhance their potential as an effective therapeutic approach.

Phage therapy has also shown significant potential for *H. pylori* treatment. The ability of bacteriophages to selectively lyse specific bacterial strains could lead to more targeted therapies that do not disrupt the microbiota. The combined use of endolysins and engineered approaches presents a compelling opportunity to overcome the challenges presented by the outer membrane barrier of Gram-negative bacteria such as *H. pylori.*

Nanotechnology has emerged as a potent tool for improved drug delivery. The properties of microparticles and nanoparticles, including their small size, high surface-to-volume ratio, and ability to overcome physiological barriers, hold significant promise for enhanced therapeutic effectiveness.

Moreover, the development of a vaccine against *H. pylori* has been considered a cost-effective method to prevent infection and associated diseases. Despite various challenges, ongoing research in multivalent epitope-based vaccines and recombinant or fusion protein-based vaccines, coupled with the use of mucosal adjuvants and nanoparticles, suggests that a successful vaccine may be within reach.

Finally, natural products also offer an appealing direction for research, with their demonstrated inhibitory effects on *H. pylori* and the potential for fewer side effects compared to synthetic drugs.

However, despite these promising alternatives, further in vivo studies and well-designed clinical trials are necessary to fully understand and realize their potential. As our understanding of *H. pylori* and its resistance mechanisms expands, these targeted therapies could provide personalized treatment options that selectively deplete *H. pylori* without disrupting the normal gastric microbiome.

In this context, these alternatives represent not only a departure from the traditional antibiotic-based treatments, but also a promising pathway towards more effective and sustainable solutions for the management and eradication of *H. pylori* infections. Such advancements could significantly improve patient outcomes, reduce the societal and economic burden of these infections, and usher in a new era of personalized and effective treatments for *H. pylori*.

In summary, the research landscape for *H. pylori* eradication is expanding, with alternative approaches like phage therapy, antimicrobial peptides, nanoparticles, vaccines, and more showing great potential. However, comprehensive in vivo studies and well-structured clinical trials are crucial to fully grasp their efficacy. These targeted therapies can provide personalized treatment options that selectively deplete *H. pylori* without disrupting the normal gastric microbiome, making them exciting alternatives to broad-spectrum antibiotics and non-selective treatments for *H. pylori* infections.

Authors' Contribution

Study Conception: SÖ; Study Design: SÖ; Supervision: SÖ; Funding: N/A; Materials: N/A; Data Collection and/or Processing: SÖ; Statistical Analysis and/or Data Interpretation: SÖ; Literature Review: SÖ; Manuscript Preparation: SÖ and Critical Review: SÖ.

Conflict of interest

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REFERENCES

1. Azevedo NF, Pinto AR, Reis NM, Vieira MJ, Keevil CW. Shear stress, temperature, and inoculation concentration influence the adhesion of water-stressed Helicobacter pylori to stainless steel 304 and polypropylene. Appl Environ Microbiol. 2006;72(4):2936-2941. doi: 10.1128/AEM.72.4.2936-2941.2006.
2. Hooi JKY, Lai WY, Ng WK, et al. Global Prevalence of Helicobacter pylori Infection: Systematic Review and Meta-Analysis. Gastroenterology. 2017;153(2):420-429. doi: 10.1053/j.gastro.2017.04.022.
3. Bik EM, Eckburg PB, Gill SR, et al. Molecular analysis of the bacterial microbiota in the human stomach. Proc Natl Acad Sci U S A. 2006;103(3):732-737. doi: 10.1073/pnas.0506655103.
4. Klymiuk I, Bilgilier C, Stadlmann A, Thannesberger J, et al. The Human Gastric Microbiome Is Predicated upon Infection with Helicobacter pylori. Front Microbiol. 2017;8:2508. doi: 10.3389/fmicb.2017.02508.
5. Parsons BN, Ijaz UZ, D'Amore R, et al. Comparison of the human gastric microbiota in hypochlorhydric states arising as a result of Helicobacter pylori-induced atrophic gastritis, autoimmune atrophic gastritis and proton pump inhibitor use. PLoS Pathog. 2017;13(11):e1006653. doi: 10.1371/journal.ppat.1006653.
6. Wang Z, Bafadhel M, Haldar K, et al. Lung microbiome dynamics in COPD exacerbations. Eur Respir J. 2016;47(4):1082-1092. doi: 10.1183/13993003.01406-2015.
7. Ferreira RM, Pereira-Marques J, Pinto-Ribeiro I, et al. Gastric microbial community profiling reveals a dysbiotic cancer-associated microbiota. Gut. 2018;67(2):226-236. doi: 10.1136/gutjnl-2017-314205.
8. Cover TL, Blaser MJ. Helicobacter pylori in health and disease. Gastroenterology. 2009;136(6):1863-1873. doi: 10.1053/j.gastro.2009.01.073.
9. Gerhard M, Rad R, Prinz C, Naumann M. Pathogenesis of Helicobacter pylori infection. Helicobacter. 2002;7 Suppl 1:17-23. doi: 10.1046/j.1523-5378.7.s1.3.x.
10. Blaser MJ. Helicobacter pylori and the pathogenesis of gastroduodenal inflammation. J Infect Dis. 1990;161(4):626-633. doi: 10.1093/infdis/161.4.626.
11. Ferreira RM, Machado JC, Figueiredo C. Clinical relevance of Helicobacter pylori vacA and cagA genotypes in gastric carcinoma. Best Pract Res Clin Gastroenterol. 2014;28(6):1003-1015. doi: 10.1016/j.bpg.2014.09.004.
12. Franceschi F, Annalisa T, Teresa DR, et al. Role of Helicobacter pylori infection on nutrition and metabolism. World J Gastroenterol. 2014;20(36):12809-12817. doi: 10.3748/wjg.v20.i36.12809.
13. Liou JM, Chen CC, Chen MJ. Sequential versus triple therapy for the first-line treatment of Helicobacter pylori: a multicentre, open-label, randomised trial. Lancet. 2013;381(9862):205-213. doi: 10.1016/S0140-6736(12)61579-7.
14. Tai WC, Liang CM, Kuo CM, et al. A 14 day esomeprazole- and amoxicillin-containing high-dose dual therapy regimen achieves a high eradication rate as first-line anti-Helicobacter pylori treatment in Taiwan: a prospective randomized trial. J Antimicrob Chemother. 2019;74(6):1718-1724. doi: 10.1093/jac/dkz046.
15. Hsu PI, Wu DC, Chen WC, et al. Randomized controlled trial comparing 7-day triple, 10-day sequential, and 7-day concomitant therapies for Helicobacter pylori infection. Antimicrob Agents Chemother. 2014;58(10):5936-5942. doi: 10.1128/AAC.02922-14.
16. Hanada K, Graham DY. Helicobacter pylori and the molecular pathogenesis of intestinal-type gastric carcinoma. Expert Rev Anticancer Ther. 2014;14(8):947-954. doi: 10.1586/14737140.2014.911092.
17. Fallone CA, Chiba N, van Zanten SV, et al. The Toronto Consensus for the Treatment of Helicobacter pylori Infection in Adults. Gastroenterology. 2016;151(1):51-69. doi: 10.1053/j.gastro.2016.04.006.
18. Malfertheiner P, Megraud F, O'Morain CA, et al. Manage-

ment of Helicobacter pylori infection-the Maastricht V/Florence Consensus Report. Gut. 2017;66(1):6-30. doi: 10.1136/gutjnl-2016-312288.
19. Molina-Infante J, Romano M, Fernandez-Bermejo M, et al. Optimized nonbismuth quadruple therapies cure most patients with Helicobacter pylori infection in populations with high rates of antibiotic resistance. Gastroenterology. 2013;145(1):121-128. doi: 10.1053/j.gastro.2013.03.050.
20. Li M, Oshima T, Horikawa T, Tozawa K, Tomita T, Fukui H, Watari J, Miwa H. Systematic review with meta-analysis: Vonoprazan, a potent acid blocker, is superior to proton-pump inhibitors for eradication of clarithromycin-resistant strains of Helicobacter pylori. Helicobacter. 2018;23(4):e12495. doi: 10.1111/hel.12495.
21. Guevara B, Cogdill AG. Helicobacter pylori: A Review of Current Diagnostic and Management Strategies. Dig Dis Sci. 2020;65(7):1917-1931. doi: 10.1007/s10620-020-06193-7.
22. Gisbert JP, Romano M, Gravina AG, et al. Helicobacter pylori second-line rescue therapy with levofloxacin- and bismuth-containing quadruple therapy, after failure of standard triple or non-bismuth quadruple treatments. Aliment Pharmacol Ther. 2015;41(8):768-775. doi: 10.1111/apt.13128.
23. Murakami K, Sakurai Y, Shiino M, Funao N, Nishimura A, Asaka M. Vonoprazan, a novel potassium-competitive acid blocker, as a component of first-line and second-line triple therapy for Helicobacter pylori eradication: a phase III, randomised, double-blind study. Gut. 2016;65(9):1439-1446. doi: 10.1136/gutjnl-2015-311304.
24. Suzuki S, Gotoda T, Kusano C, Iwatsuka K, Moriyama M. The Efficacy and Tolerability of a Triple Therapy Containing a Potassium-Competitive Acid Blocker Compared With a 7-Day PPI-Based Low-Dose Clarithromycin Triple Therapy. Am J Gastroenterol. 2016;111(7):949-956. doi: 10.1038/ajg.2016.182.
25. Gatta L, Vakil N, Vaira D, Scarpignato C. Global eradication rates for Helicobacter pylori infection: systematic review and meta-analysis of sequential therapy. BMJ. 2013;347:f4587. doi: 10.1136/bmj.f4587.
26. O'Morain NR, Dore MP, O'Connor AJP, Gisbert JP, O'Morain CA. Treatment of Helicobacter pylori infection in 2018. Helicobacter. 2018;23 Suppl 1:e12519. doi: 10.1111/hel.12519.
27. Savoldi A, Carrara E, Graham DY, Conti M, Tacconelli E. Prevalence of Antibiotic Resistance in Helicobacter pylori: A Systematic Review and Meta-analysis in World Health Organization Regions. Gastroenterology. 2018;155(5):1372-1382. doi: 10.1053/j.gastro.2018.07.007.
28. Megraud F, Coenen S, Versporten A, et al. Helicobacter pylori resistance to antibiotics in Europe and its relationship to antibiotic consumption. Gut. 2013;62(1):34-42. doi: 10.1136/gutjnl-2012-302254.
29. Savoldi A, Carrara E, Graham DY, Conti M, Tacconelli E. Prevalence of Antibiotic Resistance in Helicobacter pylori: A Systematic Review and Meta-analysis in World Health Organization Regions. Gastroenterology. 2018;155(5):1372-1382. doi: 10.1053/j.gastro.2018.07.007.
30. Malfertheiner P, Megraud F, O'Morain C, et al. Current concepts in the management of Helicobacter pylori infection: the Maastricht III Consensus Report. Gut. 2007;56(6):772-781. doi: 10.1136/gut.2006.101634.
31. Hill C, Guarner F, Reid G, et al. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol. 2014;11(8):506-514. doi: 10.1038/nrgastro.2014.66.
32. Davani-Davari D, Negahdaripour M, Karimzadeh I, et al. Prebiotics: Definition, Types, Sources, Mechanisms, and Clinical Applications. Foods. 2019;8(3):92. doi: 10.3390/foods8030092.
33. Ahmad K, Fatemeh F, Mehri N, Maryam S. Probiotics for the treatment of pediatric helicobacter pylori infection: a randomized double blind clinical trial. Iran J Pediatr. 2013;23(1):79-84.
34. Lionetti E, Miniello VL, Castellaneta SP, et al. Lactobacillus reuteri therapy to reduce side-effects during anti-Helicobacter pylori treatment in children: a randomized placebo controlled trial. Aliment Pharmacol Ther. 2006;24(10):1461-1468. doi: 10.1111/j.1365-2036.2006.03145.x.
35. Sgouras DN, Panayotopoulou EG, Martinez-Gonzalez B, Petraki K, Michopoulos S, Mentis A. Lactobacillus johnsonii La1 attenuates Helicobacter pylori-associated gastritis and reduces levels of proinflammatory chemokines in C57BL/6 mice. Clin Diagn Lab Immunol. 2005;12(12):1378-1386. doi: 10.1128/CDLI.12.12.1378-1386.2005.
36. Kulkarni T, Majarikar S, Deshmukh M, et al. Probiotic sepsis in preterm neonates-a systematic review. Eur J Pediatr. 2022;181(6):2249-2262. doi: 10.1007/s00431-022-04452-5.
37. Joshi A, Agrawal A, Bhattacharya S. Formulation and clinical advancement of nanourchins: a novel multibranched nanoparticulate drug-delivery system. Nanomedicine (Lond). 2022;17(20):1477-1499. doi: 10.2217/nnm-2022-0096.
38. Luong AD, Buzid A, Luong JHT. Important Roles and Potential Uses of Natural and Synthetic Antimicrobial Peptides (AMPs) in Oral Diseases: Cavity, Periodontal Disease, and Thrush. J Funct Biomater. 2022;13(4):175. doi: 10.3390/jfb13040175.
39. Gao JH, Guo LJ, Huang ZY, Rao JN, Tang CW. Roles of cellular polyamines in mucosal healing in the gastrointestinal tract. J Physiol Pharmacol. 2013;64(6):681-693.
40. Ruan Q, Guan P, Qi W, et al. Porphyromonas gingivalis regulates atherosclerosis through an immune pathway. Front Immunol. 2023;14:1103592. doi: 10.3389/fimmu.2023.1103592.
41. Park SC, Park Y, Hahm KS. The role of antimicrobial peptides in preventing multidrug-resistant bacterial infections and biofilm formation. Int J Mol Sci. 2011;12(9):5971-5992. doi: 10.3390/ijms12095971.
42. Jungersen M, Wind A, Johansen E, Christensen JE, Stuer-Lauridsen B, Eskesen D. The Science behind the Probiotic Strain Bifidobacterium animalis subsp. lactis BB-12(®). Microorganisms. 2014;2(2):92-110. doi: 10.3390/microorganisms2020092.
43. Michalak A, Kasztelan-Szczerbińska B, Cichoż-Lach H. Impact of Obesity on the Course of Management of Inflammatory Bowel Disease-A Review. Nutrients. 2022;14(19):3983. doi: 10.3390/nu14193983.
44. Zhang MM, Qian W, Qin YY, He J, Zhou YH. Probiotics in Helicobacter pylori eradication therapy: a systematic review and meta-analysis. World J Gastroenterol. 2015;21(14):4345-4357. doi: 10.3748/wjg.v21.i14.4345.
45. McFarland LV, Huang Y, Wang L, Malfertheiner P. System-

atic review and meta-analysis: Multi-strain probiotics as adjunct therapy for Helicobacter pylori eradication and prevention of adverse events. United European Gastroenterol J. 2016;4(4):546-561. doi: 10.1177/2050640615617358.
46. Milner E, Stevens B, An M, et al. Utilizing Probiotics for the Prevention and Treatment of Gastrointestinal Diseases. Front Microbiol. 2021;12:689958. doi: 10.3389/fmicb.2021.689958.
47. Ji J, Yang H. Using Probiotics as Supplementation for Helicobacter pylori Antibiotic Therapy. Int J Mol Sci. 2020;21(3):1136. doi: 10.3390/ijms21031136.
48. Igarashi M, Kitada Y, Yoshiyama H, Takagi A, Miwa T, Koga Y. Ammonia as an accelerator of tumor necrosis factor alpha-induced apoptosis of gastric epithelial cells in Helicobacter pylori infection. Infect Immun. 2001;69(2):816-821. doi: 10.1128/IAI.69.2.816-821.2001.
49. Guzmán-Rodríguez JJ, López-Gómez R, Suárez-Rodríguez LM, et al. Antibacterial activity of defensin PaDef from avocado fruit (Persea americana var. drymifolia) expressed in endothelial cells against Escherichia coli and Staphylococcus aureus. Biomed Res Int. 2013;2013:986273. doi: 10.1155/2013/986273.
50.Foligne B, Nutten S, Grangette C, Dennin V, et al. Correlation between in vitro and in vivo immunomodulatory properties of lactic acid bacteria. World J Gastroenterol. 2007;13(2):236-243. doi: 10.3748/wjg.v13.i2.236.
51. Hamblin MR, Hasan T. Photodynamic therapy: a new antimicrobial approach to infectious disease? Photochem Photobiol Sci. 2004;3(5):436-450. doi: 10.1039/b311900a.
52. Sokic-Milutinovic A, Todorovic V, Milosavljevic T, Micev M, Drndarevic N, Mitrovic O. Gastrin and antral G cells in course of Helicobacter pylori eradication: six months follow up study. World J Gastroenterol. 2005;11(27):4140-4147. doi: 10.3748/wjg.v11.i27.4140.
53. Kellesarian SV, Malignaggi VR, Al-Kheraif AA, Al-Askar M, Yunker M, Javed F. Effect of antimicrobial photodynamic therapy and laser alone as adjunct to mechanical debridement in the management of halitosis: A systematic review. Quintessence Int. 2017;48(7):575-583. doi: 10.3290/j.qi.a38264.
54. Calvino-Fernández M, García-Fresnadillo D, Benito-Martínez S, et al. Helicobacter pylori inactivation and virulence gene damage using a supported sensitiser for photodynamic therapy. Eur J Med Chem. 2013;68:284-290. doi: 10.1016/j.ejmech.2013.07.023.
55. Denis TGS, Hamblin MR. An introduction to photoantimicrobials: photodynamic therapy as a novel method of microbial pathogen eradication. In: Méndez-Vilas A, ed., Science against microbial pathogens: communicating current research and technological advances. Microbiology Series. No:3., Badajoz, Spain: Formatex, 2011: pp. 675-683.
56. Wilder-Smith CH, Wilder-Smith P, Grosjean P, et al. Photoeradication of Helicobacter pylori using 5-aminolevulinic acid: preliminary human studies. Lasers Surg Med. 2002;31(1):18-22. doi: 10.1002/lsm.10066.
57. Aguilera-Correa JJ, Esteban J, Vallet-Regí M. Inorganic and Polymeric Nanoparticles for Human Viral and Bacterial Infections Prevention and Treatment. Nanomaterials (Basel). 2021;11(1):137. doi: 10.3390/nano11010137.
58. Xiong MH, Bao Y, Yang XZ, Zhu YH, Wang J. Delivery of antibiotics with polymeric particles. Adv Drug Deliv Rev. 2014;78:63-76. doi: 10.1016/j.addr.2014.02.002.
59. Matricardi P, Meo CD, Coviello T, Alhaique F. Recent advances and perspectives on coated alginate microspheres for modified drug delivery. Expert Opin Drug Deliv. 2008;5(4):417-425. doi: 10.1517/17425247.5.4.417.
60. Malaekeh-Nikouei B, Bazzaz BSF, Mirhadi E, Tajani AS, Khameneh B. The role of nanotechnology in combating biofilm-based antibiotic resistance. J Drug Deliv Sci Technol 2020;60:101880. doi:10.1016/j.jddst.2020.101880
61. Mehta DK, Rai SR. Microencapsulation of Lactobacillus acidophilus NCDC 291 using emulsion technique and sensory and physico-chemical analysis of the incorporated microcapsules in dairy and non-dairy food product. In: Singhee D, Bhattacharyya K, Tuteja S, Sarkar A. eds., Reflections. JD Birla Institute: Kolkota (West Bengal), India, 2017: pp. 51-57.
62. Liang J, Yan H, Puligundla P, Gao X, Zhou Y, Wan X. Applications of chitosan nanoparticles to enhance absorption and bioavailability of tea polyphenols: a review. Food Hydrocoll 2017;69:286-292. doi:10.1016/j.foodhyd.2017.01.041
63. Gupta R, Prasad Y. Efficacy of polyvalent bacteriophage P-27/HP to control multidrug resistant Staphylococcus aureus associated with human infections. Curr Microbiol. 2011;62(1):255-260. doi: 10.1007/s00284-010-9699-x.
64. de Bortoli N, Leonardi G, Ciancia E, et al. Helicobacter pylori eradication: a randomized prospective study of triple therapy versus triple therapy plus lactoferrin and probiotics. Am J Gastroenterol. 2007;102(5):951-956. doi: 10.1111/j.1572-0241.2007.01085.x.
65. Malfertheiner P, Selgrad M, Bornschein J. Helicobacter pylori: clinical management. Curr Opin Gastroenterol. 2012;28(6):608-14. doi: 10.1097/MOG.0b013e32835918a7.
66. Dar HA, Zaheer T, Shehroz M, et al. Immunoinformatics-Aided Design and Evaluation of a Potential Multi-Epitope Vaccine against Klebsiella Pneumoniae. Vaccines (Basel). 2019;7(3):88. doi: 10.3390/vaccines7030088.
67. Chen Y, Hu H, Huang F, et al. Cocktail of isobavachalcone and curcumin enhance eradication of Staphylococcus aureus biofilm from orthopedic implants by gentamicin and alleviate inflammatory osteolysis. Front Microbiol. 2022;13:958132. doi: 10.3389/fmicb.2022.958132.
68. Abd Eldaim MA, Tousson E, Soliman MM, El Sayed IET, Abdel Aleem AAH, Elsharkawy HN. Grape seed extract ameliorated Ehrlich solid tumor-induced hepatic tissue and DNA damage with reduction of PCNA and P53 protein expression in mice. Environ Sci Pollut Res Int. 2021;28(32):44226-44238. doi: 10.1007/s11356-021-13904-8.
69. Muhammad SNH, Yaacob NS, Safuwan NAM, Fauzi AN. Antiglycolytic Activities of Strobilanthes crispus Active Fraction and its Bioactive Components on Triple-Negative Breast Cancer Cells In Vitro. Anticancer Agents Med Chem. 2022;22(7):1363-1369. doi: 10.2174/1871520621666210427104804.
70. Nzeako BC, Al-Namaani F. The antibacterial activity of honey on helicobacter pylori. Sultan Qaboos Univ Med J. 2006 Dec;6(2):71-76.
71. Viertel TM, Ritter K, Horz HP. Viruses versus bacteria-novel approaches to phage therapy as a tool against multidrug-resistant pathogens. J Antimicrob Chemother. 2014;69(9):2326-2336. doi: 10.1093/jac/dku173.

72. Casey E, van Sinderen D, Mahony J. In Vitro Characteristics of Phages to Guide 'Real Life' Phage Therapy Suitability. Viruses. 2018;10(4):163. doi: 10.3390/v10040163.
73. Uyttebroek S, Chen B, Onsea J. Safety and efficacy of phage therapy in difficult-to-treat infections: a systematic review. Lancet Infect Dis. 2022;22(8):e208-e220. doi: 10.1016/S1473-3099(21)00612-5.
74. Sousa C, Ferreira R, Azevedo NF, et al. Helicobacter pylori infection: from standard to alternative treatment strategies. Crit Rev Microbiol. 2022;48(3):376-396. doi: 10.1080/1040841X.2021.1975643.
75. Galtier M, De Sordi L, Maura D, et al. Bacteriophages to reduce gut carriage of antibiotic resistant uropathogens with low impact on microbiota composition. Environ Microbiol. 2016;18(7):2237-2245. doi: 10.1111/1462-2920.13284.
76. Elbehiry A, Marzouk E, Aldubaib M, et al. Helicobacter pylori Infection: Current Status and Future Prospects on Diagnostic, Therapeutic and Control Challenges. Antibiotics (Basel). 2023;12(2):191. doi: 10.3390/antibiotics12020191.
77. Oliveira H, Thiagarajan V, Walmagh M, et al. A thermostable Salmonella phage endolysin, Lys68, with broad bactericidal properties against gram-negative pathogens in presence of weak acids. PLoS One. 2014;9(10):e108376. doi: 10.1371/journal.pone.0108376.
78. Oliveira H, Melo LD, Santos SB, et al. Molecular aspects and comparative genomics of bacteriophage endolysins. J Virol. 2013;87(8):4558-4570. doi: 10.1128/JVI.03277-12.
79. Oliveira H, São-José C, Azeredo J. Phage-Derived Peptidoglycan Degrading Enzymes: Challenges and Future Prospects for In Vivo Therapy. Viruses. 2018;10(6):292. doi: 10.3390/v10060292.
80. Lukacik P, Barnard TJ, Keller PW, et al. Structural engineering of a phage lysin that targets gram-negative pathogens. Proc Natl Acad Sci U S A. 2012;109(25):9857-9862. doi: 10.1073/pnas.1203472109.
81. Lood R, Winer BY, Pelzek AJ, et al. Novel phage lysin capable of killing the multidrug-resistant gram-negative bacterium Acinetobacter baumannii in a mouse bacteremia model. Antimicrob Agents Chemother. 2015;59(4):1983-1991. doi: 10.1128/AAC.04641-14.

The European Research Journal 2024;10(1):144-148 Case Report

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Detection of recurrent phosphaturic mesenchymal tumors by using Ga-68 DOTATATE PET/CT

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ABSTRACT

Phosphaturic mesenchymal tumor is a rare clinical condition and often causes osteomalaciadue to tumor. Its diagnosis is often significantly delayed due to its rare occurrence in addition to the generalized and vague symptoms of their presentation. A 19-year-old female with a history of left facial nerve palsy, generalized weakness and hoarseness of voice revealed a dense mass in her brain. In this case, we reported successful application of a Ga-68 labeled DOTATATE PET/CT scan to identify the primary site and distant metastases of phosphaturic mesenchymal tumors and show the diagnostic value of Ga-68 labeled DOTATATE PET/CT imaging for the rare tumors.

Keywords: Phosphaturic mesenchymal tumor, Ga-68 labeled DOTATATE PET/CT scan, osteomalacia

Osteomalacia induced by tumor (OIT) is most often caused by a rare, benign, mesenchymal neoplasm - phosphaturic mesenchymal tumor (PMT) with overexpression of fibroblast growth factor-23 (FGF-23) [1]. PMT causes hypophosphatemia, hyperphosphaturia, bone pain, muscle weakness, and pathological bone fractures. Not more than 1000 cases are described in the literature [2]. The peak incidence is during the forties and fifties. The most frequent site of involvement is bone followed by soft tissue [3, 4]. The development of metastasis is remote possibility with PMT [5, 6] and surgical removal is the curative treatment. Commonly recommended diagnostic modalities including magnetic resonance imaging (MRI) and 18F fluorodeoxyglucose positron emission tomography / computed tomography (18F-FDG PET/CT) prove useful in the evaluation of PMT. 18F-FDG PET-CT has been associated with false-positive results [7]. The discovery of overexpression of the type 2 somatostatin receptor (SSTR) receptor by PMT cells prompted use of more specific imaging with Gallium-68, labelled DOTA-octreotate, oxodotreotide, DOTA-(Tyr3)-octreotate (Ga-68 labeled DOTATATE) [3]. Ga-68 DOTATATE PET/CT is considered more accurate technique for identifying primary tumors and distant metastases, permitting shorter image acquisition interval with reduction in radiation dose to the patient. Additionally, it provides information about the density of SSTR receptors exhibited by the tumor cells, which is a decisive factor in planning targeted radionuclide therapy [7, 8].

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CASE PRESENTATION

A 19-year-old female with a history of left facial nerve palsy and hoarseness of voice underwent a Brain CT scan. She was revealed to have a dense mass (4.0 × 3.5 cm) in the left cerebellopontine angle and cerebellum involving left mastoid air cells, eroding and destroying petrous temporal and occipital bone. In addition, the MRI revealed extra-axial abnormal signal intensity mass in the left jugular foramen region. It also indicated medial widening (4.2 × 2.7 cm) with intracranial extradural extension into cerebellopontine cistern indenting brainstem. Furthermore, ipsilateral superior and middle cerebellar peduncle were associated with perilesional edema resulting in effacement of adjacent cerebellar folia. The middle ear cavity is also compressed laterally, resulting in occlusion of the eustachian tube-posteroinferiorly, causing destruction of basiocciput along the lateral aspect of the foramen magnum. The patient underwent radiotherapy and was prescribed steroids; she remained symptom-free for one year but was readmitted with severe headache and vomiting. The CT Brain revealed obstructive hydrocephalus with cerebral edema and underwent ventriculoperitoneal shunting.

The immunohistochemistry analysis was positive for STAT6 and SATB2, tumor markers frequently reported in PMT cases [9]. MRI Brain showed an aggressive enhancing mass with infiltration into the left cerebellar hemisphere. Compared to previous scans, there was an interval progression of the disease. The Ga-68 DOTATATE PET/CT revealed a moderately avid left intracranial lesion in the left cerebellum (Maximum Standardized uptake value [SUV max] 4.9, 50 × 49 mm) involving basiocciput, occipital and temporal bone (Fig. 1).

DISCUSSION

PMTs are uncommon, benign, neoplasms of mesenchymal origin and the most frequent etiology of OIT. High amounts of the peptide hormone FGF-23 is produced and secreted by these tumors [1]. FGF-23 is a physiological phosphate regulator that minimizes the phosphate reabsorption in the proximal tubule and inhibits 1-α-hydroxylase, which causes 1-α, 25-dihydroxyvitamin D3 deficiency and hypophosphatemia [1]. There is consequent decrease in mineral density of bones leading to non-specific bone pains, pathological bone fractures, progressive functional weakness and asthenia. Most of the causative tumors for osteomalacia arise from mesenchymal cells; therefore, their usual localization is the layers of bones or soft tissues [3,4]. ~95% of PMT originate in the appendicular skeleton while only 5% occurs in the head and neck regions [10, 11]. The majority of PMTs (more than 80%) are found in the nose and paranasal sinuses [3], and origin in the jaws is exceptionally rare [11]. The diagnosis is usually based on the finding of chronic hypophosphatemia due to isolated renal phosphate depletion. This findings in combination with concomitant elevated or disproportionately normal blood levels of FGF-23 along reduced or disproportionately normal

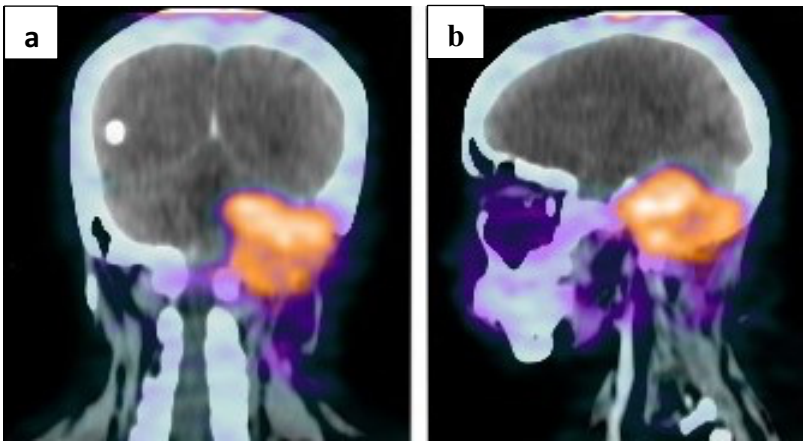

Fig. 1. Ga-68 labeled DOTATATE PET/CT scan (a) Fused coronal image shows avid mass. Occupying the left cerebellar hemisphere and infiltrating basiocciput (b) Fused sagittal image shows avidity in the same area.

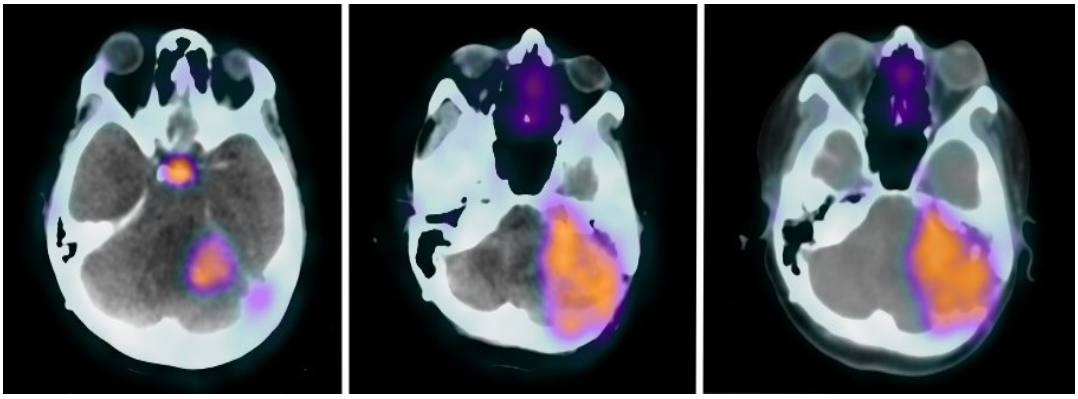

Fig. 2. Ga-68 labeled DOTATATE PET/CT scan shows uptake in the area of the left cerebellar hemisphere.

1,25-OH2-vitamin D (1,25(OH)2D) is hallmark of the diagnostic criteria [11].

This case describes the application of Ga-68 labeled DOTATATE scan that uniquely identified the primary site of PMT and excluded remotely located metastases. Localization of the site of the primary tumor is essential in the treatment of these tumors, as surgical removal of the primary tumors is often curative. Recent advances in SSTR-based functional imaging have supported the benefits of Ga-68-labeled DOTATATE scanning in tumors that over express somatostatin analogs. PMTs are known to express somatostatin receptor 2A and can therefore be visualized using radiolabeled somatostatin analogs such as In-111 pentetreotide and Ga-68 labeled DOTATATE. Ga-68-labeled DOTATATE images selectively identify SSTR2-avid tissue and are approved forclinical use. The In-111 pentetreotide scan is difficult to interpret unless SPECT-CT is performed to anatomically localize the uptake area, thus labeled Ga-68 is superior to this as tomography is inherent part of PET-CT and provides better resolution and sensitivity.

The somatostatin analogue, tyrosine 3-octreotate is combined with positron emitter (Gallium-68) using DOTATATE as a coupling agent. This Ga-68-labeled DOTATATE conjugate is administered intravenously, complexes with SSTR2, emits positrons detected by a PET scanner, and provides accurate and precise anatomical localization of the tumor. Ga-68-labeled DOTATATE scans are superior to other somatostatin receptor-based studies because they are highly sensitive, specific, accurately localize these tumors, have better spatial resolution, higher affinity for SSTR2, greater accuracy and shorter scan time, and less radiation exposure. However, a full body scan is required instead of the oncology standard body protocol used

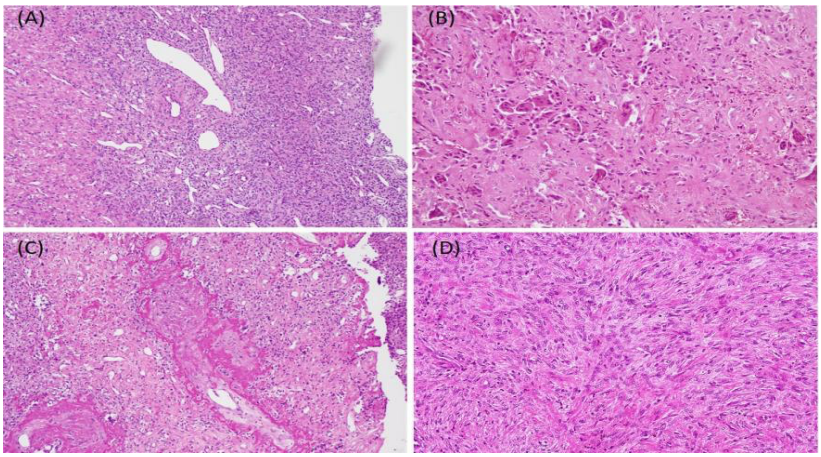

Fig. 3. (A) spindled, fusiform, hemangiopericytoma-like areas and (B) osteoclastic giant cells arranged in sheets. (C) There were also identifiable areas of calcific deposits and (D) sheets of bland spindle cells.

in most cases because PMTs can appear anywhere from head to toe.

Determining the exact location of the tumor is very challenging using conventional X-ray and nuclear medicine images, including MRI and PET/CT. False positive findings have been reported using 18F-FDG PET/CT imaging [7]. Hence use of 18F-FDG PET/CT has not gained wide acceptance in the evaluation of suspected PMT. The fact that SSTR is over expressed in mesenchymal tumors, favors use of Ga-68-labeled DOTATATE targeting SSTRs in these tumors [3]. In this report, we successfully identified a PMT (Fig.2)) using a Ga-68-labeled DOTATATE scan, which revealed a left intracranial mass avidity (SUVmax 4.9, 50×48 mm).

The accuracy of the Ga-68 labeled DOTATATE scan was also confirmed by histopathological analysis with benign features of a PMT. Microscopic findings revealed spindle-shaped, fusiform, hemangiopericytoma-like areas and osteoclastic giant cells arranged in sheets. There were also identifiable areas of calcium deposits and layers of bland spindle cells (Fig. 3), which are particularly characteristic of PMT [12]. In addition, immunohistochemical analysis was positive for STAT6 and SATB2, which are tumor markers frequently reported in PMT cases [9]. In our case, we used a Ga-68 DOTATATE scan to detect the exact anatomical location of the disease and metastatic workup. A primary site of disease was demonstrated and no metastases were detected, consistent with the patient's follow-up to date after disease-free surgical removal.

CONCLUSION

PMT is characteristically benign, extremely rare neoplasm and is known to be one of the most common causes of OIT. Identifying the location of the tumor is often very challenging using conventional radiologic techniques. This study reported the successful application of Ga-68 DOTATATE scan for identifying the primary site and exclude distant metastases of PMT that was further confirmed by histopathological analysis. The case shows the diagnostic value of Ga-68 labeled DOTATATE PET/CT imaging for rare tumors.

Informed Consent

Written informed consent was obtained from the patient for publication of this case report and any accompanying images or data.

Authors' Contribution

Study Conception: MNY; Study Design:MNY, WA, MF; Supervision: MNY, SF; Funding: N/A; Materials: SF, II, MK; Data Collection and/or Processing: WA, MK, MF; Statistical Analysis and/or Data Interpretation: WA, MK, MF; Literature Review: MK, MF, II; Manuscript Preparation: II, WA and Critical Review: SF, MNY.

Conflict of interest

The author disclosed no conflict of interest during the preparation or publication of this manuscript.

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REFERENCES

1. Ghorbani-Aghbolaghi A, Darrow MA, Wang T. Phosphaturic mesenchymal tumor (PMT): exceptionally rare disease, yet crucial not to miss. Autops Case Rep. 2017;7(3):32-37. doi: 10.4322/acr.2017.031.
2. Brandi ML, Clunie GPR, Houillier P, et al. Challenges in the management of tumor-induced osteomalacia (TIO). Bone. 2021;152:116064. doi: 10.1016/j.bone.2021.116064.
3. Lee DY, Lee SH, Kim BJ, et al. Usefulness of 68Ga-DOTATOC PET/CT to localize the culprit tumor inducing osteomalacia. Sci Rep. 2021;11(1):1819. doi: 10.1038/s41598-021-81491-2.
4. Moreno Romero M, Pérez Muñoz I, González Lizán F, Gallego Rivera JI, Valdivielso Cañas L. The phosphaturic mesenchymal tumor as a cause of oncogenic osteomalacia. Three cases and review of the literature. Rev Esp Cir Ortop Traumatol (Engl Ed). 2021:S1888-4415(21)00050-3. doi: 10.1016/j.recot.2020.12.004.
5. Qiu S, Cao LL, Qiu Y, et al . Malignant phosphaturic mesenchymal tumor with pulmonary metastasis: a case report. Medicine (Baltimore). 2017;96(17):e6750. doi: 10.1097/MD.0000000000006750.

6. Oyama N, Kojima-Ishii K, Toda N, et al. Malignant transformation of phosphaturic mesenchymal tumor: a case report and literature review. Clin Pediatr Endocrinol. 2020;29(2):69-75. doi: 10.1297/cpe.29.69.
7. El-Maouche D, Sadowski SM, Papadakis GZ, et al. 68Ga-DOTATATE for tumor localization in tumor-induced osteomalacia. J Clin Endocrinol Metab. 2016;101(10):3575-3581. doi: 10.1210/jc.2016-2052.
8. Fallahi B, Manafi-Farid R, Eftekhari M, et al. Diagnostic efficiency of 68Ga-DOTATATE PET/CT as compared to 99mTc-Octreotide SPECT/CT and conventional morphologic modalities in euroendocrine tumors. Asia Ocean J Nucl Med Biol. 2019;7(2):129-140. doi: 10.22038/AOJNMB.2019.39392.1263.
9. Chatterjee D, Bardia A, Pal R, Saikia UN, Bhadada SK, Radotra BD. Clinical, morphological and immunohistochemical analysis of 13 cases of phosphaturic mesenchymal tumor - A holistic diagnostic approach. Ann Diagn Pathol. 2021;54:151783. doi: 10.1016/j.anndiagpath.2021.151783.
10. Folpe AL, Fanburg-Smith JC, Billings SD, et al. Most osteomalacia-associated mesenchymal tumors are a single histopathologic entity: an analysis of 32 cases and a comprehensive review of the literature. Am J Surg Pathol. 2004;28(1):1-30. doi: 10.1097/00000478-200401000-00001.
11. Pelo S, Gasparini G, Garagiola U, et al. Phosphaturic mesenchymal tumor, an unusual localization in head and neck. J Surg Case Rep. 2018;2018(5):rjy091. doi: 10.1093/jscr/rjy091.
12. Richardson AL, Richardson OK. Phosphaturic mesenchymal tumor: case report. Radiol Case Rep. 2019;14(12):1518-1524. doi: 10.1016/j.radcr.2019.09.027.

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Primary pulmonary Ewing sarcoma: a rare case report

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ABSTRACT

Ewing sarcoma (ES) is a malignant soft tissue tumor that consists of undifferentiated neuroectodermal cells. The anatomical sites of ES are commonly the pelvis and long bones. Metastasis is the cause of the most prevalent pulmonary ES. The primary lung origin of ES is extremely uncommon. Here, we report a rare case of primary pulmonary ES diagnosed from cytology and biopsy material. A chest X-ray revealed a lesion with a 9×7.5 cm diameter in the pericardiac area of the right lung. Clinical and radiological examinations (computed tomography and positron emission tomography) demonstrated that the lesion was a primary lesion. No distant metastasis was detected. Bronchoscopy-guided fine-needle aspiration and cytological analysis of the lesion revealed uniformly shaped small round cell morphology. Immunohistochemistry performed on the cell block produced positive results for CD99 and FLI-1. These immunohistochemical findings support the ES diagnosis.
Keywords: Ewing sarcoma, primary pulmonary Ewing sarcoma, fine-needle aspiration, cytopathology

Ewing sarcoma (ES) is a malignant small round cell tumor that is commonly localized to the diaphysis and metaphysis of long bones. Extra-bone localization of ES is less frequently observed [1-3]. The first case of primary ES of the lung was described by Hammar *et al*. [4] in 1989, and since then, less than 40 cases have been reported in the literature [5]. It features a distinctive immunophenotype that combines diffuse and membranous CD99 expression with FLI-1 nuclear positivity [6]. Due to its extreme rarity, similar presentation to other lung tumors, and nonspecific symptoms, primary pulmonary ES can be challenging to distinguish from other pulmonary tumors [7]. Based on our best literature reviews, this is a quite rare case of ES where the diagnosis was made on fine needle aspiration cytology (FNAC) and cell block, with immunohistochemistry (IHC) [3]. Here, we presented a case of primary pulmonary ES, emphasizing its diagnosis from cytology and cell block.

CASE PRESENTATION

A 48-year-old non-smoker male was admitted with a five-month history of dry cough and no contributory past or family history. He denied any history of fever and dyspnoea.

Direct chest radiography revealed radio-opaque irregular margins mass in the para-cardiac area of the right lung (Fig. 1A). The thoracic-computed tomography (CT) scan revealed a 9 × 7.5 cm hypodense lesion that extended from the perihilar segment to the medial segment of the right middle lobe of the lung and there no chest wall invasion was detected (Fig. 2).

After such radiography and CT findings, a bron-

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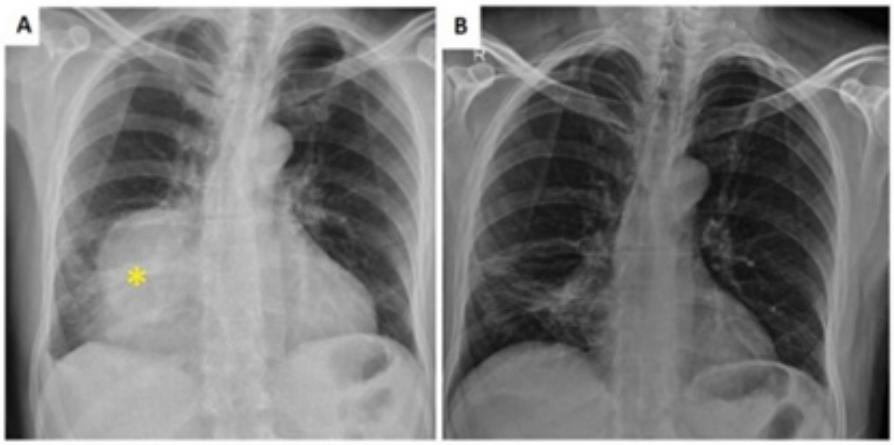

Fig. 1. **(A) A direct chest radiography revealed a radio-opaque mass in the pericardiac area of the right lung (asterisk). (B) After initiation of chemotherapy, a direct chest radiography demonstrated a response with only minimal residual disease.**

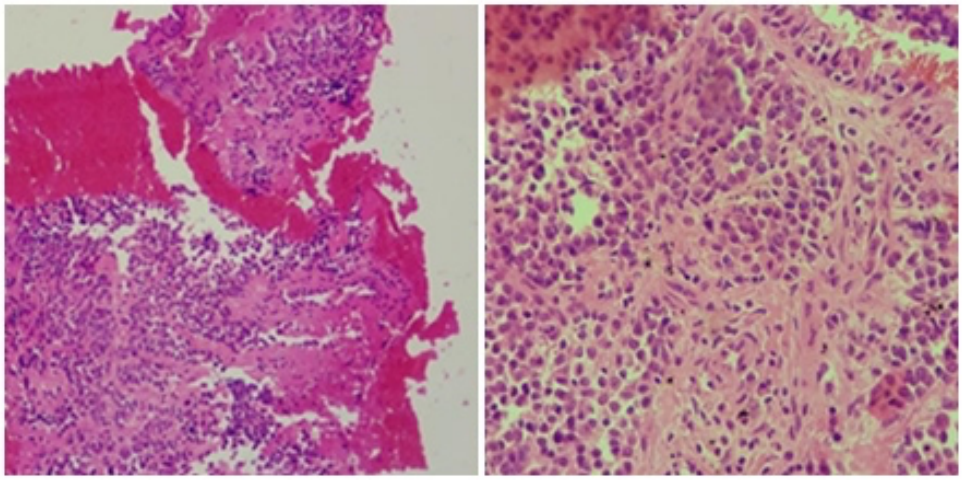

Fig. 3. **Cell block of the tumour (H&E) demonstrating atypical pleomorphic cell groups with small, round to oval nuclei and scant cytoplasm. There is respiratory epithelium in the right upper corner of the last picture. Magnification: 10× and 20×, respectively.**

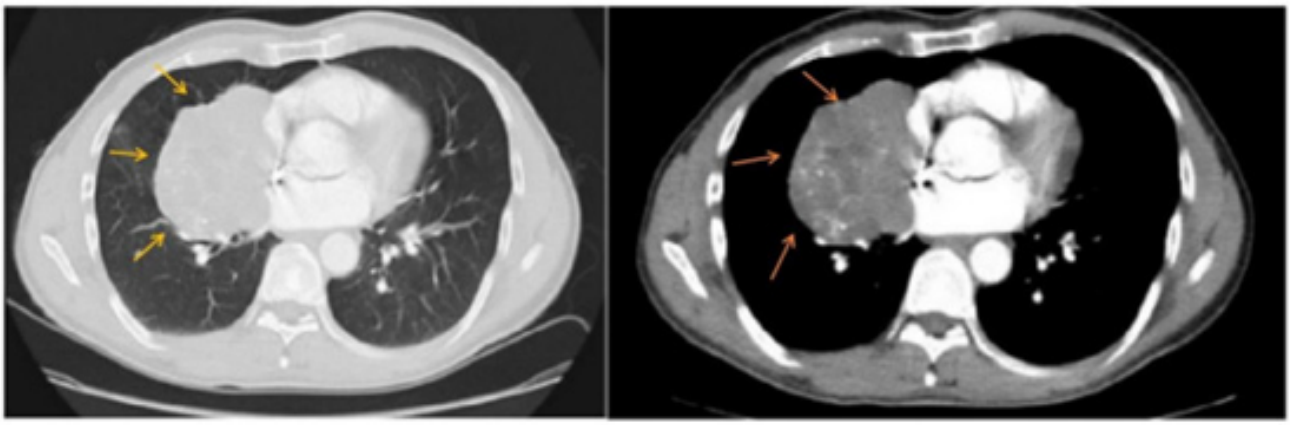

Fig. 2. **The thoracic-computed tomography (CT) scan revealed a 9 × 7.5 cm hypodense lesion that extended from the perihilar segment to the medial segment of the right middle lobe of the lung (arrows).**

choscopy detected that the middle lobe was almost completely occluded by a nodular infiltrative solid mass. From this location, FNAC, lavage, and forceps biopsy procedures were performed, and the smears were stained with hematoxylin-eosin (H&E). A cell block study was made with the remaining aspirate. The biopsy demonstrated a few atypical cells with enlargement and hyperchromatic oval nuclei with scant cytoplasm on the wide necrotic area. However, these biopsy sections were small and not diagnostic. FNAC and cell block showed atypical pleomorphic cell groups with small round to oval nuclei and scant cytoplasm was observed within the wide bleeding areas. A rosette-like pattern of arrangement was observed (Fig. 3).

Immunohistochemical analysis of the cell block revealed that the atypical cells were CD99 positive, FLI-1 focal nuclear positive, TTF-1 negative, pan CK negative, vimentin focal positive, Ki-67 positive, synaptophysin focal dot-like positive, LCA negative, CD56 negative, CD34 negative, and Melan-A negative (Figs. 4A-4F). Initial histological and immunohistochemical findings demonstrated that this was compatible with ES.

A new biopsy was attempted on the patient a month later. The repeated biopsy material was stained

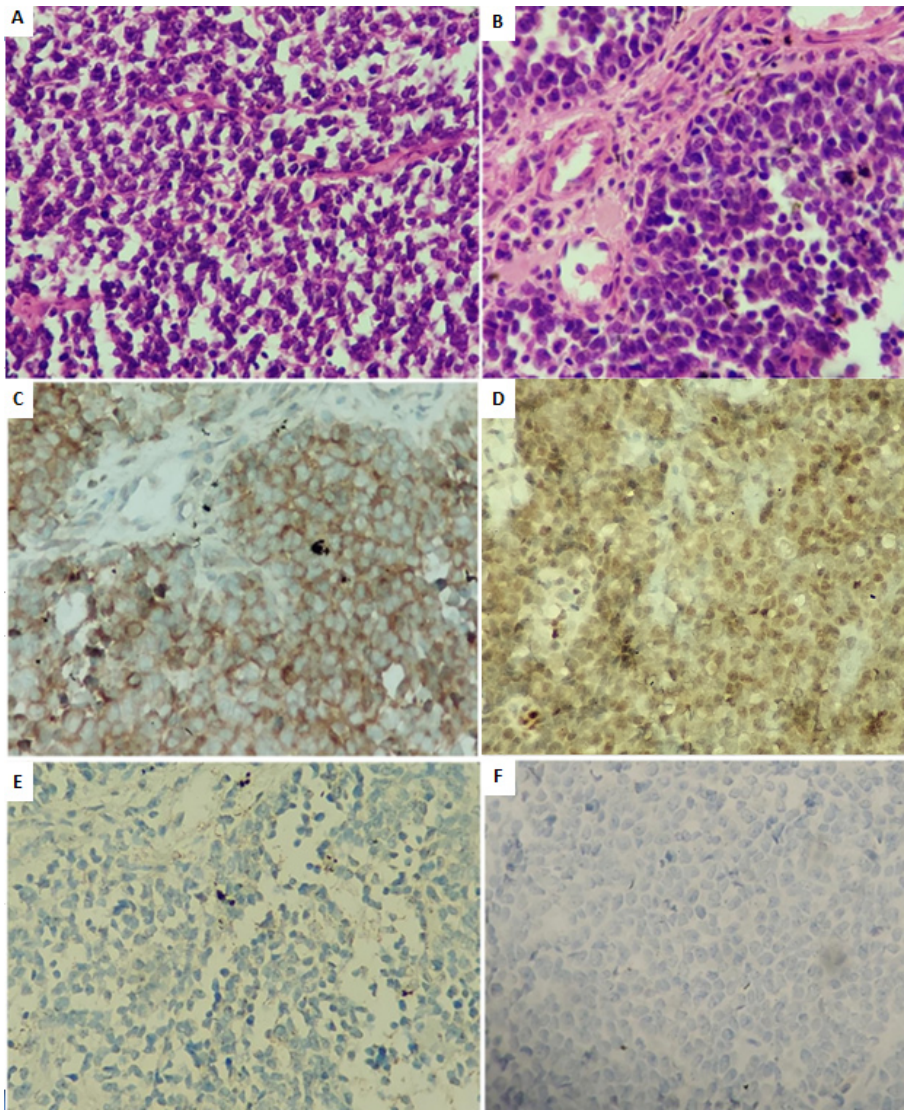

Fig. 4. (A, B) Cell block of the tumour (H&E). (C) Tumor cells showing diffuse membranous positivity for CD99. (D) Tumor cells showing focal nuclear FLI-1 positivity. (E) Immunohistochemical staining for pan CK showing negative staining. (F) Immunohistochemical staining for TTF-1 showing negative staining. Magnification: 20×, 20×, 40×, 40×, 40×, and 40×, respectively.

with H&E stains. A tumoral structure consisting of atypical cells with small round to ovoid cells, nuclear enlargement, hyperchromatic nuclei, and lots of mitotic figures was observed. A panel of IHC stains was performed; FLI1 diffuse nuclear positive, CD99 diffuse membranous positive which are characteristic findings of Ewing sarcomas, Ki-67 positive, TTF-1 negative, pan CK negative, S100 negative, synaptophysin negative, LCA negative. These findings were compatible with our previous definition of ES in the cell block and cytology material.

The patient thereafter underwent a positron emission tomography scan. As a result, neither evidence of another tumor that could be associated with the primary site nor distant metastasis was observed. The presence of any primary tumor in another focus was ruled out through radiological investigations. This confirmed the diagnosis of primary pulmonary ES. After the treatment plan, chemotherapy was initiated for the patient. After 2 months, a repeat of the chest X-ray showed a response with only minimal residual disease (Fig. 1B).

DISCUSSION

ES which were first described by James Ewing in 1921 is neuroectodermal tumors characterized by monotone small round cells arranged in sheets. ES is a rare sarcoma of bone and soft tissue and may involve any location. The most common anatomical sites of ES are the pelvis and long bones [2]. Metastasis is the cause of the most prevalent pulmonary ES. An extraskeletal primary source of this condition from the lung is extremely uncommon [1]. ES most often occurs in children and young adolescents [2].

In the differential diagnosis of pulmonary ES, small round cell malignant tumors, such as carcinoid, small cell carcinoma, desmoplastic small round cell tumor, malignant lymphoma, and poorly differentiated synovial sarcoma are included [8]. These neoplasms can be distinguished from other small round cell tumors in large part by the use of immunohistochemistry in combination with cytogenetics and molecular research [3]. On hematoxylin-eosin stain, diffuse sheets of small, round, blue cells with oval to round nuclei revealing finely dispersed chromatin and sparse, mildly eosinophilic or pale cytoplasm make up the typical histologic morphology of ES. The tumor is immunoreactive to CD99, FLI-1 [1]. In intractable cases, FLI-1 protein is more sensitive and specific and is convenient in confirming this diagnosis. Other markers (Chromogranin A, S-100 protein, CD56, Synaptophysin) are only rarely positive [9].

In this case, our patient had only a dry cough and a mass in the right lung on X-ray and CT imaging. FNAC and a cell block study showed atypical pleomorphic cell groups with small, round to oval nuclei and scant cytoplasm. When evaluating tumors with small round cell morphology presenting these histomorphological findings, ES should be included in the differential diagnosis.

To our current knowledge, extremely rare cases of ES are diagnosed by FNAC. Auxiliary tests are carried out on the cell block to establish a definitive diagnosis of ES. Most of the cases in the literature were diagnosed with tissue biopsies [3]. Here, we show that cytomorphology offers sufficient information and that IHC may be conducted on the cell block, raising the likelihood that the diagnosis is correct. We confirmed the diagnosis of ES with immunohistochemical studies performed on the rebiopsy material. After our diagnosis, the patient received chemotherapy and initially, most of his mass regressed without a surgical procedure.

CONCLUSION

Although primary pulmonary ES is a rare tumor, it should be considered in the differential diagnosis of pulmonary soft tissue tumors with small, round cell morphology. We reported a rare case of primary pulmonary ES with early detection from cytological material. This case emphasizes the importance of considering unusual differentials and the convenience of FNA with IHC techniques as the primary procedure in the diagnosis of such a rare case. With our diagnosis in the cytology material, the tumor largely regressed with chemotherapy without the need for surgery.

Authors' Contribution

Study Conception: N/A; Study Design: RA; Supervision: RA; Funding: N/A; Materials: HDT, ANT; Data Collection and/or Processing: HDT, ANT; Statistical Analysis and/or Data Interpretation: HDT,

ANT; Literature Review: HDT, ANT; Manuscript Preparation: RA, HDT, ANT and Critical Review: RA.

Conflict of interest

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REFERENCES

1. Sohn AJ, Lang B, McCarroll M, Agarwal A. Primary pulmonary Ewing sarcoma/peripheral primitive neuroectodermal tumor. Proc (Bayl Univ Med Cent). 2020;33(4):646-648. doi: 10.1080/08998280.2020.1798723.
2. Wu HH, Wingfield-Digby J, Jifon G. Primary Ewing sarcoma of the lung: a challenging case. J R Coll Physicians Edinb. 2020;50(2):141-143. doi: 10.4997/JRCPE.2020.212.
3. Dharmalingam P, Nargund A, MukundaPai M, Patil Okaly GV. Primary Ewing sarcoma of the lung diagnosed on cytology: case report with review of literature. Diagn Cytopathol. 2020;48(11):1098-1101. doi: 10.1002/dc.24458.
4. Hammar S, Bockus D, Remington F, Cooper L. The unusual spectrum of neuroendocrine lung neoplasms. Ultrastruct Pathol. 1989;13(5-6):515-560. doi: 10.3109/01913128909074534.
5. Fan YH, Ma HX, Guo SP, Chen Y, Zhang SP. Application of anlotinib combined with neoadjuvant chemotherapy in primary EWS/PNET of lung: a case report. Front Oncol. 2022;12:822469. doi: 10.3389/fonc.2022.822469.
6. Zhang J, Dong A, Cui Y, Wang Y. FDG PET/CT in a case of primary pulmonary Ewing sarcoma. Clin Nucl Med. 2019;44(8):666-668. doi: 10.1097/RLU.0000000000002659.
7. Öztürk S, Kurtulus Ozturk E, Isiksalan Ozbulbul N, Acu B, Dundar E. Primary Extraosseous Ewing's Sarcoma of the Lung: Radiologic and Pathologic correlation. Cureus. 2021;13(5):e14830. doi: 10.7759/cureus.14830.
8. Mizuguchi K, Minato H, Onishi H, Mitani Y, Kawai J. Cytopathological findings of primary pulmonary Ewing family of tumors with EWSR1 translocation: a case report. Thorac Cancer. 2016;7(5):602-606. doi: 10.1111/1759-7714.12347.
9. Andrew F, Nielsen GP (eds.). Bone and Tissue Pathology: A Volume in the Foundations in Diagnostic Pathology Series. 2nd ed. Elsevier Health Sciences, 2022.

The European Research Journal 2024;10(1):154-156 Case Report

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Type 2 biliary perforation successfully managed with early insertion of self-expandable metal stent

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ABSTRACT

Perforations after endoscopic retrograde colangiopancreatography (ERCP) are divided into four types. Type 1 refers to the duodenum, type 2 the periampullary region, type 3 the bile duct itself, and type 4 the observation of retroperitoneal free air on cross-sectional imaging. The treatment of type 2 biliary perforations remains debatable. We present a patient who was successfully treated with a self-expandable metal stent (SEMS) without the need for surgery or any complications.

Keywords: Biliary, perforation, self-expandable metal stent

Perforations after endoscopic retrograde colangiopancreatography occur between 0.3% and 1% and cause mortality between 16% and 18% [1]. They are divided into four types according to Stapfer *et al*. [2]. According to the affected side, type 1: duodenal wall (directly with duodenoscope); type 2: periampullary region (commmonly after sphincterotomy); type 3: distal bile duct (related to wire or basket instrumentation); and type 4: retroperitoneal free air with no true perforation [2]. Surgery is generally performed for type 1 perforations and conservative, endoscopic treatment for type 3 and 4 perforations. However, for type 2 perforations, which patients should be treated surgically or endoscopically is still controversial [3].

CASE PRESENTATION

An 81-year-old patient presented to the Emergency Department with epigastric colic pain a week ago. In the measured blood values, alkaline phosphatase: 331 mg/dl, gama glutamile transferase: 462 mg/dL, alanine aminotransferase:70U/L, aspartate aminotransferase: 67U/L, total bilirubin: 0.7 U/L, complete blood count was normal. In abdominal ultrasound and computerized tomography (CT), intrahepatic bile ducts and common bile duct (CBD-13 mm) were dilated and magnetic resonance colangiography was recommended (MRCP). The MRCP revealed prominent intrahepatic bile ducts and dilated common bile ducts with suspicious millimeter filling defects in the lower portion. ERCP was performed in symptomatic patients with elevated cholestatic enzymes. CBD was selectively cannulated. Cholangiography shows a considerably dilated biliary tree and the lower end of CBD noticeably narrowed. A proper sphincterotomy was carried out. Subsequently, the CBD was swept using a balloon catheter. Instead of stones, mucosal particles with papillary projections were extracted from CBD. Specimens were sent to pathology, and a 10 cm, 7 F plastic stent was implanted. Epigastric pain with radi-

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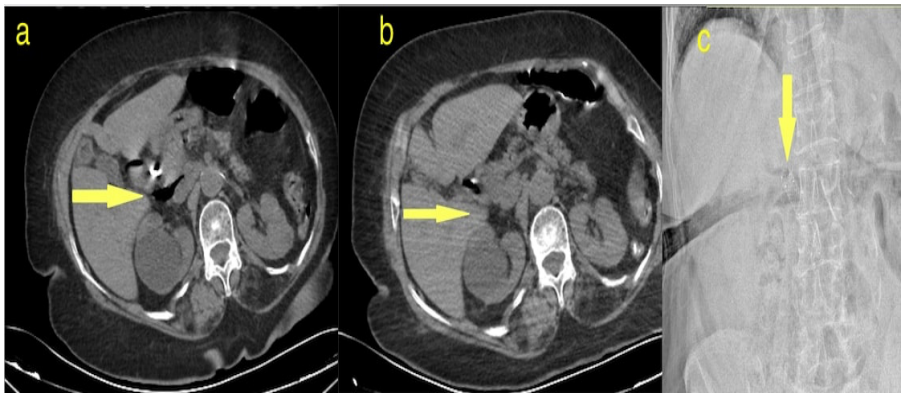

Fig. 1. (a) Type 2 perforation with free air in the anterior side of the right kidney and the inferior vena cava. CT (b) and plain radiograph (c) of perforation resolution 72 hours after SEMS insertion.

ation to the right lomber area reoccurs in the morning after the procedure on analgesics. An urgent CT scan was performed. Free air images were observed in the anterior side of the right kidney and the inferior vena cava. (Fig. 1). At the 15th hour after intervention, a 60 mm long, 10 mm wide self-expandable metal stent was placed in CBD due to clinically and radiologically confirmed type 2 biliary perforation (Fig. 2). A nasogastric tube was also inserted, and parenteral fluid support with broad-spectrum antibiotic therapy was started. The pain was immediately relieved after the metal stent was implanted. The resolution of free air was observed in the 72nd hour CT (Fig. 1). There was no significant increase in leukocyte or C reactive protein levels. The patient was discharged after enteral feeding was successfully initiated. Pathology revealed that the patient had an intraampullary tubular neoplasm with high grade dysplasia. Outpatient surgery was recommended for the patient.

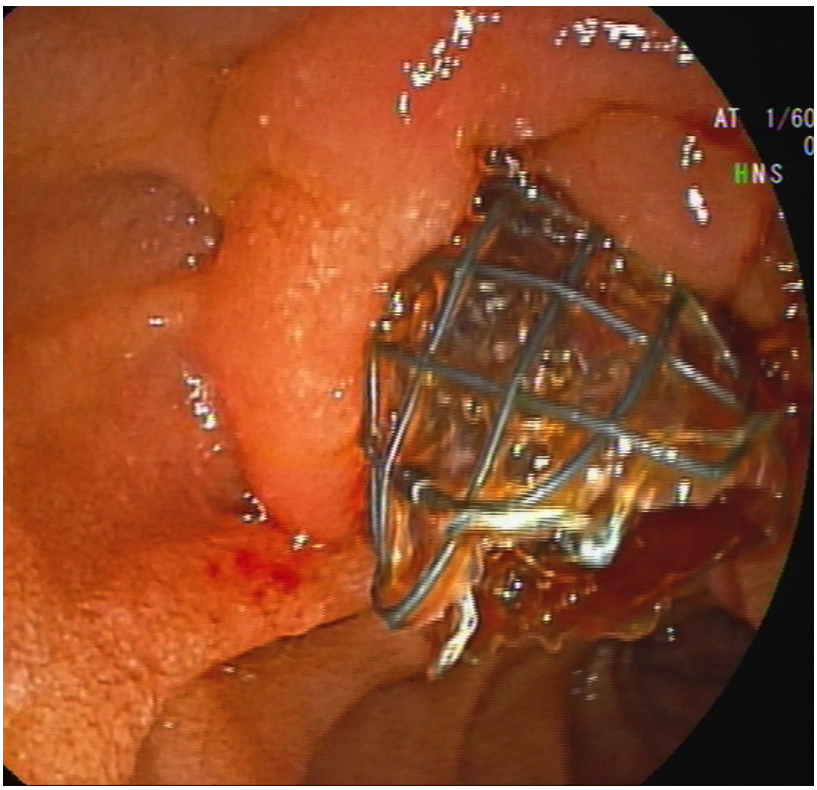

Fig. 2. Endoscopic view of a metallic stent.

DISCUSSION

In recent years, SEMS has been widely used to treat type 2 biliary perforations. Odemis *et al*. [4] compared nasobiliar drainage and SEMS in their study, they found that analgesic use, hospital stay was decreased, and leukocytosis was seen less in patients with SEMS. Type 2 perforations are not very symptomatic due to their retroperitoneal location; therefore, they are unlikely to be detected at the time of intervention. According to the current study, there was no significant difference in terms of surgery, percutaneous intervention, and mortality when a metallic stent was inserted simultaneously during the procedure or 7 to 48 hours after detection of perforation [5]. At the 15th hour of perforation detection, SEMS was successfully inserted into the common bile duct in our patient. The pain was immediately relieved after insertion, and laboratory values did not worsen. And patient was successfully discharged.

CONCLUSION

SEMS can be safely used in type 2 perforations, significantly reducing the patient's likelihood of surgery and mortality.

Informed Consent

Written informed consent was obtained from the patient for publication of this case and any accompanying pictures or data.

Authors' Contribution

Study Conception: İK; Study Design: İK; Supervision: İK; Funding: N/A; Materials: İK; Data Collection and/or Processing: İK; Statistical Analysis and/or Data Interpretation: İK; Literature Review: İK; Manuscript Preparation: İK and Critical Review: İK.

Conflict of interest

The author disclosed no conflict of interest during the preparation or publication of this manuscript.

Financing

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REFERENCES

1. Sarli L, Porrini C, Costi R, et al. Operative treatment of periampullary retroperitoneal perforation complicating endoscopic sphincterotomy. Surgery. 2007;142(1):26-32. doi: 10.1016/j.surg.2007.02.002.
2. Stapfer M, Selby RR, Stain SC, et al. Management of duodenal perforation after endoscopic retrograde cholangiopancreatography and sphincterotomy. Ann Surg. 2000;232(2):191-198. doi: 10.1097/00000658-200008000-00007.
3. Tovar FG, Mendoza SJ, Velandia CC, López L C. [Case report of rescue therapy for bile duct perforation using fully covered self-expanding metal stent after failure of conservative management]. Rev Colomb Gastroenterol 2018;33:74-79. [Article in Spanish]
4. Odemis B, Oztas E, Kuzu UB, et al. Can a Fully Covered Self-Expandable Metallic Stent be Used Temporarily for the Management of Duodenal Retroperitoneal Perforation During ERCP as a Part of Conservative Therapy? Surg Laparosc Endosc Percutan Tech. 2016;26(1):9-17. doi: 10.1097/SLE.0000000000000240.
5. Bozbiyik O, Cetin B, Gumus T, Tekin F, Uguz A. Fully covered self-expandable metal stent for intraprocedural or late-diagnosed Type-II endoscopic retrograde cholangiopancreatography-related perforations. BMC Gastroenterol. 2022;22(1):385. doi: 10.1186/s12876-022-02466-9.