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Identification of novel potential molecular targets associated with pediatric septic shock by integrated bioinformatics analysis and validation of *in vitro* septic shock model

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Ethics Committee Approval
The study is a bioinformatics study and raw data was used. The database for which we use the data, Gene Expression Omnibus (GEO), is a public database and therefore ethical approval is not required.

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

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Abstract

Background/Aim: Sepsis is a major cause of morbidity, mortality, and healthcare utilization among children all over the world. Sepsis, characterized as life-threatening organ failure, results from a dysregulated host response to infection. When combined with critically low blood pressure, it causes septic shock, resulting in high mortality rates. The aim of this study was to perform a bioinformatic analysis of gene expression profiles to predict septic shock risk.

Methods: Four datasets related to pediatric septic shock were retrieved from the Gene Expression Omnibus (GEO) database for a total of 240 patients and 83 controls. GEO2R tools based on R were used to find differentially expressed genes (DEGs). The Database for Annotation, Visualization and Integrated Discovery (DAVID) was used to examine the functional enrichment of DEGs. STRING was used to create a protein–protein interaction (PPI) network. After separately analyzing the four datasets, commonly affected genes were removed using the Venny program. Finally, human umbilical vein endothelial cells (HUVECs) were stimulated with supernatants of lipopolysaccharide (LPS)-stimulated RAW267.4 macrophage cells and expression of selected genes was confirmed by real-time reverse-transcriptase polymerase chain reaction (qRT-PCR) and used to construct an *in vitro* septic shock model.

Results: Seven-hundred seventy-one common differentially expressed genes in the four groups were found. Of these, 433 genes showed increased expression, while 338 had reduced expression. In the DAVID analysis results, DEGs up-regulated according to gene ontology results were enriched in the regulation of innate and adaptive immune responses, complement receptor-mediated signaling, and cytokine secretion processes. Down-regulated DEGs were significantly enriched in the regulation of immune response, T-cell activation, antigen processing, and presentation and integral component of plasma membrane processes. According to The Search Tool for the Retrieval of Interacting Genes/Proteins (STRING), Cystoscape Molecular Complex Detection (MCODE), nine down-regulated genes in the center of the PPI network, ZAP70, ITK, LAT, PRKCQ, LCK, IL2RB, FYN, CD8A, CD247 and four up-regulated genes, MMP9, TIMP1, LCN2, HGF, were associated with septic shock. Expressions of FYN and MMP9 genes in the *in vitro* septic shock model were consistent with the bioinformatic results.

Conclusion: Comparative bioinformatics analysis of data from four different septic shock studies was performed. As a result, molecular processes and important signal networks and 13 genes that we think will play a role in the development and risk prediction of septic shock are proposed.

Keywords: Pediatric sepsis, Septic shock, Bioinformatics, Hub gene, Biomarker, Differentially expressed genes

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Introduction

Sepsis is a major cause of morbidity, mortality, and healthcare utilization among children all over the world. Globally, 22 pediatric sepsis cases occur per 100,000 people annually, and 2,202 neonatal sepsis cases occur per 100,000 live births. This number is equivalent to 1.2 million pediatric cases annually [1]. Sepsis is defined as a life-threatening disease that causes septic shock and organ dysfunction by producing a systemic inflammatory response. Despite breakthroughs in antibiotic treatment, immunotherapy, and resuscitative treatments, sepsis remains the leading cause of mortality in critical care units [2, 3]. Despite many studies in the field of sepsis treatment in past years, studies directly targeting sepsis, those addressing treatments to ensure amelioration, and preclinical studies have not been promising. Generally, the current strategy chosen for sepsis treatment involves targeting the infective pathogen in sepsis rather than patient response. Therapeutic choices for sepsis are limited because the responsible pathogenic mechanisms are still not fully understood [4]. Septic shock is a homogeneous disease without a single cause but rather enters a broad heterogeneous disease classification that includes subclasses of several diseases. The subclass definitions of septic shock are clinically significant as they may have clear effects for design of potentially targeted treatments [5]. Studies were performed concerning the subclassifications of patients with septic shock using biological markers found in serum. Considering the complexity of septic shock, definitions of new biomarkers in biological terms is very important [6].

Microarray analysis is a broad-scope technique that can analyze all identified transcripts concurrently instead of analyzing single gene expression. The basic aim of experiments involving gene expression is to determine genes displaying different behaviors under different conditions and to identify a reliable measurement for these differentially expressed genes. Genome scale association studies use the integration method to discover new gene sets [7]. Consolidation analysis, which collects information from different studies about the same topic to reveal guiding results about genes and pathways commonly targeted in disease, has more statistical power than analyses based on a single study. Additionally, this technique is used to reveal disease subtypes, predict survival, and discover biomarkers and treatment targets in gene expression studies [8, 9].

To date, several studies about determining molecular signatures in pediatric septic shock patients have been performed. Within the scope of these studies, research has been performed to determine molecular signatures with many patients and controls by analyzing studies performed involving septic shock patients and healthy controls. Early diagnosis and accurate prognostic prediction of septic shock are very important in terms of successful disease treatment. In this context, gene expression is considered an important tool to fill a gap in the complicated network of septic shock treatment [8, 10]. The aim of this study was to determine the common role of differentially expressed genes (DEGs) in pediatric sepsis, to provide specific information for clinical sepsis treatment in children, and to research potential therapeutic targets and biomarkers during sepsis development using comprehensive bioinformatics analyses from four different datasets.

Materials and methods

Bioinformatic analysis of microarray data:

In our research, studies including all pediatric septic shock expression profiles from the publicly available functional genomic Gene Express (GEO) database were queried and four datasets (GSE26440, GSE9692, GSE26378, GSE8121) were used (http://www.ncbi.nlm.nih.gov/geo). The sample numbers in each dataset are listed: (1) GSE26440 includes 98 cases and 32 controls, (2) GSE9692 includes 30 cases and 15 controls, (3) GSE26378 includes 82 cases and 21 controls, and (4) GSE8121 includes 30 cases and 15 controls. All profiles were based on GPL570 Affymetrix Human Genome U133 Plus 2.0 Array. For bioinformatics analysis of studies performed with blood samples taken from pediatric septic shock patients and control groups, the R-based GEO2R software (http://www.ncbi.nlm.nih.gov/geo/geo2r) was used. To ensure a balance between the discovery of statistically significant genes and adjusted P value calculations to obtain |log2 (Fold Chance (FC) |, GEO2R was used to calculate adjusted P values (adjP) and Benjamini–Hochberg false discovery rates. Genes with $P < 0.001$ and $|\log2FC| \geq 1$ were accepted as DEGs. Later, overlapping results were created from different gene lists using a Venn diagram (https://bioinfogp.cnb.csic.es/tools/venny/).

Identification of DEGs: The Database for Annotation, Visualization and Integrated Discovery (DAVID) is a web-based gene function enrichment analysis software and is the main bioinformatics tool for analyzing the biological processes associated with DEGs. Statistical significance for DEGs and gene ontologies (GO) were found using DAVID. GO is a large and widely used database for categorizing gene functions into biological processes (BP), molecular functions (MF), and cell components (CC). The Kyoto Encyclopedia of Genes and Genomes ([KEGG]http://www.kegg.jp/) is a genome encyclopedia combining genomic information with more high-grade functional information to identify enriched biological paths to a significant degree. Significant genes and pathways (increasing or decreasing) with $p < 0.05$ were analyzed using gene enrichment and KEGG pathway analyses.

Protein–protein interaction analysis and Hub Gene Identification: STRING (http://string-db.org,version 11.0) is an online database providing information about protein–protein interactions (PPIs) via an analysis of functional networks between two or more proteins. The highest reliability point of 0.9 was used for interaction networks and nodes without interactions were removed. Cytoscape (version 3.8.2) software is an open access bioinformatics platform created to visualize PPI networks. Network analysis was performed by applying the Cystoscape Molecular Complex Detection (MCODE, version 1.5.1), an application within Cytoscape.

Validation of microarray results

Cell culture

In our study, two cell lines, macrophage RAW267.4 cells and human umbilical veil endothelial cells (HUVECs) were used, and the cells were obtained from the American Type

Culture Collection (ATCC) cell bank. RAW267.4 cells were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium containing 10% FBS (Hyclone) and 100 U/mL of penicillin and streptomycin (Gibco). HUVECs were grown in F12 medium (Gibco), which contained 15% FBS, antibiotics, and 30 g/mL EGFS (Sigma). Cell counts were performed on a thoma slide using trypan blue to seed 1x105 viable cells in each well before seeding into plates.

Cell viability assay: The cell viability of RAW264.7 cells after stimulation was determined using the tetrazolium (MTT) test. MTT dye was dissolved in RPMI 1640 medium to a final concentration of 2 mg/ml. After discarding the medium on the cells, 20 µl of MTT solution and 100 µl of medium were added to each well in a 96-well plate and incubated at 37 °C for 1 h in a CO_2 incubator. Absorbance was measured at 550 and 690 nm using a spectrophotometer.

***In vitro* septic shock cell model:** The septic shock cell model consisted of two steps. In the first step, the Raw267.4 cells were seeded in 6- and 96-well plates and stimulated with 10 µg/ml lipopolysaccharide (LPS) based on studies in the literature. The cells were incubated for 4, 8, and 24 h, and the culture supernatant was collected at specified hours (containing various factors secreted by cells in response to stimulation). In the second step, HUVECs were seeded in 96- and 6-well plates at a concentration of 1 x 10^5 cells and incubated for one night.HUVEC medium was then cultured with the supernatants of LPS-stimulated macrophage cells and incubated for 0, 12, 24, and 48 h. The cells were isolated and stored at –80 ºC.

Isolation of RNA and qRT-PCR

In the study, the expression of MMP9 and FYN genes was quantitatively analyzed using the real-time reverse-transcriptase polymerase chain reaction (qRT-PCR) method. Trizol was used to extract total RNA from cells (Invitrogen). Reverse transcriptase was used to synthesize complementary DNA (Roche). qRT-PCR experiments were carried out using SYBR Green PCR Master Mix (Roche). The expression of glyceraldehyde 3-phosphate dehydrogenase (GAPDH) mRNA was used as an endogenous control for normalizing mRNA expression levels. Matrix metallopeptidase 9 (MMP9) with upregulated expression and FYN Pro-to-Oncogene, Src Family Tyrosine Kinase (FYN) with downregulated expression were chosen. Primers were used as in the references provided [11–13].

Statistical analysis

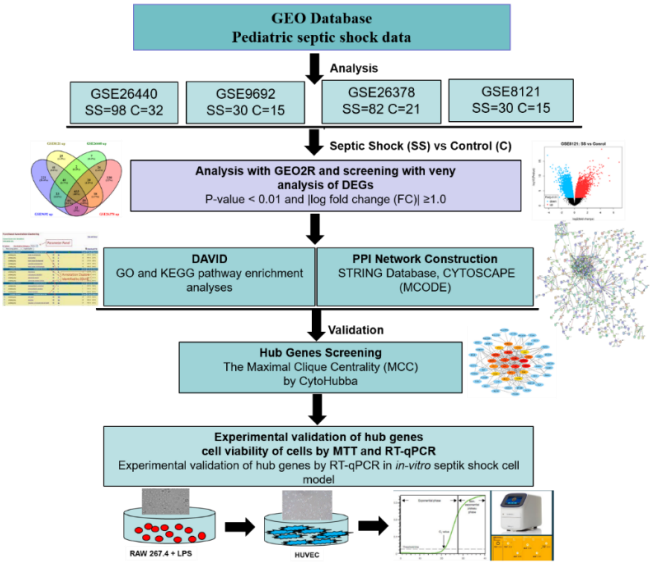
All results were presented as the mean standard error (SE) of three separate experiments. For statistical analysis, a one-way analysis of variance (ANOVA) test or an unpaired t-test was used. All statistical tests were two-tailed with a $P < 0.001$ threshold.

Results

Pre-processing of microarray data

The gene expression data for GSE26440, GSE9692, GSE26378, and GSE8121 were downloaded from the GEO general functional genomic database with the Affymetrix human genome U133 Plus 2.0 platform. Genes with *P*-value < 0.001 and |log2FC| ≥ 1 were chosen. A large group was created, with n = 240 patients and n = 83 controls (Figure 1).

Figure 1: Flow diagram of the bioinformatics analyses performed in this study (SS: Septic Shock, C: Control).



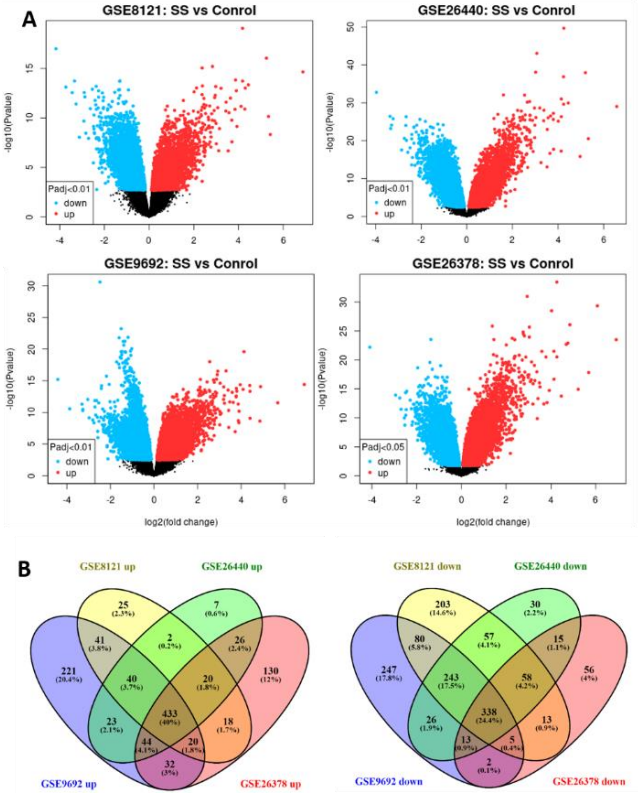
Identification of DEGs

In GSE26440 data, a total of 1375 genes had changed, with 595 genes showing increased expression and 780 genes showing decreased expression. For GSE9692 data, 1808 genes were differentially expressed, 854 of which were upregulated and 954 of which were downregulated. For GSE26378 data, the expression of 1223 genes had changed, with 723 upregulated genes and 500 downregulated genes. For GSE8121 data, expression differed for 1596 genes, with 599 upregulated and 997 downregulated genes (Table 1 and Figure 2A–B).

Table 1: Genes count with differentially expressed after bioinformatics analysis for comparison of between groups.

GEO Access Number	Number of DEGs	Upregulated gene number	Downregulated gene number
GSE26440	1375	595	780
GSE9692	1808	854	954
GSE26378	1223	723	500
GSE8121	1596	599	997

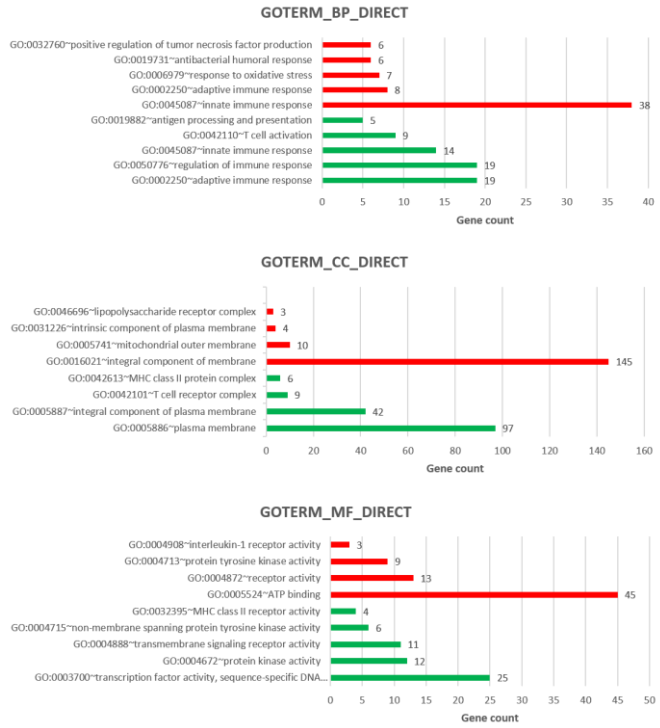
Figure 2: Venn diagram and volcano diagram of DEGs. A: Volcano diagram of 4 datasets. B: DEGs were selected with a |log2FC ≥ 1| and $P < 0.001$ among above 4 mRNA expression profiling sets datasets. The 4 datasets showed an overlap of 771 genes (433 upregulated, 338 downregulated).



After separately analyzing the four datasets, the commonly affected genes were identified using the Venny program. Seven-hundred seventy-one genes changed among the four datasets. Of these, 433 gene showed an increase in expression, and 338 had a reduction in reduced expression ($P < 0.001$).

GO Function and KEGG pathway analysis with DAVID: To better understand the functions and mechanisms of the DEGs identified after analysis of microarray data, GO and KEGG pathway analyses were completed for genes with increasing or decreasing expression levels. In GO analyses, clusters with enrichment score above 1.3 were assessed as significant clusters. According to this assessment, enrichment was observed for 27 clusters with up-regulated expression and 16 clusters with down-regulated expression based on the GO analysis of genes with common changes in the four studies. According to GO for DAVID analysis results, DEGs were collected from CC, especially in plasma membrane (97 genes) and integral membrane components (187 genes). Significant enrichment for BP in innate and adaptive immune responses and regulation of immune response (84 genes) processes and for MF in transcription factor activity, sequence-specific DNA binding (25 genes), and ATP binding (45 genes) processes was found. The GO functional enrichment analysis of up-regulated and down-regulated DEGs is shown in Figure 3 ($P < 0.001$).

Figure 3: GO functional enrichment analysis of downregulated DEGs ($P < 0.001$). The red and green colors represent upregulation and downregulation, respectively (BP indicates biological process; CC, cellular component; MF, molecular function).



For the common differentiated genes obtained from the four datasets, KEGG pathway analysis found that upregulated DEGs were basically related to metabolic pathways, neutrophil extracellular trap formation, osteoclast differentiation, transcriptional mis-regulation in cancer, and complement and coagulation cascades pathways. Additionally, the down-regulated DEGs revealed significant degrees of enrichment in a total of 10 pathways led by T-helper cells (Th) 1, 2, and 17 cell differentiation, hematopoietic cell lineage, T-cell receptor signaling pathway, and cytokine–cytokine receptor interaction pathways ($P < 0.001$) as shown in Table 2.

Table 2: KEGG pathway analysis of DEGs associated with septic shock using DAVID.

A. KEGG Pathways of Upregulated Genes

Term	Count	*P*-value	Genes
hsa01100:Metabolic pathways	55	0.005	CDA, GALNT14, PYGL, ENO1, OPLAH, HK3, SPTLC2, IMPA2, NAMPT, CA4, MAN1A1, UPP1, GYG1, ENTPD1, G6PD, DGAT2, ACSL1, ARG1, ACSL4, PGD, CHIT1, PKM, ACOX1, B3GNT5, ST6GALNAC3, BCAT1, B4GALT5, PFKFB2, PFKFB3, MGST1, ADCY3, PLD1, CYP19A1, GNS, PGS1, UGCG, FUT7, ALOX5, GPAT3, ATP6V1C1, OLAH, MGAM, GK, GALNT2, GSR, MBOAT2, BST1, VNN1, DHRS9, TBXAS1, ACER3, LPCAT2, ALPL, HPSE, GCLM
hsa04613:Neutrophil extracellular trap formation	15	0.001	CR1, ITGAM, SIGLEC9, AQP9, NCF4, C5AR1, FPR1, FPR2, MAPK14, MPO, TLR8, PADI4, TLR4, ELANE, TLR2
hsa04380:Osteoclast differentiation	14	0.001	LILRA6, SPI1, IL1R1, IFNGR1, NCF4, LILRB3, MAPK14, LILRA3, LILRA5, FOSL2, OSCAR, SOCS3, SIRPA, MAP2K6
hsa05202:Transcriptional misregulation in cancer	13	0.003	SPI1, ITGAM, BCL2A1, GADD45A, DEFA4, IL1R2, LMO2, MPO, MMP9, CCNA1, BCL6, CD14, ELANE
hsa04610:Complement and coagulation cascades	12	0.001	C1QB, C1QA, SERPINA1, CR1, ITGAM, SERPINB2, C5AR1, C3AR1, CD59, VSIG4, CD55, F5
hsa04621:NOD-like receptor signaling pathway	11	0.018	AIM2, DEFA4, NAMPT, PRKCD, CARD6, CARD16, NLRC4, TXN, MAPK14, TLR4, NAIP
hsa05150:Staphylococcus aureus infection	9	0.002	C1QB, C1QA, ITGAM, DEFA4, C5AR1, FPR1, C3AR1, FPR2, FCAR
hsa04640:Hematopoietic cell lineage	9	0.003	CSF3R, CR1, ITGAM, IL1R1, IL1R2, CD59, CD14, CSF2RA, CD55
hsa04620:Toll-like receptor signaling pathway	8	0.016	LY96, TLR8, CD14, MAPK14, TLR5, TLR4, MAP2K6, TLR2
hsa04064:NF-kappa B signaling pathway	8	0.016	BCL2A1, IL1R1, GADD45A, TRIM25, LY96, CD14, CFLAR, TLR4

B. KEGG Pathways of Downregulated Genes

Term	Count	*P*-value	Genes
hsa04658:Th1 and Th2 cell differentiation	19	0.001	MAML2, NFATC2, CD3G, GATA3, CD3E, RUNX3, CD3D, ZAP70, HLA-DMB, LCK, IL2RB, HLA-DPB1, STAT4, PRKCQ, CD247, HLA-DOA, HLA-DOB, LAT, HLA-DPA1
hsa04659:Th17 cell differentiation	18	0.001	NFATC2, RORA, CD3G, GATA3, CD3E, CD3D, IL27RA, ZAP70, HLA-DMB, LCK, IL2RB, HLA-DPB1, PRKCQ, CD247, HLA-DOA, HLA-DOB, LAT, HLA-DPA1
hsa04640:Hematopoietic cell lineage	17	0.001	MME, ITGA4, FLT3LG, CD3G, CD1C, CD3E, CD3D, CD2, HLA-DMB, CD8A, HLA-DPB1, ITGA6, HLA-DOA, IL7R, MS4A1, HLA-DOB, HLA-DPA1
hsa04660:T cell receptor signaling pathway	14	0.001	ITK, NFATC2, CD3G, CD3E, RASGRP1, CD3D, ZAP70, LCK, CD8A, CD28, FYN, PRKCQ, CD247, LAT
hsa04060:Cytokine-cytokine receptor interaction	13	0.002	CX3CR1, IL27RA, CCL5, IL2RB, TNFRSF17, XCL1, CD27, ACKR3, TNFRSF25, CCR7, CCR6, IL7R, CCR3
hsa04650:Natural killer cell mediated cytotoxicity	11	0.001	ZAP70, LCK, KLRC3, SH2D1A, SH2D1B, PRF1, NFATC2, KLRD1, FYN, CD247, LAT
hsa04514:Cell adhesion molecules	11	0.001	CD2, HLA-DMB, CD6, ITGA4, CD8A, HLA-DPB1, CD28, ITGA6, HLA-DOA, HLA-DOB, HLA-DPA1
hsa04064:NF-kappa B signaling pathway	9	0.001	CYLD, ZAP70, LCK, TRAF5, BLNK, BCL2, PRKCQ, ATM, LAT
hsa04612:Antigen processing and presentation	9	0.001	CD74, HLA-DMB, CD8A, KLRC3, HLA-DPB1, KLRD1, HLA-DOA, HLA-DOB, HLA-DPA1
hsa04062:Chemokine signaling pathway	9	0.011	CX3CR1, TIAM1, ITK, CCL5, XCL1, CCR7, CCR6, PRKACB, CCR3

Protein-–protein interaction (PPI) analysis

To research the molecular pathogenesis of septic shock, PPIs of the common differentiating genes were researched using gene expression profiling data with multiple bioinformatics methods, including gene enrichment analysis and PPI analysis. The STRING online database was used to create the PPI network using a total of 771 common DEGs (433 up-regulated, 338 down-regulated), which was analyzed by choosing the high reliability points of 0.9 for the interaction network, and then transferred to Cytoscape. The PPI network for the common down-regulated DEGs was visualized by Cytoscape and identified by the MCODE macro, an important module in Cytoscape and MCODE score >3 was chosen. Analysis of down-regulated genes found 149 edges and 64 key nodes. Three clusters with high MCODE score were investigated and hub genes were identified as ZAP70, ITK, LAT, PRKCQ, LCK, IL2RB, FYN, CD8A, CD247, CD3E, CD28, CD3D, CD3G, CD74, HLA-DPA1, HLA-DOA, HLA-DOB, HLA-DMB, HLA-DPB1, CAMK4, MEF2C, and TRAC. For up-regulated genes, 134 edges and 133 key nodes were identified. When MCODE classification is performed, MMP9, TIMP1, LCN2, HGF, PFKFB4, PFKFB2, PFKFB3, FPR1, FPR2, IL1R2, ANXA1, IL1RN, IRAK3, IL1R1, ENO1, PKM, and G6PD emerged as

hub genes (Table 3). Genes included in the first cluster with highest MCODE score for down-regulated genes were mostly associated with the T-cell receptor signaling pathways, and apart from this, the primary immunodeficiency, natural killer cell-mediated cytotoxicity, hematopoietic cell lineage, and nuclear factor kappa beta (NF-kappa B) signaling pathways were involved (Table 3 and Figure 4).

Table 3: The top significant modules in PPI networks were identified using MCODE.

Genes	Cluster	Score	Nodes	Edges	Node IDs
Downregulated	1	11	13	68	ZAP70, ITK, LAT, PRKCQ, LCK, IL2RB, FYN, CD8A, CD247, CD3E, CD28, CD3D, CD3G
	2	6	6	15	CD74, HLA-DPA1, HLA-DOA, HLA-DOB, HLA-DMB, HLA-DPB1
	3	3	3	3	CAMK4, MEF2C, TRAC
Upregulated	1	3	4	5	MMP9, TIMP1, LCN2, HGF
	2	3	3	3	PFKFB4, PFKFB2, PFKFB3
	3	3	7	9	FPR1, FPR2, IL1R2, ANXA1, IL1RN, IRAK3, IL1R1
	4	3	3	3	ENO1, PKM, G6PD
	5	3	3	3	C1QA, C1QB, VSIG4
	6	3	3	3	PRKCD, HCK, FGR

Figure 4: PPI network's MCODE components identified genes associated with septic shock. Modules discovered through the MCODE algorithm. A: 6 clusters obtained with upregulated genes. B: 3 clusters obtained with downregulated genes.

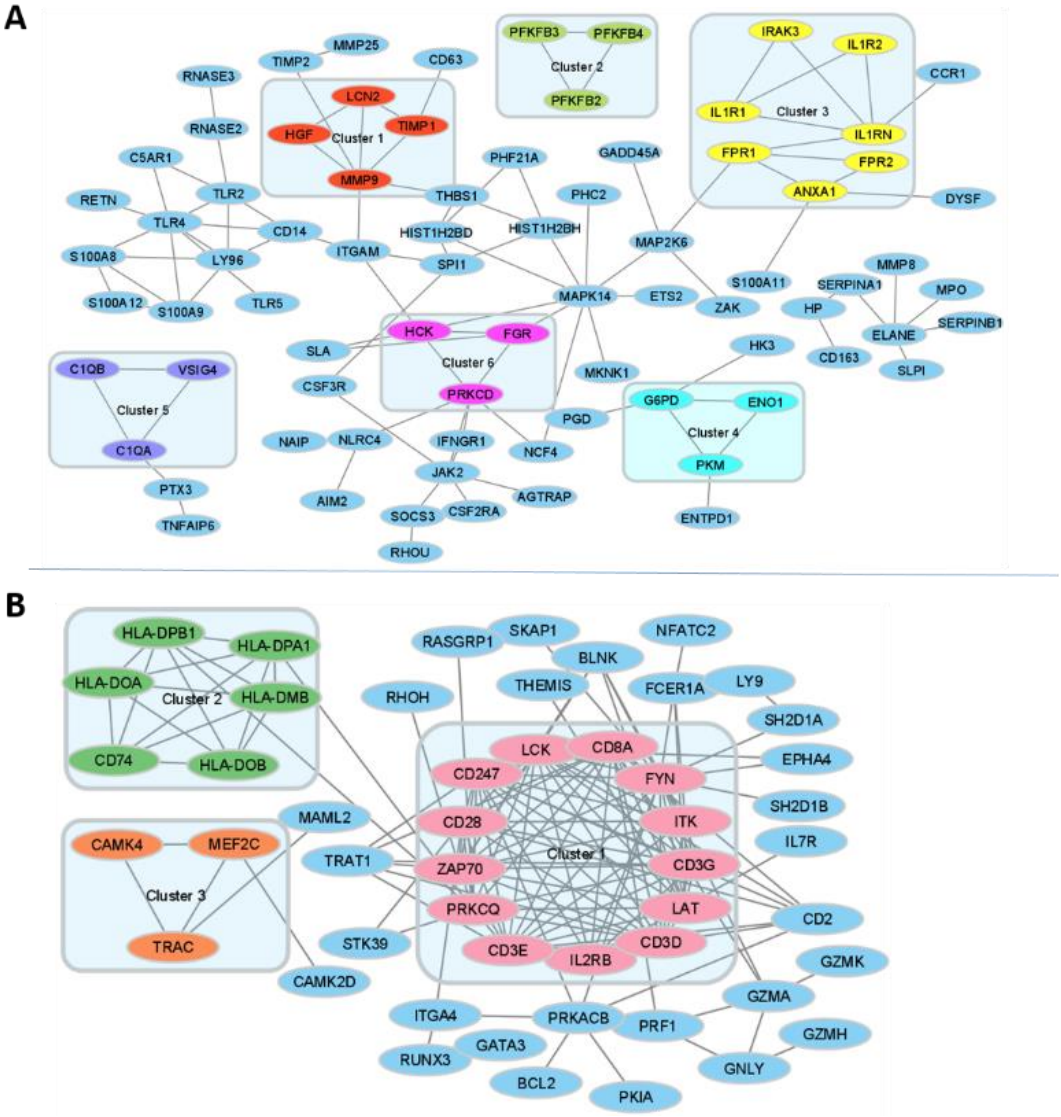
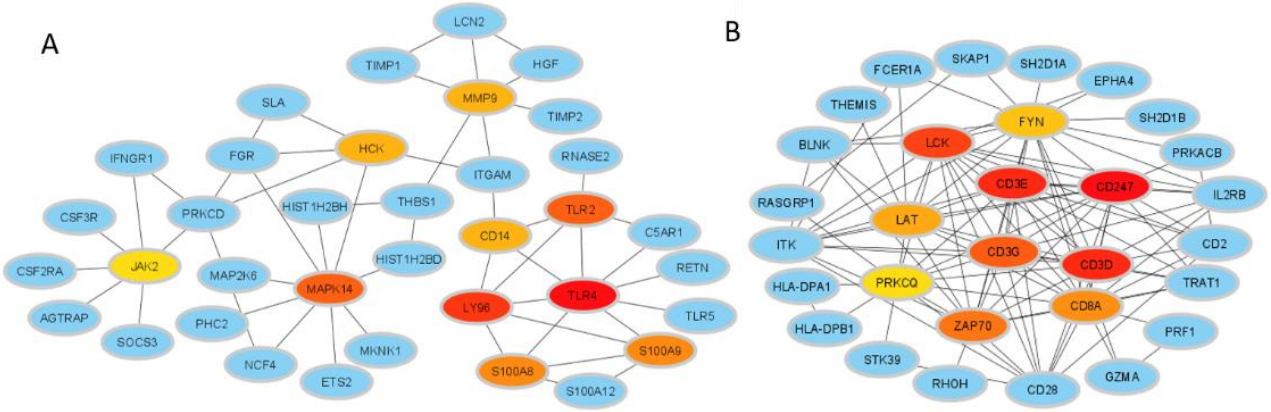


Figure 5: Visualization of the PPI network and the candidate hub genes. Identification of the hub genes from the PPI network using MCC algorithm. A: PPI network of the upregulated genes B: PPI network of the downregulated genes. The red nodes represent genes with a high MCC sores, while the yellow nodes represent genes with a low MCC score.



Identification of the Hub Gene

CytoHubba (part of Cytoscape software)was used to identify significant hub genes among the resulting DEGs. The top 10 genes in the PPI network were chosen using the MCC method. A of connectivity in the PPI network was then used to evaluate the top 10 genes for selected up- and down-regulated genes. Accordingly, down-regulated genes CD247, CD3E, CD3D, LCK, CD3G, ZAP70, CD8A, LAT, FYN, and PRKCQ and upregulated genes TLR4, LY96, TLR2, MAPK14, S100A9, S100A8, MMP9, CD14, HCK, IL1RN were prominent as hub genes. It is noteworthy that the genes with the highest degree consisted of down-regulated genes (Figure 5A and B).

Validation of Hub Gene Expression

Cell viability and qRT-PCR validation results

After using an integrated bioinformatics approach, the resulting key genes were validated in a well-established model of septic shock. qRT-PCR analysis was performed for the relative fold change in expression. We correlated qRT-PCR analysis with our findings from the bioinformatics analysis and validation study. In the first stage of the study, RAW267.4 was stimulated with 10 µg/mL LPS. The same dose was chosen based on results from the literature, and highest stimulation was observed in the 24th hour. As shown in Figure 5A, stimulation of RAW267.4 with LPS resulted in a decrease in cell viability at 24 h ($p < 0.05$). In our study, the relative cell viability began to decline in the 24th hour based on MTT results, and cell supernatant from this time was used. In the second stage, HUVEC cells were seeded at 1 x 105 cells/1 ml concentration on 96-well plates and incubated for one night. Later the 24-h culture supernatants collected from macrophages were stimulated for 0, 12, 24, and 48 h. From the 48th hour, cells were observed to have a significant degree of numerical reduction ($P < 0.001$) as shown in Figure 6A and B.

In studies performed with the aim of mimicking a septic shock model *in vitro*, samples were collected from HUVEC and treated for 0, 12, 24, and 48 h very hour with culture supernatant from the stimulated macrophages. After PCR was performed with the aim of optimizing primers designed for *MMP9, FYN*, and *GAPDH*, the resulting cDNA was subject to a qRT-PCR analysis.. The MMP9 cell model was up-regulated at 0 h, in other words, initially in the control group without application. From the 12th hour, gene expression began to decline, and this down-regulation continued until the 48th hour. The MMP9 microarray fold ratio was 6.9 with an increase in the qRT-PCR fold ratio of 3.5-fold.. As cell models with changes occurring in the same direction as array results are usable, this verifies that this is a model that can be used for drug trials mimicking septic shock or for confirming microarray studies. In the FYN cell model, down-regulation occurred starting at 0 h. The microarray fold ratio was –1.4, with qRT-PCR fold ratio of –2.4 at 0 h, 0.45 at the 12th hour, –1.6 at the 24th hour, and –1.5 at the 48th hour. Our results occurred in the same direction as the array results (Figure 7).

Figure 6: Cell viability assay results A: The MTT cell proliferation test was performed with the aim of checking cell death. RAW 264.7 cells were stimulated with 10 µg/mL LPS at 4, 8, and 24 hours. B: Delivery of supernatant from stimulated RAW 267.4 cells to HUVECs and cell viability at different time points (0, 12, 24, and 48 hours).

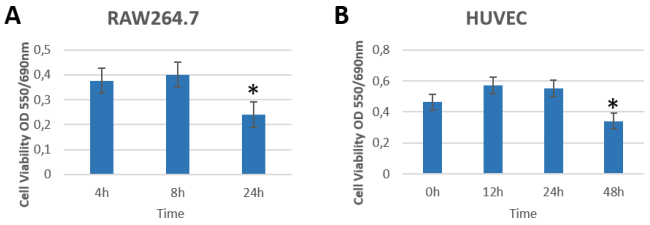
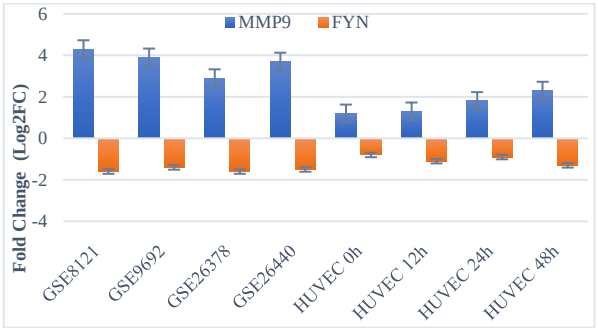


Figure 7: Relative expression of hub genes in an *in vitro* septic shock model. Quantitative real-time PCR results for validation.



Discussion

Sepsis and septic shock are leading causes of morbidity and mortality in intensive care units. Although the death rates from septic shock have improved in recent years, they still remain very high [14]. Many genes revealed in studies in recent years were reported to be associated with septic shock mortality [15]. It is difficult to determine the most important candidate genes and pathways for prognosis at present due to the complicated background of septic shock. Transcriptome studies revealed differences in gene expression profiles and responses throughout the genome [16]. With the growth of high efficiency transcriptomic data, it is possible to perform integrative analysis with multiple datasets to discover reliable candidates for prognosis and treatment. For this reason, we performed integrated analysis increasing the patient and control numbers by using four different datasets to determine potential transcriptomic markers for prognosis in pediatric septic shock and targeted the common genes and pathways playing roles in septic shock. The present study found a total of 771 differentially expressed genes (433 up-regulated, 338 down-regulated) in common in the four datasets for septic shock patients.

As a result of analysis, FYN and CD247 among the hub genes were found to be positively associated with survival in sepsis in studies. Additionally, meta analysis results showed that FYN may be beneficial for prognosis in patients and that CD247 may differentiate patients with sepsis and systemic inflammatory response syndrome. RNA sequencing using a mouse septic shock model showed that CD247 and FYN expression levels were low in this model [17]. Similar to our study, studies of these genes found similarly low expression levels.

Another study of pediatric and adult sepsis and septic shock patients identified MAPK14, FGR, RHOG, LAT, PRKACB, UBE2Q2, ITK, IL2RB, and CD247 as controls of hub genes. They also found stated irregularities between sepsis patients and septic shock patients, and also that especially expression of MAPK14, FGR and CD247 was regulated by the

methylation pathway. They stated that these findings were important to identify potential diagnostic genes associated with sepsis development and the inflammatory and metabolic response mechanisms [18].

Network analysis results found adaptive immunity was pronounced in sepsis, and a tendency to form isolated clusters with genes including CD247, CD8A, ITK, LAT and LCK was at the forefront [19].

Matrix metalloproteinases (MMPs) and metalloproteinase tissue inhibitors (TIMPs) may be promising biomarkers for prognosis during sepsis development. Hoffman et al. observed an association between mortality in septic patients and high MMP9, TIMP2, and TIMP1 plasma levels and did not find a difference in MMP while showing significantly high TIMP1 levels in those who survived compared to those who did not.. Another study found low MMP9 levels and lower MMP9/TIMP1 ratio in mortally ill septic patients [20]. In our study, MMP9 and TIMP1 expression levels were up-regulated in the septic shock group. Lipocalin-2 (LCN2) belongs to an evolutionarily preserved family comprising more than 20 members characterized by the capability of binding and transporting small hydrophobic molecules. Lipocalins are known to play a role in inflammatory diseases and cancer. Although it was determined to be a biomarker for cancer, no correlation with sepsis was found in our study. In our study, the LCN2 gene expression was high. It was first identified in cytoplasmic granules of human neutrophils [21]. It acts as an acute phase protein, and expression is induced by pro-inflammatory stimulation. The protein is included in the innate immune response. LCN2 and MMP9 genes are included among hub genes. A study of stomach cancer showed that MMP9 activity was up-regulated by LCN2, and both LCN2 and MMP9 were controlled by the NFκB pathway resulting in proliferation and invasion [21, 22]. In our results, the expression of both genes was up-regulated, leading to the notion that they are involved in inflammation and related to the NFκB pathway. This pathway can serve as a therapeutic target and will make it easier to identify other targets in sepsis and septic shock. as corrected.

The expression levels of selected genes were examined using quantitative qRT-PCR to confirm microarray data. One gene with up-regulation and one with down-regulation were chosen, and validation was performed. The qRT-PCR results showed the same correlation with those obtained from the microarray analysis. The genes have been validated in the septic shock model that we created based on the literature, but more extensive *in vitro* and *in vivo* experiments are needed to understand the pathology and investigate the gene pathways.

The PPIs and connection numbers of up-regulated and down-regulated genes are important for septic shock regulation. Strong interactions and connection power show that these genes and pathways act together. Panels created for these genes may provide a promising step forward in terms of diagnosis and treatment of septic shock. When determining these gene groups, more advanced bioinformatics analyses and functional experiments are required.

Limitations

Although our study was validated *in vitro*, it has some limitations. First, the data for analysis were downloaded from databases, and problems may arise from the differences in the experimental environments in which these studies were carried out, which may have affected the results. Second, even if molecular experiments were partially performed for validation, these preliminary results need to be performed with larger clinical samples to confirm their accuracy .

Conclusion

This study shows some genes are considered to play a role and be important for pediatric septic shock. Although many genes are known to be associated with septic shock mortality, it is of great importance to identify important candidate genes for the prognosis of the disease and the pathways in which these genes play a role. In order to evaluate the course of the disease, detecting and following the changes in terms of the amount of mRNA or mediator in the organism according to time will open new treatment avenues.. For this reason, we performed integrated analyses with multiple general microarray datasets to determine potential transcriptomic markers for prognosis of pediatric septic shock.

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Chest X-ray imaging after chest tube removal in children undergoing congenital heart surgery: May be life-saving in redo patients

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Ethics Committee Approval
The Ethical Committee of Clinical Researches (Ethics Committee of İzmir Tepecik Training and Research Hospital) granted permission for this study with the decision number 2018/22-06-112. All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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

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Abstract

Background/Aim: Parallel to the developments in congenital heart surgery, the number of children undergoing resternotomy (redo) heart surgery is increasing. In this specific group of patients, post-operative pneumothorax (PTX) and atelectasis are preventable respiratory complications. However, in the literature, pediatric data are still limited. In this study, we draw attention to the frequency and importance of PTX, a post-operative respiratory complication in redo patients. We investigate the necessity for routine chest X-rays to detect PTX following chest tube removal after closed or open-heart operations for congenital heart disease.

Methods: A total of 554 consecutive pediatric patients who underwent cardiac surgery were analyzed. The study was designed as a retrospective cohort study. The patient's demographic data, clinical characteristics with chest tube removal, and pathologies detected by chest X-ray were recorded. Patients were divided into non-redo and redo groups or subgroups. Patients who developed PTX (n = 24) were divided into subgroups: asymptomatic or symptomatic and large or small. Data analysis and statistical comparison between the groups were performed with independent-samples t-test or Mann-Whitney U test.

Results: In 24 (4.3%) of the 554 patients included in the study, PTX was detected in the post-operative evaluation after chest tube removal. Of the PTX cases, 15 (62.5%) were small, and nine (37.5%) were large. Ten (41.6%) patients were symptomatic, while nine patients had large PTX, and one patient with small PTX was identified. There were significantly more cases of large PTX in redo cases than in non-redo cases (P = 0.038). PTX was significantly more symptomatic in redo patients than non-redo patients (P = 0.031).

Conclusion: In patients undergoing cardiac surgery for the first time, a detailed clinical assessment reduces the likelihood of post-procedure PTX and makes routine chest X-ray imaging unnecessary. Conversely, clinical follow-up of these patients in terms of PTX should be essential for possible complications. However, clinical signs of late PTX development in the first 24–48 h after chest tube removal in patients undergoing redo cardiac surgery should be followed carefully by the clinician, and chest X-ray imaging should be routinely performed.

Keywords: Chest tube, Complication, pneumothorax, Redo, Radiography

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Introduction

Mediastinal and pleural tubes are routinely placed following cardiothoracic surgery to provide drainage from the chest for air, blood, fluid, or pus [1]. Chest X-rays (CXRs) have been used routinely following a chest tube's removal to detect early post-operative pathology, particularly pneumothorax (PTX). It is also essential to repeat CXRs following the chest drain insertion to confirm the tube's positioning, check the extent of lung re-expansion, and exclude the possibility of complications after removal of the chest drain [1, 2]. However, some studies have suggested that the routine use of CXRs is unnecessary for a significant portion of cardiac surgery patients [1–3].

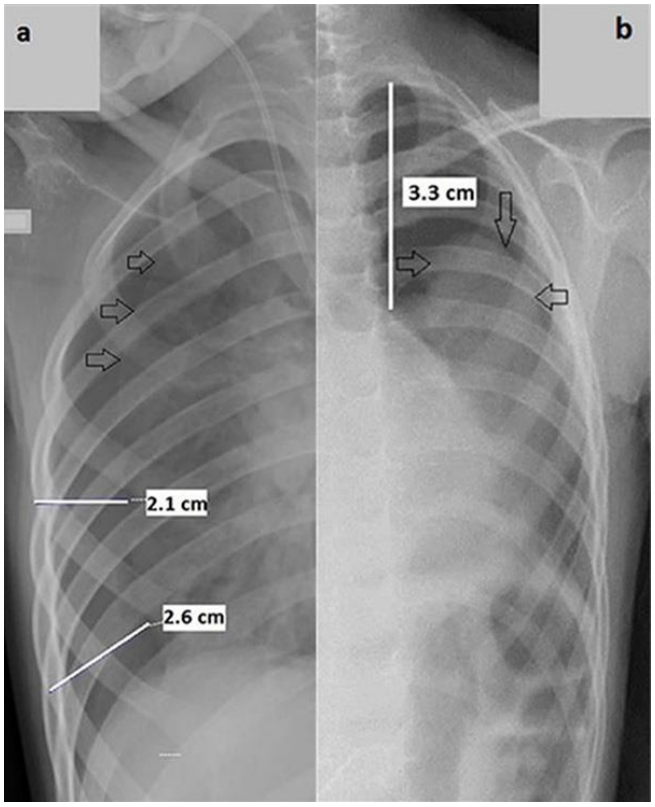
This study aims to determine if routine CXR is necessary to detect PTX following chest tube removal after closed or open-heart operations for congenital heart disease or which patients need routine CXR.

Materials and methods

Hospital records of 554 consecutive pediatric patients who underwent open-heart surgery with median sternotomy due to congenital heart disease between November 2017 and January 2019 and at Izmir Tepecik Training and Research Hospital Congenital Heart Surgery Clinic were reviewed. The exclusion criteria for the study consist of patients older than 18 years of age, those with intraoperative lung injury, air leakage from the thoracic tube in the post-operative period, those who underwent cardiopulmonary resuscitation, and those who underwent revision surgery due to hemorrhage. We used polyvinylchloride or silicone chest tubes (Bıcakcilar®, Istanbul, Turkiye) between 12 Fr and 32 Fr, depending on the patient's size. Thoracic tube drainage was sufficient in patients with a pleuro-pericardial window created intraoperatively for pericardial-pleural passage since mediastinal drainage would be sufficient for the pleura. Therefore, mediastinal tubes were not placed in these patients. Data were collected, including demographic characteristics (age, sex, and body surface area), perioperative data (cardiac complexity score [RACHS], type of surgery [redo or non-redo], position, number and duration of the drain, and number of CXR), and outcomes (duration of hospital stay, number, and size of PTX). Patients with dyspnea, tachypnea, or increased oxygen demand were symptomatic. The need for oxygen support was determined according to the patient's oxygen saturation (SaO_2) level. If the SaO_2 level was 90% and below, oxygen support was given to these patients. All patients underwent CXR in the pediatric cardiac unit after admission to the intensive care unit (ICU) and within 4 hours after chest tube removal.

A plain CXR was utilized for the quantification of the size of PTX. The British Thoracic Society guidelines divide PTX into small and large, based on the distance from the visceral pleural surface (lung edge) to the chest wall. If the PTX is greater than 2 cm, it is classified as a large PTX occupying approximately 50% of the hemithorax (Figure 1) [4]. A large PTX is an objective indication for drainage [5]. The PTX is assessed using the Picture Archive Computer Communication System. In our clinic, most patients with chest tubes removed are taken to the semi-ICU. The remaining patients are followed for one more day in the intensive care unit and then taken to the patient ward. All patients are re-evaluated with CXR imaging before admission to the patient ward.

Figure 1: (a, b) Chest X-ray images show quantification of large PTX size in different patients–the classic appearance of PTX with readily apparent visceral pleural line (black arrows). Interpleural distance at the level of the hilum (a) >2 cm represents a large PTX, and the distance from the lung apex to the cupola (b) >3 cm represents a large PTX.



The criteria for chest tube removal was less than 5 ml/kg/day drainage from the pleural space. Before removing the chest tube, it was confirmed that no PTX was observed in the CXR taken on the same day. Chest tubes were checked at the patient's bedside for fluctuation and the presence of air leaks. PTX was not detected in patients with CXR performed within 2 h after tube removal. The same surgical team removed the drains as per unit protocol. The use of ketamine is standard care in our unit for procedural sedation. The patients who received the dexmethothymidine infusion in ICU continued before and after removing the tube. Chest tubes were removed at the end of inspiration. After tube removal, patients were monitored closely for any signs of respiratory distress or cardiovascular compromise, and the drain entry area was covered with vaseline gauze to prevent air leakage in the early period.

Written informed consent was obtained from the parents of all patients. The study was conducted in accordance with the Declaration of Helsinki principles and was approved by the ethics committee. Ethics committee approval (June 22, 2018) decision number 2018/22-06-112 was obtained by the University of Health Sciences Tepecik Training and Research Hospital Clinical Research Ethics Committee.

Statistical analysis

Statistical analysis of the data was performed using SPSS 16. Mean (standard deviation) was used to summarize numerical data, whereas frequency (n) and percentage (%) distributions were used to summarize categorical data. The distribution of numerical data was evaluated using the Kolmogorov-Smirnov test. In addition, normally distributed numerical data in two independent groups was analyzed using

the independent-samples t-test, and the distribution of non-normally distributed numerical data in two independent groups using the Mann-Whitney U test at 95% confidence with a significance of $P < 0.05$.

Results

The clinical characteristics of all patients who underwent congenital cardiac surgery are shown in Table 1. The mean age of the patients was 4.1 (3.3) years, and 265 (47.8%) were males. Twenty-four patients (4.3%) developed PTX, of which 15 were small PTX while nine were large PTX. In patients with large PTX, seven (77.7%) were redo surgery patients. Twenty-six (4.69 %) were followed up as intubated while the chest tube was removed. However, PTX did not develop in any of these patients after chest tube removal.

Table 1: Demographic and clinical characteristics of patients.

Patients, n	554
Non-Redo surgery, n (%)	469 (81%)
Redo surgery, n (%)	85 (19%)
Mean age (years), mean (SD)	4.1 (3.32)
BSA (m²), mean (SD)	0.5 (0.65)
Male (%)	265 (47.8%)
RACHS score, mean (SD)	2.3 (0.75)
Drains	
Mediastinal, n	381
Pleural, n, right/left/bilateral	68/98/45
Mean duration of a drain (days), mean (SD)	2.12 (0.9)
Number of chest drains, mean (SD)	1.7 (0.3)
Mean number of CXRs, mean (SD)	2.68 (0.55)
Hospital LOS (days), mean (SD)	6.56 (1.27)
Number of PXT, n (%)	24 (4.3%)
Small, n (%)	15 (62.5%)
Large, n (%)	9 (37.5%)

BSA: body surface area, CXR: chest X-ray, LOS: length of stay, PTX: pneumothorax.

The findings and outcomes in the patients with PTX after chest tube removal are shown in Table 2. In the ten (41.6%) patients with PTX who had clinical signs or symptoms, we identified nine (37.5%) patients with large PTX and one (4.1%) patient with small PTX. The patient with a small PTX became symptomatic and had interstitial lung disease. Fourteen patients with PTX were identified to have no clinical signs or symptoms. A total of nine patients (eight with large PTX and one with small PTX, which enlarged later) underwent considerable interventions.

Table 2: Findings and outcomes in patients with PTX.

	n	%
Patients	24	4.3
Number of PXT		
Large (L)	9	37.5
Small (S)	15	62.5
Clinical findings		
Asymptomatic (L/S)	14 (0/14)	58.4
Symptomatic (L/S)	10 (9/1)	41.6
Subcutaneous emphysema	7 (3/4)	40
Tachypnea	9 (6/3)	45.8
Increased oxygen requirement	10 (8/2)	41.6
Other pathology detected on CXR		
Atelectasis	6	25
Pulmonary congestion	3	12.5
Pleural effusion (minimal, <10 mm)	10	41.6
Intervention following CXR		
Clinical observation, no drain	15	62.5
Drain reinsertion	9	37.5
Outcome		
Dissolved spontaneously	15	62.5
Drain removed on day 2	9	37.5

CXR: chest X-ray, PTX: pneumothorax.

The drains were removed on days 2–4 in all patients with PTX, and no patient needed reintervention. The clinical observation was continued in 15 patients with small PTX, and no symptoms were observed. Large PTX in redo cases was statistically significantly more than in non-redo cases ($P = 0.038$, 7 *vs.* 2 patients). In addition, the PTX of patients who underwent redo surgery was statistically more symptomatic ($P = 0.031$, 8 *vs.* 2 patients). A comparison of surgery type groups (redo and non-redo) in patients with PTX is shown in Table 3, and a comparison of PTX size and symptom status is shown in Table 4.

Table 3: Comparison of surgery type groups in patients with PTX.

	Non-Redo	Redo	*P*-value
Asymptomatic (n = 14)	9 (64.3%)	5 (35.7%)	0.064
Symptomatic (n = 10)	2 (20%)	8 (80%)	0.031
Small PTX (n = 15)	9 (60%)	6 (40%)	0.086
Large PTX (n = 9)	2 (22.2%)	7 (77.8%)	0.038

PXT: pneumothorax.

Table 4: Comparison of PTX size and symptom status.

	Small PTX	Large PTX	*P*-value
Asymptomatic (n = 14)	14 (100%)	0 (0%)	<0.01
Symptomatic (n = 10)	1 (10%)	9 (90%)	<0.01

PXT: pneumothorax.

Discussion

The current value of using CXRs following chest drain removal remains controversial [1–3]. In our clinic, as in most centers, routine CXRs are the parts of a standard practice following chest drain removal in patients undergoing cardiothoracic surgery to eliminate PTX or collection in the thoracic cavity. Although the first CXR image after chest tube removal was normal in this study, PTX was detected in 4.3% of patients on the radiograph taken 24 h later. A literature review revealed six studies detecting pathology in routine CXR ranging from 2% to 40% compared to 79% in clinically indicated CXRs [1, 6–9]. Furthermore, the incidence of therapeutic intervention requirements was about 1.5% [1–3]. In our study, the intervention rate following CXR after chest tube removal was 1.6% (9 patients), and seven were redo cases.

In most patients, clinical assessment helps recognize PTX cases where routine CXRs are not required [8, 10, 11]. Several studies have shown that routine CXRs are unnecessary regardless of whether pleural and mediastinal tubes are used [1, 7–12]. Clinical symptoms vary and may range from no symptoms to severe dyspnea depending on the PTX size, although some common signs include tachypnea, dyspnea, cough, and tachycardia [4, 5]. In addition, the patient's respiratory capacity can affect the manifestation of symptoms. In our study, at least one of the signs and symptoms of PTX was present in all patients with large PXT. The most common symptom of patients with PXT was increased oxygen requirement (10 patients, 41.6%) and tachypnea (11 patients, 45.8%).

Cardiac surgery requiring redo is technically challenging due to adhesions, scars, and previous graft placements and carries a higher intraoperative and post-operative risk. Nevertheless, redo operations are more common in pediatric patients [13]. In patients who underwent redo cardiac surgery, adhesions between the pleura, chest wall, and mediastinal structures may have caused iatrogenic lung injury. The main factors leading to the development of PTX in redo patients are the minimal injuries associated with the dissection of pleural adhesions and the disruption of respiratory function due to pain [14]. The increased respiratory capacity following clinical recovery can lead to air escaping through the lung tissue at points where the pleural adhesions are damaged. Therefore, increased respiratory capacity following the clinical improvement in redo cases, starting from the post-operative first day, can increase

PTX complications, and substantial complications may be prevented following routine CXRs in patients undergoing redo surgery. In addition, there may be areas of micro-damage in the lung tissue during mediastinal exploration and dissection of the planes in redo patients. Air leaks associated with areas of micro-injury in the lung parenchyma may be overlooked during the bedside air leak assessment. There was no considerable air leak in redo patients who developed PTX before removing the thoracic tube. A potential reason for this is that there could have been air leaks that were too small to be seen with the naked eye. Hence, we believe that routine CXR effectively increases the probability of identifying PTX in patients who underwent redo cardiac surgery due to lung injuries associated with pleural adhesions.

A higher rate of large PTX was detected in redo cases in our study, and a statistically significant difference was found. However, PTX was observed to be more symptomatic in redo cases, and also, in this respect, a statistical difference was found. Therefore, the use of routinely appropriate X-ray imaging generally outweighs the risk of possible complications or disease detection in redo cases. Therefore, the routine use of CXR should be considered in the presence of redo cardiac surgery.

In a prospective, observational study including a total of 214 consecutive patients undergoing cardiac surgery and routine CXRs within 24 hours of drain removal, Tolsma et al. [9] reported that, for the majority of the patients, it seemed reasonable to reduce the number of routine CXRs within the first 24 hours of ICU stay. However, the optimal timing for performing CXRs remains unclear [8, 9, 12]. In our study, PTX was diagnosed in all patients between post-operative days 1 and 2. Thus, these were explicitly post-operative redo cases that required intervention.

As with our study results, CXR imaging is predominant in redo patients. Children with congenital heart disease are exposed to increased amounts of low levels of ionizing radiation with each CXR, computed tomography, and cardiac catheterization procedure that they undergo, and there are risks associated with using X-ray imaging. Because the number of safe exposures to ionizing radiation is unknown, efforts should be made to minimize this risk by reducing unnecessary exposure to ionizing radiation. In addition, these patients should be protected from any unnecessary radiographs [15]. Further to the risks associated with increased exposure to ionizing radiation, routine CXRs utilize radiology, medical and nursing resources and interrupt nursing care.

Based on our results, we believe that careful observation for developing PTX is required in patients undergoing redo heart surgery. Suppose tachypnea, desaturation, and an increase in oxygen demand are observed in the post-operative follow-up of redo patients. In that case, PTX that may develop in the period after chest tube removal should be considered. Interpreting the symptoms in favor of atelectasis and applying non-invasive positive pressure ventilation to the patient may aggravate PTX. According to our results, we can say that the conventional CXR imaging after chest tube removal in the patient group undergoing non-redo cardiac surgery is unnecessary, as stated in previous publications. However, in patients with respiratory symptoms, it is undoubtedly necessary to exclude PTX with CXR in addition to other lung pathologies.

Although this research has fulfilled its aims, there were some unavoidable limitations, such as this being a single-center retrospective study and limited by the number of patients enrolled to participate. Therefore, the sample size in redo cardiac surgery was not large enough to make a definitive conclusion. Also, the size and classification of PTX were problematic in the pediatric patient group because chest size varies according to the age and physical size of the patient. While there are many methods to calculate PTX size with CXR in adult patients, there are still no cut-off values related to age and weight in the pediatric age group [16]. This topic should therefore be addressed in future studies.

Conclusions

In patients undergoing cardiac surgery for the first time, a thorough clinical assessment before chest tube removal reduces the likelihood of post-procedure PTX and makes CXR unnecessary. However, clinical follow-up of these patients in terms of PTX should be essential for possible identifying complications. According to the results of our study, the use of CXR after chest tube removal in patients who underwent surgery for the first time should be decided according to clinical indications. Based on our study results, we conclude that routine CXR should be used after chest tube removal in patients who have undergone redo cardiac surgery. When respiratory symptoms develop in the late post-operative period, especially in children who have undergone redo surgery, a possible PTX should be excluded by performing a new CXR.

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Research article

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Is it a requirement or a preference to use cross-links in lumbar instrumentation?

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Ethics Committee Approval
Ethics Committee approval was taken from the Tekirdağ Namık Kemal University Ethics Committee. (date: May 31, 2022 number: 2022.87.05.13).
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Abstract

Background/Aim: The use of cross-links (CL) is controversial due to reasons such as cost increases and instrument redundancy. While there are many biomechanical studies, the clinical data is limited. The aim of this study is to present the clinical effects of CL by putting forward postoperative clinical outcomes and long-term results of patients with (CL+) and without (CL-) CL augmentation.

Methods: In this retrospective cohort study, patients who underwent lumbar posterior instrumentation with CL+ (n = 164) and without CL- (n = 111) augmentation were evaluated. Demographic data, surgical results, preoperative and postoperative visual analogue scale (VAS), the Oswestry Disability Index (ODI) differences, and pseudoarthrosis and adjacent segment disease (ASD)-related recurrence for more than three years of follow-up were determined. Data of CL+ and CL- groups were compared.

Results: CL+ and CL- groups were similar in terms of age and gender (P = 0.319 and P = 0.777, respectively) There was no difference between the two groups in terms of bleeding amount, duration of surgery, and duration of hospitalization (P = 0.931, P = 0.669 and P = 0.518, respectively). Groups were similar in terms of VAS and ODI differences (P = 0.915 and P = 0.983, respectively), yet there was one case of infection in the CL+ group and two cases of infection detected in the CL- group. There were 13 ASDs in the CL+ group, and eight ASDs in the CL- group. Pseudoarthrosis was seen seven times in the CL+ group, while it was four in the CL- group.

Conclusion: It was observed that adding CL in patients who underwent lumbar instrumentation did not change the early period surgical results. The prevalence of complications was compatible with the scientific literature. In our study, there was no preventive advantage in terms of clinical or postoperative complications found in the use of CL.

Keywords: Internal fixators, Pedicle screws, Pseudoarthrosis, Lumbar vertebrae

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Introduction

Lumbar spinal stenosis is a gradually increasing disease in aging societies, which usually occurs due to degenerative spondylolisthesis. This disease impacts the lifestyle of middle aged and older patients, and the solution is usually surgical [1, 2]. There are limited non-surgical options, such as medication, injections, or physiotherapy; however, surgery is performed for most symptomatic patients [3]. This is always elective surgery even in severely symptomatic patients. The aim of surgery is to decompress the nerve root and spinal cord compression.

Traditional laminectomy, bilateral laminectomies, bilateral decompression through unilateral laminotomy, and different forms of laminoplasty are applied with the aim of decompression [4]. Spinal arthrodesis for spinal fusion is used in cases of spinal stenosis associated with degenerative spondylolisthesis, recurrent stenosis after previous decompression, instability, or scoliosis [5].

Dorsal instrumentation is the most frequently used arthrodesis method, and the aim is to increase the fusion rate by creating a rigid stabilization [6]. Many devices, such as plates, hooks, cerclages, CLs and interbody cages are used for the increase of rigidity. CL is a system, which is applied on contralateral rod or screw head. There are many biomechanical studies about it in scientific literature; yet, there are few clinical studies on the long-term results in the lumbar region [7]. The biomechanical advantages are controversial due to disadvantages, such as wide exposure, increase of implant load, and increase in cost and surgical period. CL, which is nowadays a method used by many surgeons, is a personal preference rather than a necessity.

We aimed in this study to present the clinical effects, patient outcomes, and long-term results of the CL use on patients with posterior instrumentation (augmentation) in the lumbar region.

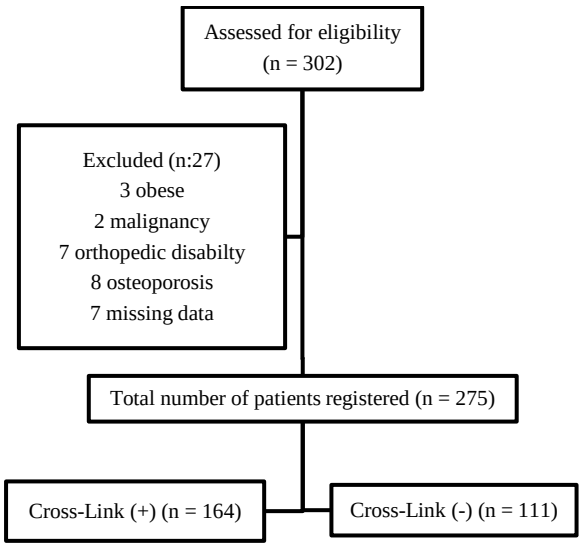
Materials and methods

Patient files and radiological images of decompression and dorsal instrumentation applied to 275 patients with degenerative lumbar spinal stenosis, ranging from 40 to 81 years of age between December 2014 and June 2019 were analyzed by the same surgeon at Tekirdağ Namık Kemal University, Neurosurgery Clinic (Figure 1). The approval for this study was granted by the Tekirdağ Namık Kemal University Ethics Committee (date: May 31, 2022 number: 2022.87.05.13).

Surgical indication criteria consisted of low back and/or radicular pain despite medical treatments. Other inclusion criteria were patients who described neurogenic claudication below 100 mt, those with a canal anterior-posterior diameter of 11.5mm in their lumbar MR images, those with a canal section area below 1.45cm², and need stabilization due to concomitant degenerative spondylolisthesis, instability, or scoliosis.

Patients with severe osteoporosis history, malignancy, advanced gonarthrosis, coxarthrosis, those with a BMI above 30, and those who had undergone interbody arthrodesis were excluded from the study.

Figure 1: Flowchart of the study



Patients were divided into two groups, one of which consisted of patients with CL use (CL+ group), and the other who had not had CL augmentation (CL- group) in their surgery. Data of the patients, such as age, gender, follow-up periods, and a three-month-period record of preoperative and postoperative visual analogue scale (VAS), and Oswestry Disability Index (ODI) were recorded. The preoperative and postoperative differences of VAS and ODI of patients were taken. ODI differences were categorically classified as very poor if below 5, poor between 6-10, fair between 5-11, good between 16-20, and excellent if above 20.

CL+ and CL- groups were analyzed in terms of infection, pseudoarthrosis, adjacent segment development, and ODI differences.

Statistical analysis

Data were analyzed by using the Statistical Package for the Social Sciences (SPSS) 24.0 (SPSS Inc., Chicago, IL, USA) statistical computer program. The Chi-square test for categorical variables was used to compare the groups with or without CL augmentation. For the comparison of continuous variables, the independent sample t-test was used. Cases in which the *P*-value was below 0.05 and the type 1 error level was below 5%, were interpreted as statistically significant.

Results

A total of 275 patients was included in the study. It was determined that 109 (66.4%) of 164 patients in CL+ were women, and 55 (33.5%) were men, while 74 (66.6%) out of 111 patients in the CL- group were women, and 37 (33.3%) were men. There was no difference between the two groups in terms of gender (P = 0.777). While the average age was 61.6 (10.6) years in the CL+ group, it was 60.2 (11.4) years in the CL- group, and both groups were similar in terms of the distribution of age (P = 0.319). Demographic characteristics of patients and surgical results are summarized in Table 1.

While the bleeding amount in the CL+ group was 285.4 (110.4) cc, it was 285.9 (109.7) cc in the CL- group, and no difference was determined between the two groups (P = 0.931). When the average surgical duration was analyzed, it was found to be 160.4 (41.8) minutes in the CL+ group, whereas it was 157.0 (38.2) minutes in the CL- group. Both groups were similar in terms of duration of surgery (P = 0.669). The duration of

hospitalization in the CL+ group was 5.4 (4.1) days, whereas it was 4.8 (2.2) days for the CL- group. Both groups were similar in terms of the duration of hospitalization (P = 0.518). When VAS differences were analyzed, it was found to be 6.81 (0.7) for the CL+ group, while it was 6.89 (0.8) for the CL- group, and the difference was insignificant (P = 0.915). ODI differences for the CL+ group were very poor for 4 patients, poor for 36 patients, fair for 89 patients, good for 30 patients, and excellent for 5 patients. ODI differences for the CL- group was very poor for 2 patients, poor for 20 patients, fair for 64 patients, good for 21 patients, and excellent for 4 patients (Figure 2). Both groups were similar in terms of ODI differences (P = 0.983). Postoperative spondylodiscitis developed in two (1.2%) patients in the CL+ group while it developed in one (0.9%) patient in the CL- group. ASD was seen in 13 (7.9%) patients in the CL+ group, while it was seen in 8 (7.2%) patients in the CL- group. When the number of pseudoarthrosis was calculated, it was determined to be seven (4.2%) for the CL+ group while it was four (3.6%) in the CL- group (Figure 3). There were no implant failures in our series.

Table 1: Demographic features and surgical outcomes of patients with and without cross-link use.

		CL + Mean (SD) / n	CL - Mean (SD) / n	*P*-value
Age		61.6 (0.6)	60.2 (11.4)	0.319
Gender	female	109	74	0.777
	male	55	37	
Bleeding amount (cc)		285.4 (110.4)	285.9 (109.7)	0.931
Duration of surgery (min)		160.4 (41.8)	157.0 (38.2)	0.669
Duration of hospitalization (day)		5.4 (4.1)	4.8 (2.2)	0.518
VAS difference		6.81 (0.7)	6.89 (0.8)	0.915

SD: Standard deviation, CL: Cross-link, ODI: Oswestry Disability Index, VAS: Visual analogue scale

Figure 2: Distribution of ODI differences between groups.

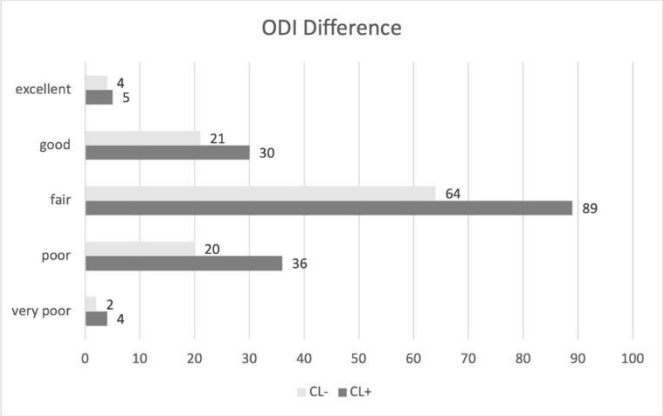
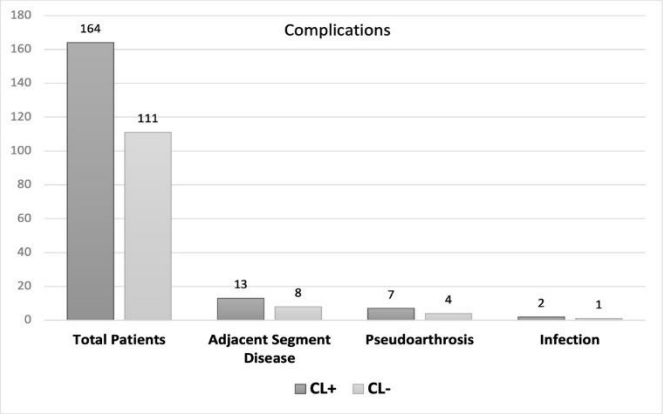


Figure 3: Distribution of adjacent segment disease, pseudoarthrosis, and infection between groups.



Discussion

Our study determined that there was no significant difference between the patients with or without CL augmentation, in terms of bleeding amount during surgery or length of postoperative hospitalization. Furthermore, there was no significant difference in the VAS and ODI, which evaluate the preoperative and postoperative functional recovery of patients. Postoperative infection, as well as ASD and pseudoarthrosis, which are late term complications in both groups, were found to be compatible with the scientific literature.

Although there have been several publications about CL augmentation for many years for patients who plan fusion surgery to increase stability, its use is still controversial [7, 8]. Most studies related to CL augmentation, which increases the instrument cost, are about biomechanical studies on synthetic materials and animal or cadaver backbones; yet, there are only few studies on clinical results [9, 10]. Most of these studies assert that CL augmentation has primarily had significant impact on axial rotation, whereas it affects lateral bending and particularly flexion extension slightly [7]. Lehman et al. [11] stated in their cadaveric study that CL augmentation in spinal fusion surgery performed between C1-C2 decreased the range of motion (ROM) at axial rotation 57%. It was also stated that this percentage decreased more between the C3-6 lower cervical area, yet the CL augmentation in the cervicothoracic region led to a decrease of 27% in the axial ROM [12, 13]. It was further argued that in the thoracic area, which already has a limited axial ROM due to the rib cage, flexion extension and lateral bending did not contribute to CL [14]. Other studies indicate that axial rotation is hindered (21%) in the lumbar region, yet it was not effective on flexion extension and lateral bending [15]. As a result, all these biomechanical studies may suggest that CL augmentation in upper cervical and cervicothoracic junction areas will accelerate fusion by preventing axial rotation; however, the advantage of CL augmentation in terms of cost-effectiveness in lumbar and thoracic areas is controversial.

Kulkarni et al. [16], in their study about CL with 208 patients and 707 fused segments excluding the cervical area, asserted that biomechanical studies did not have a clinical advantage and that the use of CL was unnecessary. In addition, Garg et al. [17] stated that CL augmentation in adolescent idiopathic scoliosis did not improve clinical or radiological outcomes. Similar findings were determined in our study for the lumbar region. When VAS and ODI scores of patients were analyzed, it was found out that CL augmentation did not provide any clinical advantage.

Following spinal fusion surgery, the reoperation percentage within five years due to infection, instrument failure, pseudoarthrosis, insufficient decompression, and adjacent segment disease is 20% [18, 19]. Of these reoperations, 51% are due to ASD, and the annual incidence for the lumbar area varies from 2% to 4% [20]. In our series within a three-year follow-up, reoperation rate due to ASD in the CL+ group was 7.9%, and it was 7.2% in the CL- group. This rate is within tolerable limits for both groups.

The prevalence of spondylodiscitis after any kind of spinal surgery varies between 0.21% and 3.6% [21, 22]. The infection ratio in the CL+ group was 1.2%, and was determined

as 0.9% in the CL- group. Looking back, it was discovered that three patients were diabetic. Although there are many publications arguing that the increased number of instruments lead to an increase in the susceptibility of infection, it is not possible to assert such an association in our study.

Pseudoarthrosis is defined as non-union in spinal surgery. It reveals itself as lack of fusion, rod breakage, or as a halo-shaped hypodense area around screws in CT images [23]. The most common reason for revision surgery in adult scoliosis is adjacent segment degeneration. This is followed by pseudoarthrosis, which is seen in 25% of all cases [24]. A pseudoarthrosis development incident in adult spine deformities is stated to be between 5% and 27% [25]. In various reviews, in which patients who underwent surgery due to deformity were analyzed, the incidence of pseudoarthrosis development was found to be between 0 and 41% [26]. In our study, the rate was 4.2% in the CL+ group, while it was determined as 3.6% in the CL- group. The development of infection, ASD, and pseudoarthrosis being within tolerable limits in both groups, indicates that the use of CL material, which leads to both additional cost and instrumental burden, should be questioned. It is obvious that CL affects the axial ROM the most. In lumbar region surgeries where the surgical aim is fusion, the contribution of preventing axial rotation, which is already low, to fusion is controversial. There are no available guidelines regarding the use of CL augmentation in recent surgical practice. It is just implemented as the choice of the surgeons. Even if some surgeons use multiple CL in order to enhance the rigidity of instruments, the economic burden of CL, the possible cause to complications such as corrosion, infection, and instrument failure should be kept in mind [16]. There are many publications indicating that the use of multiple CL leads to a decrease in the fusion area by causing instrument crowding [26].

Our clinical experience suggests that it is wrong to perform CL augmentation in cases where laminectomy is not performed, in other words, in cases where the posterior tension band has not been impaired. In such cases, it is necessary to perform osteotomies to back elements in order to be able to use CL augmentation. It should be used only in cases where wide osteotomy or facetectomy is performed, as a preference of the surgeon in order to prevent axial rotation and increase instrument rigidity. Nevertheless, in our opinion, the routine use of CL is unjustified, and should be restricted in order to avoid instrument crowding and potential risks.

Limitations

In our study, the percentage of infection, ASD, and pseudoarthrosis was lower in the CL- group; however, the scarcity of complications in this series led to an unreliable statistical comparison. This is the most important limitation in our study. We think that particularly these rare complications should be evaluated via meta-analyses.

Conclusion

While there are plenty of biomechanical studies related to CL, there are quite few clinical studies. CL, which has become habitual, and is routinely used by surgeons, is an instrument the use of which is unclear in terms of clinical benefits, yet it is certain to increase the hospital costs. According to the results of our study, in patients where fusion is aimed in the lumbar area, there are no advantages or disadvantages in CL augmentation in early or late surgical periods.

We think that particularly in surgeries where posterior elements are preserved, and in cases with no severe instability, CL augmentation should be abandoned in order to decrease both the number of instruments and the surgical costs.

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Research article

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Investigation of the relationship between serum adropin levels, oxidative stress biomarkers, and blood pressure in DOCA-salt hypertensive rats

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Ethics Committee Approval
Ethics Committee approval was taken from the Kutahya Health Sciences University Ethics Committee of Animal Care and Usage, Kütahya, Turkey (Protocol no: 2019.01.09).
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Abstract

Background/Aim: Adropin is involved in the pathophysiology and development of cardiovascular diseases, such as hypertension. The aim of this study was to investigate the effects of adropin in serum, potential use as a biochemical biomarker of oxidative stress, and effects on blood pressure in deoxycorticosterone acetate (DOCA) salt hypertensive rats.
Methods: Eighteen male Sprague-Dawley rats were divided into two groups: (1) Control (C) and (2) Hypertensive (H). Systolic and diastolic blood pressures (SBP and DBP, respectively), and mean blood pressure (MBP) were measured using the tail-cuff method. At the end of the study, serum endothelin-1 (ET-1), adropin, nitric oxide (NO), total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI) were also analyzed.
Results: Significant increases in SBP, DBP, MBP, cardiac hypertrophy index (CHI), and left ventricular hypertrophy index (LVCI) in the H group compared with the C group were found. Serum levels of ET-1, TOS, and OSI were significantly higher in the H group and serum levels of NO, adropin, and TAS were lower than in the C group. A negative correlation between serum adropin levels and the variables SBP, DBP, MBP, TOS, OSI, CHI, and LVHI was found. Adropin levels were positively correlated positively with serum NO levels in both groups.
Conclusion: Serum adropin levels decreased in hypertensive DOCA-salt rats. Lower serum adropin levels were found to be significantly associated with hypertension and may play a role in this disease. However, further comprehensive and diverse studies are needed.

Keywords: Adropin, Oxidative stress, DOCA-salt hypertension, Blood pressure

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Introduction

Adropin has been detected in a variety of tissues and organs, including the pancreas, liver, brain, kidneys, endocardium, myocardium, and epicardium [1]. Adropin is capable of controlling lipid metabolism, reducing insulin resistance, and improving vascular endothelial cell function, and has preventive effects on the pathogenesis and progression of cardiovascular disease. It also has anti-inflammatory effects [2]. In the study by Aydın et al., it was shown that the level of adropin is a prognostic marker rather than a treatment [3].

Hypertension is one of the most common progressive diseases that has significant public health implications. It is an independent and important risk factor for several cardiovascular diseases characterized by elevated blood pressure, such as arterial aneurysms, strokes, heart failure, and atherosclerosis [4]. Deoxycorticosterone acetate (DOCA), an inactive acetate, is experimentally used as a mineralocorticoid to produce hypertension and cardiovascular pathology [5]. The DOCA-salt-induced hypertensive rat is a popular experimental model for use in studying antihypertensive effects. DOCA in combination with saline treatment leads to an increase in inflammation and overactive sympathetic tone [6].

Therefore, I aimed to investigate whether serum adropin levels are effective in the development of DOCA-salt-induced hypertension. I examined the relationship between hemodynamic, and hypertrophic parameters, serum adropin levels, and markers of oxidative stress.

Materials and methods

Animals and experimental protocol

Adult male Sprague-Dawley rats weighing 250–350 g were fed standard rat chow and water ad libitum and maintained under well-controlled conditions, namely, a temperature of 22 2 °C, humidity of 55 5%) and a 12-hour light/dark cycle. The rats were randomly divided into two groups of nine rats each: (1) C-control and (2) H-hypertensive). The protocol was approved by the Ethics Committee of Kutahya Health Sciences University of Animal Care and Usage, Kütahya, Turkey (Protocol numbered: 2019.01.09).

DOCA (25 mg/kg subcutaneously, twice weekly, dissolved in 0.4 ml of dimethylformamide) and salt solution were administered to rats for four weeks to induce hypertension [7]. DOCA-treated animals received 1% NaCl solutions instead of drinking water, while the control group received normal drinking water.

Blood pressure measurements and hemodynamic parameters

On the day before DOCA treatment and on the 28th day of DOCA treatment, SBP, DBP, and MBP levels were measured between 9 am and 12 pm. An indirect-tail-cuff method, monitored with a Biopac Student Lab PRO 3.7 software (Model No. MP36, AD Instruments Co.) and a pneumatic pulse generator (MAY NIBP200-A, Ankara, Turkey), was used. The rat was fixed in a plastic holder (Kursunluoglu Metal Co., Denizli, Turkey), and a tail cuff was attached to the rat's tail to obtain blood pressure measurements. Each measurement was taken three times in a quiet room at room temperature without anesthesia, and the average was taken as the value at each time point.

Blood sample collection

All animals were anesthetized with Ketamine/Xylazine HCl (10–90 mg/kg intraperitoneally) at the end of the study. Serum was collected by centrifuging blood samples from the aorta for 10 min at 4,000rpm. This serum was then stored at −20 °C until further biochemical examination.

Hypertrophic parameters

Hearts were removed, washed with saline, and weighed. The left ventricles, including the interventricular septum, were divided and the weight determined. The cardiac hypertrophy index (CHI) was calculated as the ratio of heart weight (HW, mg) to body weight (BW, g), and the ratio of the left ventricular weight (LVW, mg) to BW (g) represented the left ventricular hypertrophy index (LVHI).

Biochemical parameters

Serum concentrations of endothelin 1(ET-1), nitric oxide (NO), and adropin were determined using rat enzyme-linked immunosorbent assay (ELISA) kits (Cusabio, China). Serum concentrations of total antioxidant status and total oxidant statuses (TAS and TOS, respectively) which are oxidative stress parameters, were measured using a commercial Rel Assay kit (Mega Tip, Gaziantep, Turkey) according to the manufacturer's protocol [8, 9]. Changes in absorbance of serum samples were determined using an enzyme microplate reader (Thermo Multiscan GO, 1510, Thermo Fisher Scientific Inc., Finland). The oxidative status index (OSI) in arbitrary units was calculated as shown: OSI (arbitrary unit) = ([TOS, mmol/L]/ [TAS, mmol Trolox equivalent/L)]/100).

Statistical analysis

All values were expressed as mean (standard error of the mean (SEM) Statistical analysis was performed using the Mann–Whitney U test and the SPSS program 16.0 (SPSS Inc., Chicago, USA). The Spearman test was used for correlation analysis. A significance level of $P \leq 0.05$ was considered statistically significant.

Results

Before DOCA administration, there was no statistically significant difference in SBP, DBP, or MBP of the rats in any of the groups studied. After 4 weeks, the H group had significantly higher SBP, DBP, and MBP than the C group due to DOCA-salt treatment (Table 1).

Table 1: Hemodynamic parameters in the control and hypertension groups

	Before DOCA treatment			After DOCA treatment		
	Control (n=7)	Hypertension (n=7)	*P*-value	Control (n=7)	Hypertension (n=7)	*P*-value
SBP	121.3 (2.06)	121.7 (1.08)	0.730	122.0 (1.23)	167.4 (0.80)	<0.001
DBP	79.1 (1.87)	81.8 (0.79)	0.161	80.9 (1.49)	95.9 (0.64)	<0.001
MBP	101.6 (1.76)	101.1 (1.17)	0.931	102.1 (0.99)	122.9 (1.18)	<0.001

Continuous data are expressed as the mean (SEM), categorical variables are expressed as a percentage, and values of $P \leq 0.05$ are considered statistically significant. SBP: systolic blood pressure, DBP: diastolic blood pressure, and MBP: mean blood pressure.

Serum ET-1 levels were significantly higher in the H group than in the C group ($P = 0.014$), but serum adropin and NO levels were significantly lower ($P = 0.003$ and $P < 0.001$, respectively) as shown in Table 2. The serum TAS level of the H group was statistically lower than that of the C group ($P = 0.04$). The serum levels of TOS and OSI in the H group were statistically higher than those of the C group ($P = 0.001$ and $P <$

0.001, respectively). In addition, after DOCA injection, the CHI and LVHI values of the H group increased more than those of the C group ($P < 0.001$) as shown in Table 2.

Table 3 shows a negative correlation between serum adropin levels and SBP (r = −0.77; $P < 0.001$), DBP (r = −0.88; $P < 0.001$), MKB (r = −0.82; $P < 0.001$), CHI (r = −0.86; $P < 0.001$), LVHI (r = −0.77; $P < 0.001$), ET-1 (r = −0.48; $P = 0.04$), TOS (r = −0.54; $P = 0.01$), and OSI (r = −0.68; $P = 0.002$). In addition, a positive correlation between serum levels of adropin and NO in all groups was found (r = 0.65; $P = 0.003$).

Table 2: Biochemical and hypertrophic parameters in the control and hypertension groups

Variable	Control	Hypertension	*P*-value
ET-1	3.40 (1.00)	13.3 (2.99)	0.014
NO	2.67 (0.74)	0.88 (0.18)	0.003
Adropin	40.0 (0.99)	24.9 (1.15)	<0.001
TAS	0.63 (0.12)	0.33 (0.08)	0.040
TOS	13.8 (0.83)	25.0 (3.49)	0.001
OSI	2.84 (0.52)	11.0 (2.05)	<0.001
CHI	2.27 (0.24)	3.26 (0.14)	<0.001
LVHI	1.02 (0.13)	1.90 (0.08)	<0.001

Continuous data are expressed as the mean (SEM), and values of $P \leq 0.05$ are considered statistically significant. ET-1: Endothelin-1, NO: Nitric oxide, TAS: Total antioxidant status, TOS: Total oxidant status, OSI: Oxidative stress index, CHI: Cardiac hypertrophy index (HW/BW: heart weight/body weight), LVHI: Left ventricular hypertrophic index (LVW/BW: left ventricular weight/body weight).

Table 3: Spearman correlation between adropin and other clinical characteristics

	All groups (n = 18)		Control (n = 9)		Hypertension (n = 9)	
	r	*P*-value	r	*P*-value	r	*P*-value
ET-1	-0.48	0.040	0.09	0.803	0.16	0.668
NO	0,65	0.003	-0.06	0.871	0.88	0.452
TAS	0.40	0.090	-0.08	0.830	-0.13	0.732
TOS	-0.54	0.010	0.07	0.847	0.43	0.244
OSI	-0.68	0.002	-0.07	0.847	0.11	0.765
SKB	-0.77	<0.001	-0.12	0.744	-0.05	0.881
DKB	-0.88	<0.001	-0.87	0.002	-0.22	0.569
MKB	-0.82	<0.001	-0.39	0.288	-0.15	0.695
CHI	-0.86	<0.001	-0.11	0.762	-0.76	0.010
LVHI	-0.77	<0.001	-0.04	0.915	-0.26	0.488

$P \leq 0.05$ was considered to be statistically significant. ET-1: Endothelin-1, NO: Nitric oxide, TAS: Total antioxidant status, TOS: Total oxidant status, OSI: Oxidative stress index, SBP: Systolic blood pressure, DBP: Diastolic blood pressure, MBP: Mean blood pressure, CHI: Cardiac hypertrophy index (HW/BW: heart weight/body weight), LVHI: Left ventricular hypertrophic index (LVW/BW: Left ventricular weight/body weight).

The serum levels of adropin and DBP in the C group presented a significant negative correlation (r = −0.87; $P = 0.002$). In the H group, a negative correlation between CHI and serum adropin levels (r = −0.76; $P = 0.01$) was found. In the H group, no correlation between serum adropin and blood pressure variables was noted (Table 3).

Discussion

Arterial blood pressure has been shown to increase dramatically when rats are injected subcutaneously with DOCA and NaCl is administered in their drinking water [10]. In this study, SBP, DBP, MBP, and serum level ET-1 increased in hypertensive DOCA-salt rats when compared with controls as shown in our previous studies [11, 12]. These findings may serve as precursors for the development of hypertension in rats. In this study, a decrease in serum NO levels was observed in hypertensive DOCA-salt rats when compared with the controls. One reason for the decrease in serum levels NO could be the higher levels of ET-1 in H group. Schiffrin et al. [13] demonstrated that renin secretion from the kidney decreases when ET-1 levels increase. The DOCA-salt is a model for hypertension with low renin levels [14]. Cheng et al. [15] reported that production of NO causes a reduction in hypertension by activating the angiotensin II type 1/phosphoinositide 3 kinase/protein kinase B/endothelial nitric oxide synthase (AT1/PI3K/Akt/eNOS) pathway after renin administration. Thus, another reason for the decrease in serum NO levels could be the decrease in renin in the H group. In my previous study, I showed that serum renin levels were low in rats with DOCA-salt hypertension [11].

Sato et al. [16] showed that activation of eNOS via the PI3K/Akt pathway was impaired in DOCA-salt hypertensive rats. Lovren et al. [17] observed that adropin promotes eNOS expression via upstream activation of vascular endothelial growth factor receptor 2 (VEGFR2), which in turn activates the PI3K/Akt and ERK1/2 pathways. The reduced NO production in parallel with eNOS formation may be due to the lower adropin levels in DOCA-salt hypertensive rats. In this study, it was not possible to investigate the activation of eNOS. Allcock et al. [18] showed that the production of NO by the kidney was increased in DOCA-salt hypertensive rats. Han et al. [19] observed that plasma levels of NO were not altered by DOCA-salt hypertension. The discrepancies between these studies could be due to the temporal and spatial specificity of eNOS expressions and other upstream signaling pathways.

In this study, serum concentrations of adropin were found to decrease in hypertensive DOCA-salt rats. Topuz et al. [2] discovered low adropin concentrations in patients with endothelial dysfunction. Gulen et al. [20] demonstrated that adropin levels were significantly lower in hypertensives when compared with the normotensive group. Bolayır et al. [21] discovered that patients with non-dipping hypertension had lower serum adropin levels than hypertensives and normotensives, indicating that SBP at night was strongly negatively related to adropin. Gu et al. [22] showed that lower plasma adropin levels were related to higher blood pressure in hypertension, and ET-1 and adropin were negatively correlated. In another study, it was shown that lower adropin concentration was measured in obese children, and no correlation was found between adropin concentration and hypertension was found [23]. In this study, no correlation between serum adropin level, ET-1 level, and SBP in the H group was found. Lower serum adropin levels are associated with higher blood pressure and may not be protective against endothelial damage in DOCA-salt hypertensive rats.

It has been shown that CHI and LVHI are higher in spontaneously hypertensive rats [24]. In this study, treatment with DOCA caused a significant increase in CHI and LVHI in the H group when compared with the C group. In the H group a negative correlation between serum adropin levels and CHI was detected. No study in the literature comparing serum adropin and CHI with the results of this study has been published.

In this study, TAS decreased in the H group when compared with the C group, whereas TOS and OSI increased significantly. These findings were consistent with those from previous studies [25, 26]. Accordingly, the decrease in serum TAS and increase in TOS and OSI could lead to an increase in blood pressure in hypertensive rats. Moreover, the serum levels of TAS negatively correlated with the levels of OSI, and no significant correlations between blood pressure, adropin, TAS, and TOS in the H group were found. No study in the literature comparing adropin, TAS, and TOS in hypertension that can be compared with the findings of this study is available.

Conclusion

Although results are contradictory, studies on the association between adropin and hypertension have been published. This study is the first one in the literature to examine the levels of serum adropin in DOCA-salt hypertensive rats. As a result, serum adropin levels are reduced in DOCA-salt hypertensive rats. Lower adropin may directly affect eNOS, cause a reduction in NO production, and be associated with hypertension. These data suggest that adropin is an endogenous hypertensive factor that plays a role in hypertension onset and development.

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Electrophysiological profile of serum vitamin B12 levels, correlation with serum methylmalonic acid levels, and determination of subclinical peripheral nerve involvement

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Ethics Committee Approval
This study was approved by the Ethics Committee of Kirikkale University (date: 01.10.2018, App Nb:2018/15 Project No: 15/01).
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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

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Abstract

Background/Aim: Vitamin B12 is essential for normal neural conduction in peripheral nerves. This study aimed to investigate the electrophysiological profile for varying degrees of serum B12 levels and to determine whether a correlation existed between electrophysiological profiles and serum methylmalonic acid (MMA) levels. Also, determination of subclinical peripheral nerve involvement with cold administration was planned in serum B12 levels.

Methods: A total of 101 (63 females, 38 males) subjects with known serum vitamin B12 levels were selected randomly from the neurology outpatient clinic for this study. The subjects were divided into three groups based on the serum total Vitamin B12 levels: (1) B12 deficiency (<126 pg/mL), (2) low B12 (126-250 pg/mL), and (3) normal B12 (250–500 pg/mL). Serum MMA and nerve conduction studies (NCS) were assessed and compared between the three groups. After the cooling procedure was applied to the ulnar and sural nerves, NCS was repeated.

Results: There were 13 subjects in the B12 deficiency group, 44 subjects in the low B12 group, and 44 subjects in the normal group. We found that ulnar sensory nerve action potential amplitudes were significantly decreased (P = 0.009), ulnar F latency (P = 0.003; P < 0.001)) was prolonged, and peroneal combined muscle action potential amplitudes decreased (P = 0.026) in the B12 deficiency when compared with the low and normal B12 groups. Sural nerve amplitude and conduction velocities were found to be significantly abnormal after the cold application in all groups (P < 0.001). The increase in sural nerve sensory nerve amplitude potentials (SNAP) amplitudes was higher in the B12 deficiency group than in the other groups. Mean serum MMA levels were high in all groups. A correlation of nerve conduction study (NCS) changes with serum vitamin B12 and MMA was not observed in the groups after cold application.

Conclusion: Vitamin B12 deficiency may cause subclinical sensorial and motor axonal nerve conduction changes. Nerve conduction changes may not always reach pathological values based on electrophysiological studies but may be detected after cooling administration even in the normal serum B12 levels. A correlation between serum MMA and vitamin B12 levels was found. Therefore, serum levels of vitamin B12, which is important for nerve conduction, should be carefully evaluated in clinical practice.

Keywords: Vitamin B12, Neuropathy, Cold, Biomarkers

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Introduction

Vitamin B12 (cobalamin) is a water-soluble vitamin that has a role in cellular and mitochondrial metabolism. The most common reason for its deficiency is inadequate dietary vitamin B12 intake in adults and malabsorption in the elderly [1]. In the laboratory, it is usually diagnosed through measurements of serum vitamin B12 levels. Although no agreed-upon cut-off points for B12 deficiency and low serum B12 levels have been established, values of 148 pg/mL for B12 deficiency and 260 pg/mL for low B12 levels are frequently used [2]. Holotranscobalamin (holoTC), homocysteine, and methylmalonic acid (MMA) are other markers of vitamin B12 deficiency [3]. The occurrence of the typical neurological symptoms may be expected when serum vitamin B12 levels are generally lower than 200 pg/mL, but they may also occur at levels up to 400 pg/mL [4]. Symptoms and serum vitamin B12 levels may not be correlated, and neurological manifestations might be seen before hematological disorders are detected [5].

Vitamin B12 acts as a coenzyme in the synthesis of myelin and plays a role also in the conversion of methylmalonyl-CoA into succinyl-CoA in the nervous system. In cases of vitamin B12 deficiency, this reaction is insufficient and leads to elevated MMA and impairment in the structure of myelin. Thethe methionine synthase reaction in DNA synthesis is also impaired, which leads to elevated levels of homocysteine [6]. Vitamin B12 also has a neuroprotective effect via the action of reactive oxygen species, thereby causing an increase in axonal regeneration and facilitating the repair of neuronal damage [7]. Peripheral neuropathy may be seen in approximately 25% of subjects with B12 deficiency that may be associated with various pathologies. Neuropathy is mostly axonal. Symptoms are usually symmetrical, painless, and sensorial. Some subjects may also have subclinical involvement based on electrophysiological studies [8].

The effects of temperature were examined using electrophysiological studies based on conduction physiology in peripheral nerves, a technique that could be used to reveal some conduction pathologies or clinical findings [9]. The cooling procedure might reveal subclinical peripheral nerve involvement that is associated with the effects of different serum B12 levels on peripheral nerve conduction.

The aims of our study were to investigate the electrophysiological profiles for varying degrees of serum B12 levels ranging from deficient/low to normal and to determine whether a correlation exists between electrophysiological profiles and serum MMA levels, which is known as a functional marker of B12 deficiency. Also, determination of subclinical peripheral nerve involvement after cold administration was planned in serum B12 levels in this study.

Materials and methods

Study design and subjects

Subjects who presented to the neurology outpatient clinic to nonspecific headache and dizziness without neuropathic symptoms and known serum vitamin B12 levels were enrolled and categorized into three groups based on serum vitamin B12 levels. First, the subjects were separated into those with B12 deficiency (<126 pg/mL) and those with serum B12 levels within laboratory range (reference range in our laboratory: 126–500 pg/mL). Subjects who had serum B12 levels within the laboratory range were further divided into two groups: (1) low B12 (126–250 pg/mL) and (2) normal B12 (250–500 pg/mL). Venous blood samples were taken from three groups to measurement MMA. Electrophysiological studies and neurological examinations were performed by a neurologist. Results of nerve conduction studies (NCS) were compared between the three groups by the same neurologist.

Exclusion criteria for subjects consisted of plexopathy, radiculopathy, diabetes mellitus, acute/chronic renal dysfunction, thyroid disease, metabolic disorders, malignancy, history of chemotherapy and/or radiotherapy, vasculitis, alcohol/substance addiction, neurodegenerative diseases, presence of diagnosis of polyneuropathy, and/or symptoms suggesting neuropathic pains.

This study was approved by the institutional ethics committee (15/01-01.10.2018). Informed consent was waived from all subjects before the study.

Electrodiagnostic methods and cooling

NCS were performed on a Medelec Synergy electromyography (EMG) device at room temperature (25 °C) in the neurophysiology laboratory of a tertiary center. The skin and electrode felts were cleaned before the measurements were taken to minimize skin resistance. In each subject, NCS of the median (right), ulnar (left), tibial (right), peroneal (left), and sural (left) nerves were performed using superficial bipolar electrodes. The amplitude and distal motor latency (DML) of compound muscle action potentials (CMAP) and nerve conduction velocities (NCV) were measured after stimulation of the median nerve (wrist/elbow), ulnar nerve (wrist, lower and upper elbow), common peroneal nerve (ankle/fibula head), and tibial nerve (ankle/knee) after supramaximal stimulation in motor conduction studies. The amplitude of the sensorial nerve action potential (SNAP) and sensorial NCV were evaluated using orthodromic methods in the median nerve (second finger interphalangeal joint space and palm), ulnar nerve (fifth finger- wrist with a distance of 12 cm), and sural nerve (lateral malleolus-foreleg with a distance of 14 cm) conduction studies. F-wave latency of each motor nerve was evaluated. Amplitude measurements were calculated from baseline to negative peak, and sensory and motor distal latency measurements were also calculated from baseline to the first negative peak. The laboratory reference values were considered a sural sensorial nerve velocity of > 40 m/s and amplitude > of 5 μV [10].

Cooling was carried out at 18 °C with a freezing air-free ice pack on the skin over the ulnar nerve and sural nerve tract for 10 min after which skin temperature (< 25 °C) was confirmed with a superficial heat meter oof an electromyography (EMG) device. It was confirmed that the EMG device was cooled to lower 25 °C. Ulnar nerve motor conduction, F-wave latency, and sural nerve conduction studies were repeated after cold application.

Measurement of serum methylmalonic acid (MMA)

Serum MMA levels were measured in venous blood samples using micro enzyme-linked immunosorbent assay ([ELISA] Andy Gene Biotechnology Co. Ltd, Serial number: AD20022Hu). Biochemical tubes without preservatives were

filled with 5 mL of blood. centrifuged for 30 min at 2000 rpm, and stored at –80 °C until used for analysis.

All samples were studied concurrently. The measurement range of the reagent was 0.5 to 40 pg/mL Samples were studied by making 5-fold dilutions with sample diluents. Samples over 200 pg/mL were studied with 10-fold dilutions. The sensitivity of the reagent was 0.1 pg/mL. The results of test were expressed in nmol/L (laboratory range: 0.05–0.26 nmol/L, cutoff point for diagnosing functional cobalamin deficiency was taken as 0.376 nmol/L) [11].

Statistical analysis

Based on the research of Leishear and colleagues, sample number was calculated with G.Power3.0.10. This study was designed as cross-sectional in a similar way with his study as a reference.. Leishear and his colleagues carried out a cross-sectional research study on B12 vitamin levels and peripheral nerve function involving 2287 participants. In this study, the vitamin B12 deficiency was found in0.069% of the participants and an additional 10.1% had low serum B12 levels. Also, in this research, B12 deficiency was associated with worse sensory and motor peripheral nerve function (odds ratio [OR]: 1.50;95%confidence interval [] 1.06–2.13). With the resulting data, the sample that was calculated with α err prob = 0.05, Power (1-β err prob) = 0.80 was found to be 101.

Study data were evaluated using the SPSS 22.0 statistical package program. Descriptive statistics were expressed as means (standard deviations), medians (min–max), frequency distributions, and percentages. For categorical variables, the difference between the groups in terms of frequency was evaluated using a chi-squared test. The suitability of continuous variables to normal distribution was evaluated using the Kolmogorov–Smirnov or Shapiro–Wilk test. Numerical variables showing normal distribution among the groups were evaluated using one-way analysis of variance (ANOVA), and non-normally distributed numerical variables using the Kruskal–Wallis test. If a statistically significant difference was found, the origin of the difference was determined using post hoc tests (Bonferroni test/Mann–Whitney U test). The evaluation of conduction changes before and after cold application was performed using the paired t-test for data with normal distribution and Wilcoxon's signed-ranks test for data without normal distribution. The relationship between vitamin B12 and MMA levels was evaluated using Spearman's correlation test. Statistical significance was accepted as $P < 0.05$.

Results

A total of 101 (63 females, 38 males) subjects with a mean age of 30.8 (10.24) years were included in the study. Thirteen subjects were included in the B12 deficiency group (serum levels <126 pg/mL), 44 subjects in the low B12 group (serum levels: 126–250 pg/mL), and 44 subjects in the normal group (serum levels: 250–500 pg/mL). Adverse effects were not observed due to the cold application.

The characteristics of the groups are shown in Table 1.

Table 1: Characteristics of groups

Data	B12 deficiency (n = 13)	Low B12 (n = 44)	Normal B12 (n = 44)	*P*-value
Age, years	32.69 (14.38)	28.72 (9.27)	32.31 (9.62)	0.191
Gender, female/male, n	2 /11	29/15	32/12	0.001[2]
Vitamin B12 (pg/mL)	104.91 (12.26)	200.22 (37.94)	335.71 (60.90)	<0.001[1]
Serum MMA level (nmol/L)	148.84 (230.4)	88.79 (103.4)	84.68 (125.6)	0.952

Data shown are mean (SD) unless otherwise specified. [1] Kruskal–Wallis test, [2] Chi-squared test

Results of nerve conduction studies

Motor nerve conductions

Motor nerve conduction values were found to be were normal according to age and length in all groups. However, we found statistically significant differences between the groups.

In the upper extremities, ulnar nerve F latency was found to be significantly between the groups ($P = 0.001$). It was prolonged only in the low B12 group (mean 28.01 [1.8] ms in B12 deficiency, 26.51 (1.64) ms in low B12, and 26.10 [1.41] ms in normal B12, $P = 0.001$). The statistical differences were significant between the B12 deficiency and low B12 ($P = 0.003$) and the B12 deficiency and normal B12 ($P < 0.001$) groups. Other parameters, such as compared motor action potential and distal motor latency (CMAP and DML, respectively) for ulnar and median nerve motor conduction studies did not show statistically significant differences.

Tibial nerve DMLs were found to be different in the groups ($P = 0.026$). DMLs for tibial nerve were found to be significantly prolonged in the B12 deficiency and low B12 groups when compared with the normal B12 (4.58 [0.50] ms in B12 deficiency, 4.49 [0.50] ms in low B12, and 4.26 [0.43] ms in normal B12; $P = 0.024$ and $P = 0.038$, respectively). Other parameters for peroneal and tibial nerve motor conduction studies showed no statistically significant differences in the lower extremities.

Sensorial nerve conductions

Ulnar (digit V) SNAP amplitudes were found to be significantly lower in the B12 deficiency versus the low B12 and normal B12 groups (12.53 [3.02] μV in B12 deficiency, 24.95 [2.3] μV in Low B12, and 25.02 [14.74] μV in normal B12; $P = 0.009$). No statistically significant differences between the groups according to sural nerve sensorial conduction studies were detected ($P > 0.05$).

Cooling procedure

In all groups, no significant differences in CMAP amplitudes (distal) and NCV (proximal) parameters of the ulnar nerve (before and after cold) were found ($P > 0.05$). However, DML and F-wave latency led to a significant prolongation and NCV (distal) was significantly decreased in the ulnar nerve after cold application ($P < 0.001$, $P < 0.001$, and $P < 0.05$, respectively). CMAP amplitude (proximal) significantly decreased only in the low B12 group.

For the sural nerve, significant increases in SNAP amplitude and a decrease in NCV in all groups were found ($P < 0.001$). The increase in SNAP amplitude of the sural nerve was higher in the B12 deficiency group than in the other groups (B12 deficiency group versus low B12 group; $P = 0.049$, B12 deficiency group versus normal B12 group; $P = 0.154$, and low B12 group versus normal B12 group 3; $P = 0.478$). Although the mean sural NCV of all groups was found to be <40 m/s, no significant differences among the groups in terms of sural NCV ($P > 0.05$) were detected.

Table 2: Pre- and post-cooling nerve conduction parameters of groups

Parameter	B12 deficiency (n = 13)			Low B12 (n = 44)			Normal B12 (n = 44)		
	Pre-cooling	Post-cooling	*P*-value	Pre-cooling	Post-cooling	*P*-value	Pre-cooling	Post-cooling	*P*-value
L, Sural nerve									
SNAP amplitude (μV)	19.88 (9.31) (5–40.3)	26.26 (12.91) (7.8-54.2)	0.003[1*]	17.25 (8.36) (5-43.3)	19.75 (7.71) (5.6–39.7)	0.004[1]	17.17 (7.6)	20.83 (8.17)	0.001*
NCV (m/sn)	50.39 (7.72) (37.5–67.6)	33.59 (7.90) (25-52.1)	0.001[2*]	50.82 (5.17) (40-65.3)	32.24 (6.1) (19.7–48)	<0.001[2]	49.74 (4.63)	32.99 (5.6)	<0.001[2]
L, Ulnar nerve									
DML (ms)	2.5 (0.51) (1.88–4.01)	2.65 (0.55) (2.08-4.27)	0.007[2]	2.47 (0.32) (1.93-3.28)	2.7 (0.42) (1.98-3.65)	<0.001[2]	2.55 (0.34)	2.78 (0.41)	<0.001[2]
CMAP amplitude									
Distal (μV)	11.59 (2.07) (8.2–15.4)	11.28 (2.24) (7.9-16)	0.16[2]	13.04 (1.62) (10.3-17.5)	12.92 (1.96) (9.2–18.5)	0.87[2]	12.77 (2.36)	12.8 (2.44)	0.73
Proximal (μV)	10.94 (2.18) (7.3–15)	10.26 (2.5) (6.2-15.9)	0.05[1]	12.3 (1.73) (9.8-17.8)	11.82 (2.06) (7.8–18.2)	0.01[1]	11.88 (2.33)	11.58 (2.26)	0.08
F wave latency (ms)	28.01 (1.81) (25.16–32.03)	29.84 (2.42) (25.31-33.34)	0.002[2*]	26.51 (1.64) (23.49-29.9)	28.13 (2.19) (24.48-33.18)	<0.001[2]	26.10 (1.41)	27.66 (1.95)	<0.001[2]
NCV, Distal (m/sn)	54.47 (3.83) (47.5–61.8)	48.32 (3.93) (43.7-56.4)	0.002[2*]	56.77 (4.08) (50.3-69.8)	51.12 (5.27) (37.9–64.5)	<0.001[2]	56.75 (40.17)	51.35 (4.87)	<0.001

SNAP: Sensorial nerve action potential, NCV: Nerve conduction velocity, DML: Distal motor latency, CMAP: Compound muscle action potential, Data as mean (SD) or median (min–max) in B12 deficiency and Low B12, mean (SD) in Normal B12, [1] Paired T test, [2] Wilcoxon Signed Ranks test

The pre- and post-cooling nerve conduction parameters of the groups are shown in Table 2.

Correlation between MMA and vitamin B12 levels

Mean MMA levels were high in all groups:(1) 17.56 (27.18) nmol/L (min–max: 0.37-93.83) in the B12 deficiency group, 10.47 (12.20) nmol/L (min-max:1.55-58.41) in the low B12 group, and 9.99 (14.82) nmol/L (min-max:0.35-75.24) in the normal B12 group. However, no significant correlation between MMA and vitamin B12 levels between the groups was found. These results are presented for the three groups:: (1) B12 deficiency group (r = –0.084; *P* = 0.79); (2) low B12 group (r = 0.027; *P* = 0.86); and (3) normal B12 group (r = –0.017; *P* = 0.91). No significant correlations between MMA and vitamin B12 in the B12 deficiency group (r = –0.084; *P* = 0.79), the low B12 group (r = 0.027; *P* = 0.86), and normal B12 group (r = –0.017; *P* = 0.91) were found.

Correlation between serum MMA, B12 levels, and alterations in nerve conduction parameters after the cooling procedure

Nerve conduction parameter alterations after the cooling procedure were not found to be correlated with any of the serum levels of vitamin B12 or MMA (*P* = 0.89, *P* = 0.734).

Discussion

Vitamin B12 is one of the neurotropic vitamins and contributes to optimal nerve function in the peripheral nervous system. Deficient or functionally deficient levels of the B12-dependent metabolites despite normal serum vitamin B12 levels may affect nerve conduction, which could result in neuropathy [6]. The findings that could be expected based on the electrophysiological studies are slowing of the conduction velocity of the sensory nerves and/or a decrease in SNAP amplitude in the presence of neuropathy due to B12 deficiency although such changes may or may not be correlated with serum B12 levels [12, 13].

In this study, we did not find significant NCS changes, which would be indicative of peripheral neuropathy, in any vitamin B12 level groups even in those with B12 deficiency. Furthermore, sural nerve conduction parameters, which are important parameters for the diagnosis of peripheral neuropathy, were within normal ranges even in the deficiency group [14]. However, we found that a decrease in sensorial (median and ulnar) nerve amplitude, prolonged ulnar F-wave latencies, and tibial DMLs in addition to a decrease in peroneal CMAP amplitudes in B12 deficiency. With this evidence, we suggest that these findings indicate axonal involvement in sensorial fibers and mixed-type involvement in motor nerve conduction parameters in the B12 deficiency group. These findings were found to be statistically significant when compared with the NCS of other serum B12 level groups. It has been previously reported that the electrophysiological findings of subjects with asymptomatic/symptomatic vitamin B12 deficiency (mean serum level <200 pg/mL) revealed inconsistent pathological and electrophysiological findings. Neuropathy has not been observed electrophysiologically by researchers in any subjects with B12 deficiency [8, 15, 16]. A comparison of symptomatic subjects with B12 deficiency and healthy controls showed that NCS of asymptomatic subjects with vitamin B12 deficiency were not different from healthy subjects [17]. It is also known that nerve conduction changes do not correlate with vitamin B12 levels. However, we observed that sensory nerves might have a tendency to axonal-type involvement in B12 deficiency although not at a neuropathic level.

In this study, another important finding was that sural NCVs achieved neuropathic ranges in all groups after cooling, but no statistically significant differences in terms of the decrease in sural nerve NCVs between the groups after the cooling procedure were detected. In fact, a linear curve between the decrease in temperature and NCV was found, and it is generally reported that a 1 °C variation in surface temperature causes a reduction in NCV of about 1.5 to 2 m/s [18–21]. However, we observed a more apparent reduction in sural NCVs after the cooling procedure. We suppose that myelin might have had an effect on sensory nerves at serum B12 levels of <500 pg/mL. Previous research has shown that electrophysiologically, the underlying demyelinating pathology results from cold application in clinical carpal tunnel syndrome cases with normal nerve conduction [22]. In vitamin B12 deficiency, axonal-weighted features are generally expected in NCS, but an effect of Schwann cells in the peripheral nervous system, which has a closer relationship with axons than oligodendrocytes in the central nervous system, has been shown in animal experiments. Accordingly, Schwann cell activation and intra-myelin edema has been observed to develop without demyelination or remyelination with little or no axonal change [23–25]. After cooling, we also observed a significant increase in sural SNAP amplitudes in all three groups. A remarkable increase in the B12 deficiency group was observed. The effect of temperature on neuromuscular electrophysiology has been reported as an increase in amplitudes and decrease in NCVs, and the change in amplitude is much greater than that seen in normal subjects with axonal disorders [9]. We suggest that the B12 deficiency group might have axonal involvement in sensory nerves as we previously concluded. Asymptomatic subjects with B12 deficiency who had normal NCS at the first examination were found to have abnormal NCS findings after cooling, indicating axonal neuropathy.

We chose the sural and ulnar nerves to reveal silent changes in the peripheral nerves after cold treatment in this study. It is known that cold applications administered via various methods (ice pack, ice massage, cold water immersion) have a more complex effect, particularly on the sensory nerves [9]. We also observed the expected physiological effects on ulnar nerve conduction parameters (amplitude, velocity, F-wave latency, and DML) after cold application, but the changes were not pathological.

In clinical practice, investigation of serum levels of vitamin B12 is the most common approach in the assessment of vitamin B12 deficiency. The levels of homocysteine, holoTC, and MMA can also be used in the laboratory although a lack of consensus on the best marker in this regard exists. Harrington et al. reported that vitamin B12 serum levels lacked the necessary sensitivity for indicating actual deficiencies [26]. In previous studies, Nexo et al. [27] and Herrmann et al. [28] reported that holoTC was being more sensitive if creatine levels were within the normal range. Serum MMA levels may be more reliable for assessing vitamin B12 deficiency in conjunction with holoTC, another metabolite, and for investigating MMA in the urine. MMA is biochemically more stable in urine than in serum in which concentrations are 40 times greater than in serum [29]. Sun et al. reported that the urine levels of MMA may be a marker in the assessment of B12 serum levels in polyneuropathy in diabetic patients [30]. We found high serum MMA levels according to our laboratory range, but no relationship between serum MMA and B12 levels and also NCS was found. Serum MMA levels were not found to have a predictive value for electrophysiological results.

Limitations

This study has some limitations. The B12 deficiency group consisted of a small number of subjects, and we did not examine homocysteine levels. The B12 laboratory reference range in our study appears slightly different from the literature, but this difference did not constitute a limitation. A larger study group and urinary MMA levels might be recommended for future research.

Conclusion

In a summary, we found that subjects who had vitamin B12 deficiency have a tendency to develop axonal/demyelinating-type involvement in both sensory and motor nerve fibers. Nerve conduction changes may not always reach pathological values in electrophysiological studies but may detected after cooling treatment even in those with normal serum B12 levels. Cooling is an easy test to perform. A correlation between serum MMA and vitamin B12 levels was found. Serum levels of vitamin B12, which is important for nerve conduction, should be carefully evaluated in clinical practice.

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Evaluation of the effectiveness of quick COVID-19 Severity Index and COVID-GRAM Critical Illness Risk Score in determining mortality and severity in COVID-19

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Ethics Committee Approval
Ethics Committee approval was taken from the İzmir Katip Çelebi University Local Ethics committee, 25/05/202, Decision number: 0288. All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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

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Abstract

Background/Aim: With the COVID-19 pandemic, the increase in the number of patients admitted to the emergency department has led to an increase in the need for intensive care and mechanical ventilation. Methods that can predict the development of serious disease will allow for a more accurate use of resources. This study was conducted to test the ability of the Quick COVID-19 Severity Index and the COVID-GRAM Critical Illness Risk Score to predict serious disease development and mortality.
Methods: This is a prospective cohort study. Among the patients admitted to the emergency department, those hospitalized due to COVID-19 were included in the study. The Quick COVID-19 Severity Index and COVID-GRAM Critical Illness Risk Scores of the patients were calculated, and the ability of these scores to predict serious illness and mortality was investigated.
Results: A total of 556 patients were included in this study. Development of critical illness, described as the need for non-invasive / invasive ventilation or the need for intensive care unit admission, was found significant when the Quick COVID-19 Severity Index was above 5 and the COVID-GRAM Critical Illness Risk Score showed high risk (AUC: 0.927; $P < 0.001$, AUC: 0.986; $P < 0.001$, respectively). A Quick COVID-19 Severity Index over 6 and COVID-GRAM Critical Illness Risk Score indicating high risk were found to be associated with mortality (AUC: 0.918, $P < 0.001$, AUC: 0.982, $P < 0.001$, respectively).
Conclusion: Both the Quick COVID-19 Severity Index and the COVID-GRAM Critical Illness Risk Score can be used to assess severity in COVID-19 patients in the emergency room. However, the COVID-GRAM Critical Illness Risk Score was more successful in differentiating low- and high-risk patients.

Keywords: COVID-19, Severity, Mortality, Emergency department

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Introduction

COVID-19 is a serious health problem that may cause critical illness and even death. In COVID-19, critical illness is generally associated with multi-organ failure and pneumonia that can progress to acute respiratory distress syndrome ARDS [1]. With the onset of the pandemic, hospitals around the world have faced an influx of COVID-19 patients, and a serious workload and resource shortage has developed. Therefore, early recognition of COVID-19 patients at high risk of critical illness and death, as well as the prevention of unnecessary hospitalization of low-risk patients to the intensive care unit (ICU) and unnecessary resource consumption has become a serious necessity [2]. Some early warning scores, such as the quick sepsis-related organ failure assessment (qSOFA), the Rapid Emergency Medicine Score (REMS), the Modified Early Warning Scores (MEWS), the National Early Warning Score (NEWS) and the National Early Warning Score-2 (NEWS-2) were evaluated for use in patients with COVID-19 and found to be beneficial [3, 4]. However, these scorings are not specific for COVID-19 and are suitable for the general patient population.

Recently, Quick COVID-19 Severity Index (qCSI), and COVID-GRAM Critical Illness Risk Score (COVID-GRAM), specific to COVID-19, were developed to assess disease severity. Developed by Haimovich et al. [5], qCSI is a simple scoring that assesses the probability of severe shortness of breath in a COVID-19 patient at 24 hours. The COVID-GRAM, developed by Liang et al. [6], evaluates the risk of developing critical illness and mortality. Few studies have been conducted that evaluate the efficacy of both scorings.

The aim of this study is to assess the effectiveness of qCSI and COVID-GRAM to evaluate the risk of critical illness and mortality in subjects diagnosed with COVID-19 in the emergency department (ED).

Materials and methods

Study design

This is a prospective observational cohort study. Approval was obtained from the Izmir Katip Celebi University non-interventional clinical studies ethics committee with the application number 2021- GOKAE - 0346 and the decision number 0288. Written consent was obtained from all subjects to participate in the study.

Setting

The study was carried out in the ED of a tertiary hospital receiving 400,000 admissions annually, from Jan. 6, 2021 to Dec. 31, 2021. In the ED, there are two main sections, the isolated area where patients with a diagnosis of COVID-19 are treated, and the clean area where patients other than COVID-19 are treated. In the isolated area, one nurse, one emergency medicine specialist, and one emergency medicine resident doctor work in each shift. This study was conducted in an Celebi University non-interventional clinical studies ethics committee isolated area.

Participants

Patients over the age of 18 presented to the ED with a confirmed diagnosis of COVID-19 or whose diagnosis of COVID-19 was confirmed by RT-PCR after applying to the ED were included in the study. Pregnanacy, trauma, presence of intubation and/or cardiopulmonary arrest at the time of admission were determined as exclusion criteria.

Variables

The primary outcome of the study was the development of critical illness, which was defined as presence at least one of the following [7];

1. The need for non-invasive ventilation
2. The need for invasive ventilation
3. The need for ICU admission.

Mortality was the secondary outcome of the study.

Data sources

Age, gender, comorbid diseases, history of hemoptysis, cancer history, presence of dyspnea, Glascow coma scale, respiratory rate, SO2, O2 flow rate, neutrophil/lymphocyte ratio, lactate dehydrogenase, and direct bilirubin level were recorded for the subjects who met the inclusion criteria. Using the recorded data, qCSI and COVID-GRAM were calculated for each one of the subjects. The qCSI is a scale calculated using respiratory rate, SO2, and O2 flow rate and scored between 0 and 12 points [5]. The qCSI is also available as a web-based risk calculator (https://www.mdcalc.com/calc/10304/quick-covid-19-severity-index-qcsi#evidence. Access date: Sept. 13, 2022). The COVID-GRAM is calculated by using the data of abnormal radiological findings, age, hemoptysis, dyspnea, altered consciousness, number of comorbid diseases, presence of cancer, neutrophil/lymphocyte ratio, lactate dehydrogenase level, and direct bilirubin level It is a scoring that categorizes the risk as low, moderate and high [6]. COVID-GRAM is also available as a web-based risk calculator (https://www.mdcalc.com/covid-gram-critical-illness-risk-score#next-steps. Access date: Jan. 13, 2022).

Bias

Study data were collected by a nurse working outside the ED who was blinded to the study to avoid potential bias, as it may influence the decisions of the patient's primary physician.

Study size

The sample size was calculated using the computer program G*Power 3.1.9.2. When calculating the sample size, according to the data obtained from a previous similar study, H1: 15%, H0: 56%, and the odds ratio was 9.4 [8]. The calculated sample size was 402, with an alpha value of 0.05 and a power of 0.95.

Statistical analysis

Data obtained in the study were analyzed using IBM SPSS Statistics for macOS, Version 26.0. Armonk, NY: IBM Corp. Categorical variables were expressed as numbers and percentages, while numerical variables were expressed as mean and standard deviation when presenting the descriptive statistics. ROC analysis was used to evaluate the power of the scales to predict the risk of critical illness and mortality and to determine the appropriate cut-off values. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were used to evaluate the success of the tests in predicting critical illness and mortality. The Chi-square test was used for the comparison of two categorical variables. The results were expressed at a 95% confidence interval. *P* value, and less than 0.05 was considered statistically significant.

Results

A total of 556 patients diagnosed with COVID-19 were included in the study. The mean age of the patients, of whom 286 (51.4%) were male, was 48 (19) years. Other socio-demographic data of the subjects are presented in Table 1.

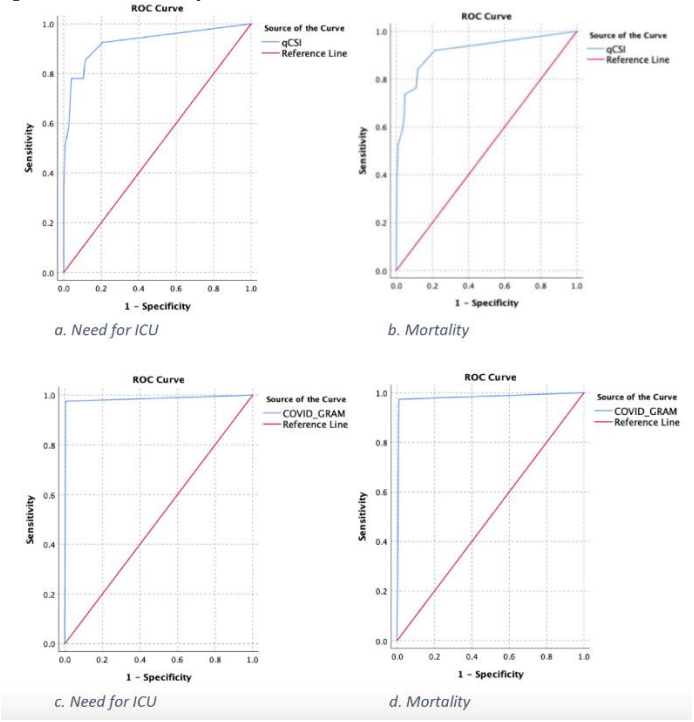
Table 1: Socio-demographic characteristics of subjects

		Mean	SD
Age		48	19
Respiratory rate		16	4
Oxygen saturation (%)		93	13
		Number	%
Gender	Female	270	51.4
	Male	286	48.6
Admission	Discharged	380	68.3
	Admitted to ward	140	25.2
	Admitted to ICU	36	6.5
Mortality	No	518	93.2
	Yes	38	6.8

SD: standard deviation, ICU: Intensive care unit

In the ROC analysis to evaluate the power of the COVID-GRAM and qCSI to predict critical illness and mortality, the area under the curve (AUC) of qCSI in predicting the development of critical illness was 0.927% (0.874-0.979). T and the cut-off value was 5. The AUC of qCSI to predict mortality was found to be 0.918% (0.861-0.975) with a cut-off value of 6 ($P < 0.001$, $P < 0.001$, respectively). In the ROC analysis for the COVID-GRAM, the AUC of the risk for development of critical illness was 0.986% (0.958-1.013), and the AUC for the risk of mortality was 0.982% (0.952-1.012). The score indicates high risk ($P < 0.001$, $P < 0.001$, respectively). The ROC curves of COVID-GRAM and qCSI scores for estimating mortality and critical illness risk are given in Figure 1.

Figure 1: ROC curves for qCSI



It has been observed that the COVID-GRAM is associated with the development of critical illness and mortality, and this relationship is due to the high rate of critical illness and high mortality in the high-risk group. In low and medium risk groups, critical illness and mortality rates were found to be similarly low ($P < 0.001$, $P < 0.001$, respectively). Considering the relationship of qCSI with critical illness and mortality, it was seen that a score above 5 was significant in terms of the development of critical illness, and a score above 6 was significant in terms of mortality ($P < 0.001$, $P < 0.001$ respectively) (Table 2).

Table 2: Association of COVID-GRAM critical illness risk score and qCSI with mortality

		Mortality			
		No	Yes	Total	*P*-value
COVID-GRAM	Low risk	375 (100.0%)	0 (0.0%)	375	<0.001
	Medium risk	138 (99.3%)	1 (0.7%)	139	
	High risk	5 (11.9%)	37 (88.1%)	42	
Total		518 (93.2%)	38 (6.8%)	556	
qCSI	≤6	<0.001	10 (2%)	503	<0.001
	>6	<0.001	28(52.8%)	53	
Total		518 (93.2%)	<0.001	556	
		Development of critical illness			
		No	Yes	Total	*P*-value
COVID-GRAM	Low risk	375 (100.0%)	0 (0.0%)	375 (100.0%)	<0.001
	Medium risk	138 (99.3%)	1 (0.7%)	139 (100.0%)	
	High risk	2 (4.8%)	40 (95.2%)	42 (100.0%)	
Total		515 (92.6%)	41 (7.4%)	556 (100%)	
qCSI	≤5	461 (98.1%)	9 (1.9%)	470 (100%)	<0.001
	>5	54 (62.8%)	32(37.2%)	86 (100%)	
Total		515 (92.6%)	41 (7.4%)	556 (100%)	

COVID-GRAM: COVID-GRAM Critical Illness Risk Score, qCSI: quick COVID-19 severity index

It was observed that qCSI, with a cut-off value of 5 could predict the development of critical illness with a sensitivity of 78% and a specificity of 90% (PPV: 37, NPV: 98). Since the low- and medium-risk scores were similar according to the COVID-GRAM, both were considered low risk for Intensive Care Unit (ICU) admission. Thus, two risk categories were obtained as low/medium risk and high risk. According to these two categories, this scoring can predict the development of critical illness with 98% sensitivity and 99% specificity (PPV: 95, NPV: 99) (Table 3).

Considering the diagnostic value of the tests in terms of mortality, it was seen that qCSI with a cut-off value of 6 could predict mortality with 74% sensitivity and 95% specificity (PPV:53, NPV:98). Since the low- and medium-risk scores were similar according to the COVID-GRAM, both were accepted as low risk in terms of mortality. Thus, two risk categories were obtained as low/medium risk and high risk. According to these two categories, this scoring can predict mortality with 97% sensitivity and 99% specificity (PPV: 88, NPV: 100) (Table 4).

Table 3: Predictive value of qCSI and COVID-GRAM critical illness risk score regarding critical illness development

Test	Risk category	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
qCSI	≤5 (low)	78	90	37	98
	>5 (high)	(62.3-89.4)	(86.54-92.02)	(30.5-44.4)	(96.64-98.92)
COVID-GRAM	Low/medium	97.6	99.6	95	99.8
	High	(87.1-99.9)	(98.6-99.9)	(83.4-98.8)	(98.7-99.9)

COVID-GRAM: COVID-GRAM Critical Illness Risk Score, qCSI: quick COVID-19 severity index, PPV: Positive predictive value, NPV: Negative predictive value

Table 4: Predictive value of qCSI and COVID-GRAM critical illness risk score regarding mortality

Test	Risk category	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
qCSI	≤6 (low)	74	95	53	98
	>6 (high)	(56.9-86.6)	(92.9-96.85)	(42.2-63.2)	(96.7- 98.8)
COVID-GRAM	Low/medium	97	99	88	99.8
	High	(86.2-99.9)	(97.8-99.7)	(75.5-94.7)	(98.7- 99.9)

COVID-GRAM: COVID-GRAM Critical Illness Risk Score, qCSI: quick COVID-19 severity index, PPV: Positive predictive value, NPV: Negative predictive value

Discussion

In this study, which was conducted to evaluate the success of COVID-GRAM and qCSI in determining the severity of COVID-19 patients admitted to the ED, both scores were found to be successful in predicting both need for ICU and mortality.

According to Armiñanzas et al. [8], COVID-GRAM was more successful than the CURB-65 score in estimating the severity of COVID-19 disease, but both scorings can be used for

risk classification. In the study conducted by Doğanay et al. [9], the CURB-65 score was found to be more successful than the COVID-GRAM. Rodriguez-Nava et al. [10] found that qCSI was successful in predicting ICU hospitalization in COVID-19 patients. However, to our knowledge, this study is the first to compare the COVID-GRAM and qCSI in predicting the development of critical illness in COVID-19 patients. In the present study, we found that a high COVID-GRAM and a qCSI above 5 were significant in predicting the risk of developing critical illness in patients with COVID-19. However, the COVID-GRAM Critical Illness Risk Score was found to be more successful than qCSI in both identifying and ruling out critical illness (Sensitivity: 97.56 vs. 78, Specificity: 99.61 vs. 90, PPV: 95 vs. 37, NPV: 99.81 vs. 98).

In a study by Martin-Rodriguez et al. [11], CURB-65 and qCSI were compared to predict mortality in COVID-19 patients, and CURB-65 was found to be more successful. Armiñanzas et al.'s [8] results indicated that the COVID-GRAM was effective in showing 30-day mortality and was more successful than CURB-65 in this regard. A study by Covino et al. [12] found that the ISARIC-4C score, COVID-GRAM, NEWS, and qCSI had similar success in predicting in-hospital mortality in COVID-19 patients. In the present study, we found that the COVID-GRAM indicating high risk and qCSI above 6 were significant in predicting the risk of mortality in patients with COVID-19. However, the COVID-GRAM was found to be more successful than qCSI in both identifying and ruling out risk of the development of critical illness (Sensitivity: 97 vs. 74, Specificity: 99 vs. 95, PPV: 88 vs. 53, NPV: 99.81 vs. 98).

This study has some limitations. Vaccination information of patients for COVID-19 was not questioned. Therefore, the possible effects of the vaccine on the development of critical illness or mortality may have affected our results.

Conclusion

COVID-GRAM and qCSI appear to be promising tools for predicting critical illness development and mortality in patients with COVID-19. However, this still needs to be confirmed by further studies.

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Paraoxonase 1 (PON1) gene Q192R polymorphism in patients with vitiligo

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Ethics Committee Approval
The approval was taken from the Kutahya Health Sciences University Ethics Committee, Turkey, March 6, 2019 (2019/03-5).
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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

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Abstract

Background/Aim: Vitiligo is a prevalent inflammatory illness that can affect the skin and mucosal surfaces and is characterized by patchy loss of skin pigmentation. Paraoxonase1 (PON1) is an esterase enzyme with antioxidant properties that binds to high-density lipoproteins. We examined whether the PON1 gene Q192R polymorphism is a risk factor for vitiligo among Turkish people.
Methods: The study included 70 controls and 60 vitiligo cases. Polymerase chain reaction and the restriction fragment length polymorphism technique were used to genotype the PON1 gene Q192R polymorphism.
Results: PON1 gene Q192R genotype distribution was 66.7% QQ, 33.3% QR, and 0% RR in the vitiligo and 81.4% QQ, 18.6% QR, and 0% RR in the control (P = 0.05). When vitiligo patients were compared with controls, the prevalence of the PON1 QR genotype was substantially higher and was linked to a 2.19-fold greater risk of developing vitiligo (odds ratio: 2.19, 95% confidence interval (CI): 0.97–4.91).
Conclusion: These findings imply that Q192R polymorphisms in the PON-1 gene may be linked to vitiligo in the Turkish population. The PON1 QR genotype may be a major genetic risk factor for vitiligo susceptibility and progression. Further studies with larger populations should more thoroughly clarify the association.

Keywords: Vitiligo, PON1 gene Q192R, Polymorphism

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Introduction

Vitiligo is a common dermatological illness characterized by skin lesions that are confined and depigmented [1, 2]. The exact etiology of vitiligo is still unknown but is thought to involve interactions between multiple genes and immunological and environmental events [3, 4]. An imbalance between the synthesis and accumulation of oxygen-reactive species (ROS) in cells and tissues causes oxidative stress. DNA damage, lipid and protein peroxidation are all caused by increased oxidative stress [1–5]. Recent research has highlighted the importance of oxidative stress in vitiligo [1–3].

Paraoxonase 1 (PON1) is an antioxidant enzyme that is linked to high density lipoprotein regulation and has been reported to lead to a reduction in oxidative stress and protect low- and high-density lipoproteins against oxidation [5, 6]. The PON1 gene is situated on chromosome 7 in the q21-q22 region. PON1 activity shows individual and ethnic differences due to genetic polymorphisms. The first polymorphism occurs when glutamine (Q genotype) is replaced with an arginine (R genotype) at locus 192 [6]. Some previous studies have reported that the PON1 Q192R gene polymorphism is associated with diseases, such as chronic obstructive pulmonary disease [6], coronary artery disease [7], and ischemic stroke [8].

To our knowledge, the PON1 Q192R gene polymorphism has not been studied in patients with vitiligo. Based on these observations, the purpose of this study was to determine the potential role of the PON1 Q192R gene polymorphism in vitiligo and its relationship to vitiligo susceptibility in a group of Turkish patients.

Materials and methods

Study design and study population

The research was conducted at Kutahya Health Sciences University's Department of Dermatology, Faculty of Medicine. In this study, 60 people with vitiligo (23 females, 37 males; mean age, 41.9 [17.1] years) and 70 ethnically matched, healthy volunteers from our healthcare workers were selected as controls (42 females, 28 males; mean age, 39.4 [16.8] years). Vitiligo was detected based on clinical symptoms and a Wood lamp examination. Additional parameters, such as demographics, family history, duration of sickness, and autoimmune diseases, were included in all patients' data. Healthy controls were selected from the same hospital if they had not been diagnosed with vitiligo based on a comprehensive physical examination by a dermatologist and had no personal or family history of autoimmune or inflammatory illnesses. Ethical approval for the research was obtained from the Ethics Committee of the Kutahya Health Sciences University, Turkey, on March 6, 2019 (2019/03-5), all procedures were performed in accordance with the Helsinki Declaration, and all patients provided informed consent.

Genomic DNA extraction

Venous blood samples were obtained from all individuals and then placed in tubes containing the anticoagulant ethylenediaminetetraacetic acid (EDTA). Samples were used to isolate genomic DNA using the phenol–chloroform method. Following extraction, the concentration and purity of DNA were evaluated spectrophotometrically. DNA quality was also evaluated on a 0.7% agarose gel electrophoresis, and the DNA was stored at −20°C until further analyses.

Genotyping of PON1 Q192R polymorphism

PON1 Q192R polymorphism was genotyped using polymerase chain reaction (PCR) and fragment length polymorphism (PCR–RFLP) based on the protocol described previously by Öktem et al. [9]. The primers used for PCR are mentioned in Table 1. In a total volume of 20 mL, the reaction mixture contained 3 mL (100 ng) of genomic DNA, 10.7 mL nuclease-free H_2O, 2 mL 10X PCR buffer, 1 μL $MgCl_2$ 25 mM, 2 mL 2 mM dNTPs (abm, Canada), 1 mL (each) of 10 pM corresponding forward and reverse primers (GenScript, USA), and 0.3 mL (3U/mL) Taq Polymerase (abm, Canada). With the aid of a thermal master cycler gradient (Thermo Scientific, EU Lithuania), PCR products were amplified and then digested with BspI (New England Biolabs, Ipswich, MA, USA) after which they were electrophoresed on 4% agarose gel and stained with ethidium bromide (Table 1).

Statistical analysis

The frequency distributions of each allele and genotype of the PON1 Q192R polymorphism were compared between the cases and controls using the $\chi 2$ and Fisher's exact tests for calculating odds ratios (OR) with 95% confidence intervals (CI). The association between PON1 genotypes and the clinical characteristics of the vitiligo patients were assessed using a $\chi 2$ test for categorical variables and an analysis of variance (ANOVA) for continuous variables. Statistical significance was considered a P-value ≤ 0.05. The SPSS software was used to conduct the statistical analysis (version 24.0).

Results

Characteristics of vitiligo patients and controls

Among the patients who visited the dermatology clinics of the Medicine Faculty, Kutahya Health Sciences University, a total sample of 60 unrelated patients with generalized vitiligo and 70 unrelated controls without a history of autoimmune and/or inflammatory illnesses were enrolled in the study. Age and gender variations between the case and control groups were insignificant (P = 0.34 and P = 0.65, respectively). Table 2 shows the demographic and clinical characteristics of the vitiligo cases.

Table 1: Summary of conditions for the PON1 Q192R genetic analyses

SNP	Prime sequence (5'-3')	Tm (°C)	PCR product size	Restriction Enzyme	Genotyping
PON1 Q192R	F-TATTGTTGCTGTGGGACCTGAG R-GACATACTTGCCATCGGGTGAA	60 °C	199 bp	BspI	QQ: 199 bp QR: 135 bp - 199 bp RR: 135 bp

PON 1: Paraoxonase 1, SNP: single nucleotide polymorphism, Tm: primer annealing temperature, bp: base pairs

Table 2: Demographic and clinical characteristics of vitiligo and control subjects

Parameter	Vitiligo (n = 60)	Controls (n=70)
Gender n (%)		
Female / Male	23 (38.3) / 37 (61.7)	42 (60) / 28 (40)
Age, years	41.9 (17.1)	39.4 (16.8)
Mean duration of the disease, months	87.5 (145.6)	-
Age of vitiligo onset, years	34.7 (18.1)	-
Age of vitiligo onset, n (%)		
Early-onset, (< 30 years)	15 (25)	-
Late-onset, (≥ 30 years)	45 (75)	-
Type of vitiligo, n (%)		
Generalized	46 (76.7)	-
Localized	10 (16.7)	-
Acrofacial	4 (6.7)	-
Skin type, n (%)		
2	7 (11.7)	-
3	47 (78.3)	-
4	6 (10)	-
Initial location, n (%)		
Head neck	17 (28.3)	-
Upper extremity	2 (3.3)	-
Body	20 (33.3)	-
Lower extremity	6 (10)	-
Hands	15 (25)	-
With/without Koebner phenomenon, n (%)	5 (8.3) / 55 (91.7)	-
With/without Halo nevi, n (%)	5 (8.3) / 55 (91.7)	-
With/without leukotrichia, n (%)	17 (28.3)/43 (71.7)	-
With/without repigmentation, n (%)	17 (28.3)/43 (71.7)	-
With/without Family history of vitiligo, n (%)	18 (30.5)/41 (69.5)	-
With/without other skin disease, n (%)	5 (8.3)/55 (91.7)	-
With/without other autoimmune disease, n (%)	10 (16.7)/50 (83.3)	-
With/without stress, n (%)	46 (76.7)/14 (23.3)	-

Association between PON1 Q192R genotypes and alleles with the risk of developing vitiligo

Table 3 shows the allele and genotype frequencies for the distribution of the PON1 Q192R gene polymorphism. The genotype frequencies of the vitiligo and control groups were in Hardy–Weinberg Equilibrium (P = 0.12 and P = 0.39, respectively).

The frequencies of each PON1 gene Q192R genotype were revealed as 66.7% for QQ (n = 40), 33.3% for QR (n = 20), and 0% for RR (n = 0) in the vitiligo patient; 81.4% for QQ (n = 57), 18.6% for QR (n = 13) and 0% for RR (n = 0) in the control. The distribution of the PON1 gene Q192R genotypes was found to be significantly different between groups ($\chi2 = 3.71$; df = 1; P = 0.05). A positive association between the risk of developing vitiligo and the QR genotype of PON1 was also noted (OR = 2.19, 95% CI 0.97–4.91; P = 0.05) as shown in Table 3.

The Q allele was reported in 83.3% (n = 100) of the vitiligo and 89.3% (n = 125) of the controls. The R allele was observed in 16.7% (n = 20) of the vitiligo and 10.7% (n = 15) of the controls. No discernible variations in the PON1 gene Q192R allele distributions between patients and controls were found ($\chi2 = 1.96$; df = 1; P = 0.16) as shown in Table 3.

Table 3: Distributions of PON1 Q192R polymorphism genotype and allele frequencies in the study populations and risk of vitiligo

PON1 Q192R	Genotype/Allele	Vitiligo (n = 60) n (%)	Controls (n = 70) n (%)	OR	95% CI	*P*-value
	QQ	40 (66.7)	57 (81.4)	0.45	0.20 - 1.02	0.05
	QR	20 (33.3)	13 (18.6)	2.19	0.97 - 4.91	0.05
	RR	-	-	-	-	-
	$\chi2 = 3.71$, df = 1, P = 0.05					
	Q	100 (83.3)	125 (89.3)	0.60	0.29 - 1.23	0.22
	R	20 (16.7)	15 (10.7)	1.66	0.81 - 3.42	0.22
	$\chi2 = 1.96$, df = 1, P = 0.16					

OR: odds ratio, CI: confidence interval

Association between PON1 Q192R genotypes and clinical characteristics of the vitiligo patients

Patients who had a history of vitiligo in their families had more QR genotypes than QQ genotypes on average (47.4% versus 22.5%; P = 0.05) as shown in Table 4.

Table 4: Baseline clinical and demographical features of the study patients with vitiligo stratified according to PON1 Q192R gene polymorphisms

Characteristic	QQ	QR	RR	*P*-value
Gender, male/female, n (%)	17/23 (42.5/57.5)	6/14 (30/70)	-	0.975
Age (years)	39.3 (17.0)	47.1 (16.7)	-	0.08
Disease duration (months)	79.6 (144.6)	103.2 (150.0)	-	0.604
Age of vitiligo onset (years)	32.7 (18.7)	38.7 (16.6)	-	0.224
Age of vitiligo onset, n (%)				
Early-onset, (< 30 years)	12 (30)	3 (15)	-	0.206
Late-onset, (≥ 30 years)	28 (70)	17 (85)	-	
Type of vitiligo, n (%)				
Generalized	30 (75)	16 (80)	-	0.511
Localized	8 (20)	2 (10)	-	
Acrofacial	2 (5)	2 (10)	-	
Skin type, n (%)				
2	6 (15)	1 (5)	-	0.123
3	32 (80)	15 (75)	-	
4	2 (5)	4 (20)	-	
Family history of vitiligo, n (%)				
Yes	9 (22.5)	9 (47.4)	-	0.05*
No	31 (77.5)	10 (52.6)	-	

Data were analyzed by analysis of variance and $\chi2$ test. Mean plus standard deviation of the mean values are presented for age, disease duration, age of vitiligo onset. * P value ≤ 0.05 was considered significant.

Discussion

Vitiligo is a multifactorial polygenic skin disorder with a complicated pathogenesis that is caused by both genetic and non-genetic factors. This disorder may involve genes related to melanin manufacturing, the antioxidant system, and the control of autoimmune diseases [10]. PON1 is involved in both inflammation and lipid metabolism. This enzyme leads to a reduction in low-density lipoprotein (LDL) oxidation and has been shown to be deficient in many disease states. PON1 gene polymorphisms are linked to the metabolic syndrome [11]. The full etiology and pathogenesis of vitiligo is yet unknown. Consequently, in light of these findings and those in the literature, PON1 may play a significant role in the pathogenesis of vitiligo by protecting cells from oxidative stress. For understanding the etiopathogenesis of vitiligo and creating viable treatment options, genetic research is crucial.

To the best of our knowledge, only a few studies concerning the PON1 Q192R gene polymorphism in vitiligo have been published. As a result, this study is the first to use the PCR–RFLP method to assess the link between the PON1 Q192R gene polymorphism and vitiligo. The current study sought to determine the relationship between PON1 Q192R gene polymorphism and the risk of developing vitiligo. An independent case-control study was conducted using a sample of 60 vitiligo patients and 70 matched controls. Seçkin et al. [12] found no significant differences between vitiligo and PON1 Q192R gene polymorphism using LightCycler PCR technology [12]. Although no discernible variation in the allele frequencies of the PON1 gene Q192R polymorphism in this investigation was found, the vitiligo and control groups were found to have significantly different genotype distributions. The PON1 Q192R genotype frequency was statistically and substantially higher in the vitiligo patient when compared with the controls. In individuals with the QR genotype, we found a 2-fold relative risk increase for the onset of vitiligo. In addition, patients with vitiligo in their families had a higher prevalence of the QR genotype than the QQ genotype. The PON1 QR genotype was thus found to be strongly related to the chance of developing vitiligo and may therefore contribute to vitiligo according to our findings.

PON1 is an antioxidant enzyme related to high-density lipoprotein (HDL) regulation in the blood and is a very important ester hydrolase that protects lipoproteins from oxidation [13].

Serum PON1 activity has been examined in various dermatological conditions, including Behcet disease [14] and psoriasis [15]. Age, dyslipidemia, diabetes mellitus, smoking, hypertension, and increased oxidative stress have all been linked to low PON1 activity [16]. Ramadan et al. [17] demonstrated that both tissue and serum levels of PON1 were significantly reduced in the vitiligo patients when compared with the controls [17]. In this study, it was not possible to examine serum PON1 levels. Pola et al. [18] suggested that serum levels of the PON1 protein are influenced by the 192 Q/R polymorphism of the PON1 gene [18]. No studies in the literature addressing serum PON1 levels and the PON1 gene Q192R polymorphism in vitiligo are available.

Limitations

The fact that we were unable to precisely gauge the serum levels of the PON1 gene was one of the study's drawbacks. Highlighting the consequences of the Q192R variation in the PON1 gene and correlating the genotyping results would have been helpful. Very little evidence in the literature describing the relationship of vitiligo to the PON1 gene variation exists. As a result, comparing the findings of this study to vitiligo and PON1 gene polymorphism is not conceivable. Therefore, this study needs to be supported by further, research including a larger sample size of patients.

Conclusion

Consequently, a significant difference between the vitiligo and PON1 gene Q192R polymorphism in the Turkish population was found. The QQ genotype in controls and the QR genotype in vitiligo were found to be abundant. Additionally, it was discovered that patients with a family history of vitiligo had a much higher frequency of the PON1 QR genotype. The relative risk of developing vitiligo was shown to have increased 2-fold in those with the QR genotype. Interestingly, it has been discovered that the PON1 gene Q192R polymorphism is linked to vitiligo, and more research in this area will shed light on the function of PON1 gene polymorphism in vitiligo pathogenesis, treatment, and prevention.

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Effects of pneumoperitoneum and patient position on intracranial pressure in obese patients undergoing laparoscopic cholecystectomy

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Ethics Committee Approval
The approval was taken from the Necmettin Erbakan University Ethics Committee of Non-Pharmaceutical and Non-Medical Device Researches (Decision No: 2018/1494).
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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

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Abstract

Background/Aim: Optic nerve sheath diameter (ONSD) measurement is one of the non-invasive techniques used for intracranial pressure (ICP) measurement. ICP changes have been evaluated based on ONSD measurements during many laparoscopic surgeries. However, such analyses in the obese patient populations are limited. This study aimed at investigating the effects of pneumoperitoneum and reverse Trendelenburg and head-up position on ICP based on ONSD measurements in obese patients undergoing laparoscopic cholecystectomy.

Methods: This observational study included 60 female patients who were scheduled for laparoscopic cholecystectomy. Obese patients with a body mass index (BMI) of 30 and above were assigned to Group 1, while BMI < 30 patients were assigned to Group 2. The first ONSD measurement was performed just before insufflation (T1). The second measurement was taken 5 min after insufflation (T2), the third measurement 5 min after placing patients in the reverse Trendelenburg and head-up position (T3), and the last measurement 5 min after the deflation while the reverse Trendelenburg and head-up position was maintained (T4).

Results: ONSD measurements at the T2 and T3 time points in Group 1 patients were higher than in Group 2 patients ($P = 0.012$ versus $P = 0.020$). Both measurement values were higher in obese patients. In Group 1 patients, T2 and T3 measurements were significantly higher than T1 and T4 measurements (T2 > T1; $P < 0.001$, T2 > T4; $P < 0.001$, T3 > T1; $P < 0.001$, and T3 > T4; $P < 0.001$). No significant difference between T2 and T3 and between T1 and T4 measurements were found. In Group 2 patients, T2 measurements were significantly higher than the T1, T3, and T4 measurements, while T3 measurements were significantly higher than T1 and T4 measurements (T2 > T1; $P < 0.001$, T2 > T3; $P = 0.022$, T2 > T4; $P < 0.001$, T3 > T1; $P < 0.001$, and T3 > T4; $P = 0.048$). No significant difference between T1 and T4 measurements was noted.

Conclusion: Laparoscopic cholecystectomy does not cause an increase in ICP of obese patients with limited pneumoperitoneum pressure, reverse Trendelenburg and head-up position, and controlled anesthesia.

Keywords: Optic nerve, Obesity, Laparoscopy, Cholecystectomy

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Introduction

Cholelithiasis is one of the most prevalent gastrointestinal diseases in adults, and obesity is one of the important risk factors associated with an increase in the risk of developing cholelithiasis [1]. Obesity is defined as a body mass index (BMI) greater than 30 kg/m^2 with an increasing prevalence among all racial groups, genders, and people of different educational levels [2]. Laparoscopic cholecystectomy is a surgical technique frequently used for cholelithiasis treatment. For performing this surgery, pneumoperitoneum is created by the insufflation of CO_2. While obesity was formerly considered a contraindication for laparoscopic surgery, nowadays, laparoscopy is considered to be a safe, effective, and the procedure of choice for obese patients when it is performed by experienced surgeons [3].

Pneumoperitoneum and the resulting increase in intra-abdominal pressure cause decreased venous return, and CO_2 absorption from the peritoneal surface leads to hypercapnia and respiratory acidosis. Hemodynamic and respiratory side effects are moderate and often well-tolerated. While the results concerning the effects of pneumoperitoneum on intracranial pressure (ICP) are limited, a positive correlation has been shown between intra-abdominal pressure and ICP [4]. Intra-peritoneal pressure causes an increase in intra-thoracic pressure by causing an elevation of the diaphragm and an increase in central venous pressure with compression of the inferior vena cava. Elevated central venous pressure results in an increase in cerebrospinal fluid pressure, thereby leading to an increase in ICP [5]. Another reason for an increase in ICP is arterial vasodilation caused by a CO_2 increase [6].

Although invasive techniques have been indicated to be the gold standard for intracranial pressure measurements, the literature data also show the feasibility of non-invasive techniques. Ultrasonographic measurement of optic nerve sheath diameter (ONSD) is one such technique [7]. Changes in ICP have been analyzed using ONSD measurements during many laparoscopic surgeries and in different patient positions [8,9]. However, analyses in the obese patient populations are limited, and as far as we know, no study in the literature that analyzes the effect of pneumoperitoneum on ICP in the reverse Trendelenburg and head-up position has been published. Obesity is associated with an increase in intra-abdominal pressure, which can become chronic without noticeable clinical signs and result in intracranial hypertension [5]. Patients undergoing laparoscopic surgery are at risk of an increase in ICP, and ICP monitoring is important for critically ill patients.

This study aimed to investigate the effects of pneumoperitoneum and reverse Trendelenburg and head-up position in laparoscopic cholecystectomy surgery based on non-invasive ONSD measurements on ICP in obese patients.

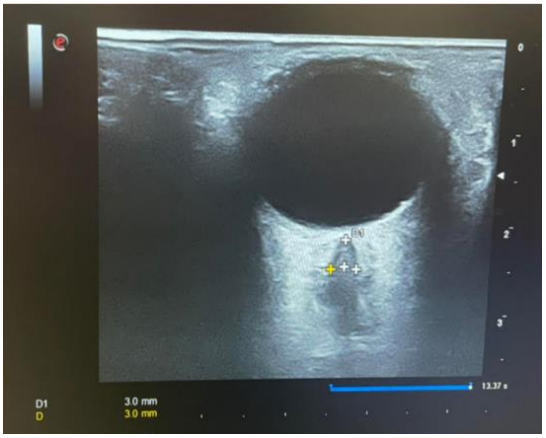
Materials and methods

This prospective observational study was conducted in an academic university hospital between September 2019 and October 2020 in accordance with the principles of the Declaration of Helsinki after obtaining approval from the University Ethics Committee (Decision Number: 2018/1494, Approval Date: 14/09/2018). A written informed consent form was obtained from all patients who agreed to participate in the study. The study included female patients aged 18–65 years with an American Society of Anesthesiologists (ASA) score of I/II who were scheduled for elective laparoscopic cholecystectomy surgery. Patients with ASA III/IV class, intracranial pathology, a history of cerebrovascular disease, a history of ocular pathology or disease, and pregnant patients were excluded from the study. Patients were monitored using an electrocardiogram (ECG), pulse oximeter, and noninvasive arterial pressure measurement. Muscle relaxation of patients was monitored with a neuromusculer transducer (NMT; SJC17200038HA, GE Healthcare, Helsinki, Finland). Anesthesia was induced with 1 mcg/kg remifentanil (Rentanil, VEM, Tekirdağ, Turkey), 2 mg/kg propofol (Propofol %1 Fresenius, Fresenius Kabi AB, Uppsala, Sweden) and 0.6 mg/kg rocuronium (Myocron, VEM, Tekirdağ, Turkey). When the train of four (TOF) count was 0, patients were intubated and anesthesia was maintained with desflurane (Suprane, Baxter Healthcare, Puerto Rico, USA) inhalation in 50% oxygen–air mixture and intravenous (iv) remifentanil infusion. Desflurane and remifentanil were titrated to maintain vital values within a range of 20% of the baseline values. Tidal volume and respiratory rates were adjusted to maintain end-tidal CO_2 levels between 35 and 40 mmHg. Patients were given iv 2 mg/kg tramadol (Tradolex, Mefar, İstanbul, Turkey) for post-operative analgesia and iv 4 mg ondansetron (Zofran, GlaxoSmithKline, Research Triangle Park, England) for nausea and vomiting. In the pneumoperitoneum procedure, the pressure was limited to 12 mmHg. Age, ASA score, BMI, pneumoperitoneum time, and post-operative nausea and vomiting were recorded for all patients.

Ultrasonographic measurement of optic nerve sheath diameter

The ONSD values of patients were measured by ultrasonography (USG) at four different time points. Measurements were taken by a trained anesthesiologist who was not involved in the study. A 12.0 MgH linear USG probe (Esaote MyLab Six CrystaLine, Genova, Italy) with a thin layer of gel was carefully placed on the closed upper eyelid. Avoiding excessive pressure, the probe was adjusted to display the optic nerve insertion into the eyeball in two-dimensional (2D) mode. After providing optimal contrast enhancement between the hypoechoic image of the optic nerve and the echogenicity of the retrobulbar adipose tissue, the image was frozen. ONSD was measured at 3 mm behind the optic disc using the electronic caliper of the USG (Figure 1).

Figure 1: Ultrasonographic image of optic nerve sheath diameter (ONSD) measurement



The measurement was repeated four times for a single eye, two times horizontally and two times vertically, and the average of these four measurements was considered the ONSD. Measurements were obtained at four different time points from the same eye. The measurement time points were defined as T1 (before pneumoperitoneum when the patient was in the supine position), T2 (5 min after pneumoperitoneum when the patient was in the supine position), T3 (pneumoperitoneum and after the patient was placed in the reverse Trendelenburg and head-up position), and T4 (5 min after pneumoperitoneum termination when the patient was in the reverse Trendelenburg and head-up position). At these four time points, the heart rates, noninvasive mean arterial pressures, and end-tidal CO_2 values of patients were also recorded in addition to the ONSD measurements. Moreover, heart rates and noninvasive mean arterial pressures were recorded when patients were first monitored, after induction of anesthesia, and after intubation. Patients were placed in the reverse Trendelenburg and head-up position by the anesthesiologist, who would measure the ONSD under the guidance of the surgeon.

Statistical analysis

The data obtained in the study were analyzed using the Statistical Package for the Social Sciences (SPSS) software, version 23.0 (IBM SPSS 23.0 for Windows, Armonk, New York, United States). Frequencies of general demographic characteristics and descriptive statistical values of all time-dependent measurements were indicated. When examining the normality of values between the groups, the Shapiro–Wilk test was applied if n was < 30, and the Kolmogorov–Smirnov test was applied if n was > 30. The values were considered to be non-normally distributed between the groups if P was < 0.05 and normally distributed between the groups if $P > 0.05$. As a result of the normality test, the Mann–Whitney U test was used for non-normally distributed variables, while the independent samples t-test was used for normally distributed variables to examine intergroup differences. A chi-squared test was used when examining intergroup dependency in categorical data. When examining the intergroup difference and dependency, the level of statistical significance was set at 0.05, and a $P < 0.05$ was considered to indicate a significant difference between the groups, while a $P > 0.05$ was considered to indicate none. The Wilcoxon signed-rank test was used to examine the differences between the intra-group time-dependent measurement values. $P < 0.05$ was considered to indicate a change in measurement values over time, while a $P > 0.05$ was considered to indicate no change over time.

Sample size

The sample size of the study was calculated using the G*Power software for Windows, version 3.1.9.4 (Universität Düsseldorf, Düsseldorf, Germany) based on the data set consisting of 10 patients (obese: 5, non-obese: 5). The results of the pilot study showed that the ONSD measured 5 min after pneumoperitoneum while the patient was in the supine position was 4 (0.3) (mean [standard deviation {SD}]) in the obese group, while it was 3.8 (0.3) in the non-obese group. The sample size required to detect a 30% difference was determined as 30 patients for each group, 60 patients in total with 80% power and 0.05 margin of error. Obese patients were classified as Group 1, while non-obese patients were classified as Group 2.

Results

The study included 60 female patients. No patient was excluded from the study, and data from a total of 60 patients were analyzed (Figure 2). Considering the distribution of demographic data among the groups, the mean weight and BMI values of the patients in Group 1 were higher than the patients in Group 2 ($P < 0.001$ versus $P < 0.001$). In addition, while all of the patients in Group 1 were ASA 2, only 20 of the patients in Group 2 were ASA 2 patients ($P = 0.001$). The distribution of demographic data between the two groups is given in Table 1.

Figure 2: Flowchart of the study

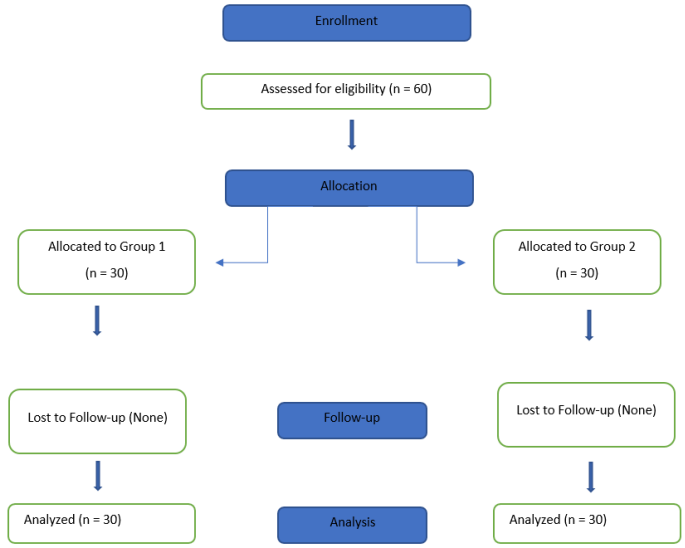


Table 1: Distribution of demographic data (mean [SD])

	Group 1 (n = 30)	Group 2 (n = 30)	*P*-value
Age (year)	48.77 (10.37)	42.97 (14.11)	0.075
Weight (kg)	91.37 (10.07)	68.33 (8.49)	< 0.001
Height (cm)	161.17 (5.87)	164.27 (7.84)	0.122
BMI (kg/m^2)	35.19 (3.28)	25.28 (2.19)	< 0.001
ASA (I/II)	0 / 30	10 / 20	0.001

SD: standard deviation, BMI: Body mass index, ASA: American Society of Anesthesiologists

The pneumoperitoneum time was 40.2 (9.0) min in Group 1 and 40.6 (7.9) min in Group 2. No statistically significant difference between the groups in terms of pneumoperitoneum time ($P = 0.855$) was found. Considering the presence of post-operative nausea/vomiting, 20 (66.7%) patients in Group 1 and 27 (90%) patients in Group 2 had nausea/vomiting; however, the difference was not statistically significant ($P = 0.060$).

ONSD measurements at the T2 and T3 time points in Group 1 patients were higher than Group 2 patients ($P = 0.012$ versus $P = 0.020$). Both measurement results were higher in obese patients than in non-obese patients (Table 2).

Table 2: Differences in ONSD measurements between the groups (mean [SD])

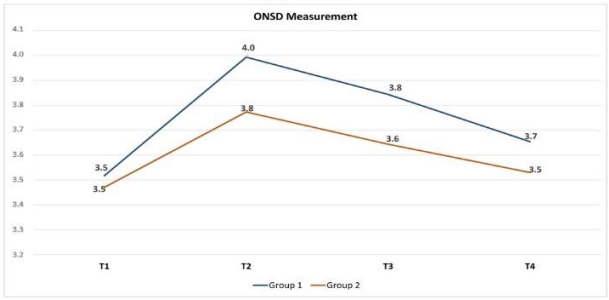
	Group 1 (n = 30)	Group 2 (n = 30)	*P*-value
ONSD T1 (mm)	3.5 (0.31)	3.5 (0.30)	0.556
ONSD T2 (mm)	4.0 (0.30)	3.8 (0.29)	0.012
ONSD T3 (mm)	3.8 (0.32)	3.6 (0.28)	0.020
ONSD T4 (mm)	3.7 (0.32)	3.5 (0.26)	0.260

SD: standard deviation, ONSD: Optic Nerve Sheath Diameter

The analysis of the changes in ONSD measurements of Group 1 revealed significantly higher T2 and T3 measurements than T1 and T4 measurements (T2 > T1; $P < 0.001$, T2 > T4; $P < 0.001$, T3 > T1; $P < 0.001$, and T3 > T4; $P = 0.001$), but no significant differences between T2 and T3 measurements and between T1 and T4 measurements were found. In Group 2 patients, T2 measurements were significantly higher than T1, T3, and T4 measurements, while T3 measurements were significantly higher than T1 and T4 measurements (T2 > T1; $P < 0.001$, T2 >

T3; $P = 0.022$, T2 > T4; $P < 0.001$, T3 >T1; $P < 0.001$, and T3 > T4; $P = 0.048$). No significant difference between T1 and T4 measurements was noted (Figure 3).

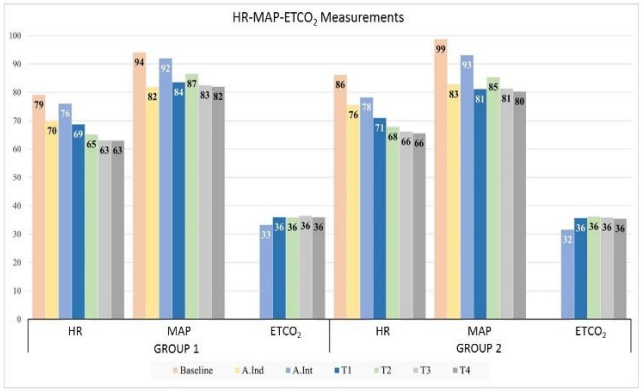
Figure 3: Changes in ONSD measurements by time (mm)



ONSD: Optic Nerve Sheath Diameter

No statistically significant difference between the groups in terms of changes in heart rate, mean arterial pressure, and end-tidal CO_2 values over time were found ($P > 0.05$) as shown in Figure 4.

Figure 4: Changes in HR, MAP, and $ETCO_2$ values by time



HR: Heart rate, MAP: Mean arterial pressure, $ETCO_2$: End-tidal CO_2, A.Ind: After Induction, A.Int: After Intubation

Discussion

This study investigated the effects of pneumoperitoneum and reverse Trendelenburg and head-up position on ICP in obese patients undergoing laparoscopic cholecystectomy. The results of the study demonstrated that ONSD was increased by pneumoperitoneum in both obese and non-obese patients, while this increase was more significant in obese patients. The reverse Trendelenburg and head-up position after pneumoperitoneum caused a significant decrease in ONSD values of non-obese patients. The reverse Trendelenburg and head-up position maintained after the termination of pneumoperitoneum caused ONSD measurements to approach baseline values in both obese and non-obese patients. The measurements showed no ONSD value suggestive of an increased ICP in any patient.

Laparoscopic cholecystectomy is the gold standard treatment for benign gallstone disease [10]. The requirement for creating pneumoperitoneum may cause various intra- and post-operative hemodynamic and metabolic complications in these patients. A definite positive correlation between increased ICP and high intra-abdominal pressure has been reported in the literature [11]. Obesity is associated with an increase in intra-abdominal pressure and ICP, and this relationship may lead to an increase in the side effects caused by pneumoperitoneum [5]. The gold standard for ICP measurement is invasive measurement techniques. However, these techniques may cause serious complications, such as bleeding and infection, and their use is difficult in routine practice for short-term surgical procedures [12]. It has been shown that ONSD is affected by ICP changes and can be used as a non-invasive measurement technique [13]. The optic nerve is part of the central nervous system and is surrounded by the dural sheath. In cases of increased ICP, the sheath expands, and changes in the diameter of the nerve sheath can be visualized by transocular USG [14].

In the literature, no consensus on the threshold for ONSD measurement value for increased ICP can be found. Shirodkar et al. [15] found high sensitivity and specificity for cut-off values of 4.6 mm in women and 4.8 mm in men. Amini et al. [16] found that values greater than 5.5 mm showed an increase in ICP with 100% sensitivity and specificity. Another study suggested that values greater than 5 mm indicated an elevation in ICP values that may require intervention [17]. Therefore, a broad variation in the optimal cut-off values of ONSD compared to invasive ICP monitoring exists [18]. Because of ethnicity and gender differences, ONSD measurement values and cut-off values for ICP increase may be different. The study by Goeres et al. [19] with a healthy cohort determined a mean basal value of 3.78 mm for men and 3.60 mm for women. The same study found a cut-off value of 4.11 mm for an increase in the ICP increase with a 95% confidence interval in women, arguing that it might have more clinical benefits than the generally accepted level of 5 mm. This value is also the lowest cut-off value we have found in the literature. Most of the ONSD studies on laparoscopic surgery have baseline values of 4.00 mm and above. For our country's population, no study to determine the mean ONSD and cut-off value for ICP in healthy individuals is available. In our study, the mean baseline ONSD was found to be 3.5 mm in both groups. Since the initial measurements were performed after the induction of anesthesia, these values could be attributed to the presence of deep anesthesia. The highest mean value was found to be 4.0 (0.3) in obese patients and 3.8 (0.29) in non-obese patients. Therefore, these results show that 12 mmHg pneumoperitoneum and laparoscopic cholecystectomy performed in the reverse Trendelenburg and head-up position do not lead to an increase in ICP in either obese or non-obese patients even if the lowest cut-off value reported in the literature is used. Considering the fact that all patients included in the study were female, the reason for the absence of significant increases in ONSD measurements, including obese patients, may be due to the effect of previous pregnancies and the presence of higher abdominal wall compliance with the effect of increased volume in obese patients. A need for further studies to clarify the physiopathology of these results exists.

Obese patients tolerate laparoscopic procedures very well. The normal intra-abdominal pressure of non-obese individuals is 5 mm Hg or lower [20]. In contrast, morbidly obese patients have chronically high intra-abdominal pressures of 9 to 10 mmHg [21]. In general, CO_2 absorption and excretion in morbidly obese people are similar to non-obese people. Changes in the cardiac index have also been found to be similar [22]. In our study, no statistically significant differences between the groups in terms of changes in heart rate, mean arterial pressure, and end-tidal CO_2 values over time were found. Similar results in terms of hemodynamic parameters with non-obese patients and even in morbidly obese patients are in line with our results, and it is not

surprising to find no differences in hemodynamic parameters of obese patients. Considering the relationship of high intra-abdominal pressure with high ICP, we anticipated encountering higher ONSD measurements in obese patients. The study of Dip et al. [5] comparing ONSD in obese and non-obese patients found a significant difference 30 min after pneumoperitoneum. In laparoscopic procedures, a minimum of 10 to15 min is required for an increase in partial pressure of carbon dioxide ($PaCO_2$) after pneumoperitoneum, and the increase in ICP by small volume increments with involved homeostatic mechanisms is kept within a narrow range [23]. When this compensatory effect is exhausted, substantial increases are observed in ICP in direct proportion to the increase in volume [24]. The measurements at the T2 and T3 time points in the study were obtained in the presence of pneumoperitoneum and during the first 10 min. If measurements were performed after longer periods, perhaps higher values would have been found. Moreover, the Dip study was conducted on both male and female patients who underwent different surgical procedures under 14 mmHg pressure, and ONSD measurements were found that suggested high ICP of 86%. The authors stated that the incidence of ICP increase was significant in obese and non-obese patients who underwent laparoscopic procedures. No such result was found in our study. We believe that the reason for this different result is due to the fact that the pneumoperitoneum pressure, which was 14 mmHg in the Dip study, was 12 mmHg in our study. Furthermore, the study by Dip also included morbidly obese and super-obese patients. Our study, on the other hand, included only obese patients. Another reason for the different results may be the existing increased chronic intracranial pressure in morbidly obese and super-obese individuals.

In laparoscopic cholecystectomy, appropriate surgical conditions are provided by pneumoperitoneum and reverse Trendelenburg and head-up position. One of the most studied parameters associated with ICP in laparoscopic surgeries is patient position. Numerous studies showing that the Trendelenburg position causes an increase in ONSD have been published [7, 25]. A study on anesthetized neurosurgery patients revealed that the head-down position and head rotation can lead to significant increases in ICP [22]. An animal study by Halverson et al. [26] revealed that increased ICP resulting from CO_2 insufflation was aggravated in the Trendelenburg position and not relieved when the reverse Trendelenburg position was used. However, the animal study of Rosenthal [27] reported that the highest and lowest ICP values during CO_2 pneumoperitoneum were observed in the Trendelenburg and reverse Trendelenburg positions, respectively. Our study results are consistent with the study results of Rosenthal as the reverse Trendelenburg and head-up position led to a reduction in ONSD in the presence of pneumoperitoneum in non-obese patients and after deflation in both groups. The study of Sahay et al. [8], which investigated the effects of the Trendelenburg and reverse Trendelenburg positions in non-obese female patients found that pneumoperitoneumn in addition to both positions led to an increase in ONSD measurements with the increase being more pronounced in the Trendelenburg position. The measurements did not approach baseline values even at 5 min after deflation. The pneumoperitoneum pressure in the study was the same as in our study, and a 20° reverse Trendelenburg position was used. In our study, we attempted to provide a 30° reverse Trendelenburg position. For this reason, the effects of the reverse Trendelenburg position may have been found to be different. In our last measurement study, the reverse Trendelenburg and head-up position was used after deflation. In the study of Sahay, on the other hand, the last measurement was performed in the supine position after deflation. We believe that the reverse Trendelenburg and head-up position we maintained after deflation has an effect on reaching the baseline values in as short a time as 5 min.

Studies investigating the effect of the 10° reverse Trendelenburg position in craniotomy surgery have found a decrease in ICP without deterioration of cerebral perfusion pressure [28–30]. It has been found that the 30° reverse Trendelenburg position provides a reduction in ICP of approximately 1 mmHg [31]. The 30° head-up position has been shown to cause a significant reduction in cerebral blood volume in healthy surgical patients based on a near-infrared spectroscopy reading [32]. The study of Demirgan et al. [33] found that placing the patient in the reverse Trendelenburg position before establishing pneumoperitoneum at a pressure of 15 mmHg in patients undergoing laparoscopic cholecystectomy prevented the increase in ONSD. The Trendelenburg position causes an increase in preload due to an increase in venous return from the lower limbs. This position causes a cephalic shift of the internal organs, which leads to an increase in the pressure on the diaphragm. In the reverse Trendelenburg position, the internal organs shift caudally, causing a reduction in the pressure on the diaphragm and improvement in respiratory function. Therefore, we expect the side effects of increased intra-thoracic pressure, one of which is increased ICP, to be reversed. From this point of view, we anticipated that the reverse Trendelenburg and head-up position used in cholecystectomy surgery would lead to a reduction in ONSD measurements with the opposite effect. As we predicted, in both obese and non-obese patients, the position produced a decrease in ONSD measurements compared to measurements taken after pneumoperitoneum. However, this decrease was statistically significant in non-obese patients. In both groups, the position did not cause a return to the baseline measurement values after anesthesia induction. This return to baseline was achieved when the position was maintained after the termination of pneumoperitoneum in both groups. Therefore, we believe that the reverse Trendelenburg and head-up position should be maintained in obese and non-obese patient groups undergoing laparoscopic surgery until the recovery process is completed unless a surgical contraindication exists. However, more studies are needed to explain the background of the fact that the effect of position in the presence of pneumoperitoneum in obese patients is not as pronounced as in non-obese patients.

Another well-studied parameter associated with ICP in laparoscopic surgeries is pneumoperitoneum pressure. A study investigating the effects of pneumoperitoneum created with 8 and 14 mmHg pressure emphasized the significance of low pressure to reduce changes in ICP [34]. The study was performed on patients undergoing laparoscopic cholecystectomy under propofol anesthesia in a 30° reverse Trendelenburg position, and ONSD measurements above 5.2 were considered a significant change, while ONSD measurements above 5.7 were considered the threshold value to terminate pneumoperitoneum. In the study with a mean pneumoperitoneum time of 78 min, a value above 5.2 was

mostly determined in the high-pressure group. Another study investigating the effect of pneumoperitoneum pressure in laparoscopic cholecystectomy compared the effects of 10, 12, and 14 mmHg pressure [35]. The study was performed under sevoflurane anesthesia and found that ONSD measurements increased as the applied pressure increased. Both studies were performed with non-obese patients. In our study, we used a pressure of 12 mmHg and our mean pneumoperitoneum time was 40 min. We did not find any measurement values suggestive of increased ICP values in either the obese or non-obese groups. We think that in addition to the reverse Trendelenburg and head-up position, the use of 12 mmHg pressure and the duration of pneumoperitoneum significantly contributed to this result. We are of the opinion that a pneumoperitoneum pressure of 12 mmHg should be preferred to higher values during short-term laparoscopic surgeries of obese patients. However, a need for studies comparing ONSD measurements in laparoscopic surgeries performed at different times and under different pressure values to determine the safe pressure value in obese patients is present.

Volatile anesthetics have vasodilatory activity on vascular smooth muscle. This vasodilatory activity is evident above 1.0 MAC and can cause a significant increase in cerebral blood flow, resulting in an increase in ICP. In a pig model, this increase was shown to be higher in desflurane compared to sevoflurane and isoflurane [36]. Verdonck et al. [37] reported that ONSD remained stable during robot-assisted laparoscopic radical prostatectomy and ONSD measured 10 min after pneumoperitoneum combined with the Trendelenburg position did not increase under general anesthesia maintained with sevoflurane. The study of Kim et al. [9] using desflurane showed a slight but controllable increase in ONSD measured after induction of anesthesia and Trendelenburg position without pneumoperitoneum. The reason for this result was explained by the authors as the maintenance of anesthesia with desflurane at 1 to 1.5 MAC in all registered patients. Desflurane at more than 1 MAC was not used in any patient in the study. It would not be correct to speculate on the clear effect of desflurane anesthesia since no measurement was taken before anesthesia induction. However, we believe that the use of desflurane at 1 MAC or below does not cause a significant increase in ICP in obese and non-obese patients based on measurements made at other time points.

Carbon dioxide (CO_2), a potent cerebral vasodilator, is associated with an increase in ICP [6]. Therefore, strict control of $PaCO_2$ will prevent an increase in ICP. End-tidal CO_2 concentration is a non-invasive parameter used as an indirect indicator of $PaCO_2$. $PaCO_2$ increases due to the diffusion of CO_2 from the peritoneum during pneumoperitoneum thus leading to an increase in ICP [27]. However, the dynamic response of ONSD associated with acute $PaCO_2$ change, which may affect ICP, is unclear. The study of Kim et al. [38] in which the effects of short-term hyperventilation on ONSD in patients under general anesthesia were. investigated found that ONSD diameters, which were measured as 0.5 mm with 40 mmHg, decreased to 4.0 mm at 30 mmHg. End-tidal CO_2, which was measured at different time points in the study, was maintained in the 36 mmHg range in both groups and never exceeded 40 mmHg at any time interval. We believe that continuous monitoring of end-tidal CO_2 concentration and maintaining this value within certain limits have an effect on the absence of an increase in ICP in both groups and the ONSD approach to baseline values after deflation.

Limitations

The present study has some limitations. The preferred position of surgeons for laparoscopic cholecystectomy in our institution is the 30° reverse Trendelenburg position. No electronic inclinometer was used while positioning. The position was given by the anesthesiologist, who would measure ONSD under the guidance of the surgeon. Although the anesthesiologist was the same, different surgical teams performed surgeries in the study. This may have prevented the complete standardization of the given position and our ability to achieve objective results. If we had monitored the depth of anesthesia using bispectral index and entropy and had seen the $PaCO_2$ values concurrently with the end-tidal CO_2, we could have interpreted the effects of the anesthesia parameters that we used on the measurement results more objectively. The lack of concurrent invasive measurement with the transocular USG may have caused us to fail to exclude an increase in ICP. No threshold value was defined for ONSD when detecting an increase in ICP. This lack of definition prevented us from achieving a clear result regarding an increase in ICP. ONSD ultrasound is operator-dependent and therefore subject to variability [39]. Measurements were performed by one person to minimize variability. Due to the single-observer strategy, we could not examine the inter-observer variability described and suggested by Dubourg et al. [40]. Another limitation of this study is the lack of capability to compare ONSD measurements with a standard imaging modality, such as computed tomography or magnetic resonance imaging. Not measuring ONSD before induction of anesthesia when the patients were awake, after intubation, and after extubation in the recovery room may have prevented us from detecting possible intubation- and extubation-related effects, both of which may cause a change in ICP. Moreover, if the measurements were taken in the supine position after deflation, the effect of the reverse Trendelenburg position on the restoration of ONSD measurements could be shown more clearly. The sample size and 80% power of the study can be indicated as another limitation.

Conclusion

Laparoscopic cholecystectomy does not cause an increase in ICP of obese patients with a limited pneumoperitoneum pressure, reverse Trendelenburg and head-up position, and controlled anesthesia. It would be beneficial to maintain the reverse Trendelenburg and head-up position after deflation, especially in patients in whom increased ICP would pose a risk.

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Is arthroplasty necessary for three and four-part proximal humerus fractures in elderly?

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Ethics Committee Approval
Ethics approval for this study was obtained from Lokman Hekim University Ethics Committee with the decision no:2021/068(code no:2021066) and approval was given on 15/06/2021.
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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Abstract

Background/Aim: Proximal humerus fractures are common in elderly patients. Treatment of three and four-part fractures is especially controversial in these patients. In recent years, surgical options have been widely used, especially among shoulder surgeons. The purpose of this study was to compare clinical results of conservative and arthroplasty methods.

Methods: Between 2016 and 2020, 30 patients who were treated for Neer type 3 and type 4 proximal humeral fractures were included in the study. Patient data were evaluated retrospectively and then divided into three groups. Group 1 was treated conservatively, group 2 underwent hemiarthroplasty; and group 3 underwent reverse total shoulder arthroplasty. CONSTANT and visual analog scale (VAS) scores and radiological results at six months were evaluated retrospectively from patient records. At the last control they have been evaluated with CONSTANT, University of California/Los Angeles. (UCLA), and VAS scores.

Results: Twenty-three (76.7%) of the patients included in the study were females. The mean age was 73.5 (5.7) years. The mean follow-up period was 33 (2.5) months. The mean follow-up periods in groups 1–3 were 33.3 (2.9), 32.8 (2), and 32.2 (2.9) months, respectively. When the CONSTANT scores of the patients were compared at the sixth month, they were observed to be better in the reverse total shoulder arthroplasty group (P = 0.001). Final control CONSTANT scores in the hemiarthroplasty group were lower than in the other groups (P = 0.001) and similar in the reverse shoulder prosthesis and conservative treatment group (P = 1). When the UCLA scores of all groups were compared, the mean UCLA scores were found to be significantly higher in groups 1 and 3 compared to group 2 (P = 0.001). When the VAS scores of the patients were compared, a significant difference between all groups was detected (P < 0.05). The highest VAS scores were observed in group 2, the second highest in group 1, and the lowest in group 3.

Conclusion: For treatment of proximal humerus fractures in the elderly, patients should be evaluated according to activity levels and expectations, and surgical treatment should be suggested rather than ordered.

Keywords: Humerus fractures, Elderly patients, Revers shoulder arthroplasty

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Introduction

Proximal humeral fractures account for 60% of fractures in adults, and 75% of the proximal humeral fractures are observed in individuals over 60 years of age. These fracture are the third most common osteoporotic fractures after hip and wrist fractures [1–3]. After the age of 60, the incidence is higher in women than in men [3]. The presence of additional diseases, such as diabetes mellitus, neuromuscular weakness, and dementia, leads to an increase in the the risk of occurrence. Such fractures are an important cause of morbidity in elderly patients, and treatment of these fractures is regarded as time-consuming and expensive [4, 5].

Eighty-five percent of proximal humeral fractures are usually non-displaced or slightly displaced fractures and are treated conservatively. However, in comminuted fractures, a classification method should be used for treatment selection of these fractures. The most widely used method for classifying proximal humeral fractures is the Neer classification system. This classification is based on the number of pieces and amount of displacement when defining a fracture. Four anatomical segments of proximal humerus are evaluated primarily. These segments are humeral head, greater tuberosity, lesser tuberosity, and humeral shaft [6]. A separation of more than 1 cm between fragments and an angulation of more than 45 degrees with the humeral shaft is defined as a four-part fracture and constitutes 3% of all proximal humeral fractures [7].

Although most fractures are treated conservatively, the choice of treatment is more complex and still unclear for four-part fracture. Surgical options include percutaneous fixation, open reduction and internal fixation, and arthroplasty. While surgical treatment options show good clinical results in young patients, they may show variable results and higher complication rates in elderly patients [8]. Poor bone quality in elderly patients leads to difficulties in internal fixation and causes complications, such as loss of reduction and avascular necrosis [9, 10]. Studies showing that internal fixation is not superior to conservative treatment are have been published [11].

Also, another treatment modality, arthroplasty, is available when open reduction and internal fixation can not be used. In studies comparing open reduction and internal fixation with arthroplasty, open reduction and internal fixation yields lower results in terms of quality of life and clinical scores. This difference leads to more frequent revision surgery after complications such as nonunion, screw penetration, and implant failure [9, 10]. Arthroplasty is applied using two different treatment methods: (1) hemiarthroplasty and (2) reverse total shoulder prosthesis.

Considering the studies conducted in recent years, the surgical option is increasingly preferred in the treatment of proximal humeral fractures [12, 13]. In our study, we aimed to investigate the clinical and radiological results of the patients to whom three different treatment options were applied for proximal humeral fractures.

Materials and methods

Ethics committee approval was obtained for our study from the non-interventional ethics committee of Lokman Hekim University Faculty of Medicine on June 15, 2021 with document number 2021/068, and all patients gave written informed consent for the use of their data in the study. Thirty patients who were treated for proximal humeral fractures between 2016 and 2020 and whose full records could be accessed were included in the study. Patients were treated in two different centers. Inclusion criteria were determined as having a three and four-part proximal humerus fracture according to the Neer classification system, being over 65 years old, being able to read, write, and cooperate. Exclusion criteria were defined as having ipsilateral upper extremity pathology, multitrauma, age less than 65 years old, and/or history of open reduction-internal fixation, revision surgery, and/or pathological fracture.

Patient data were accessed through the hospital automation system. Age, gender, American Society of Anesthesiology (ASA) score at the time of surgery, dominant extremity, mechanism of fracture, joint range-of-motion (ROM) level in controls, and clinical scores were evaluated over the records. They were divided into three groups according to the treatment methods. Group 1 was treated conservatively, Group 2 w patients who underwent hemiarthroplasty, and group 3 were determined as patients who underwent reverse total shoulder arthroplasty. CONSTANT and Visual Analogue Scale (VAS) scores and radiological results at six months were evaluated retrospectively from the records of the patients. Each patient was called for the final follow-up and evaluated with CONSTANT, University of California at Los Angeles (UCLA), and VAS scores.

Conservative treatment protocol

It was observed that the patients in group 1 were treated conservatively because of the risk in surgery due to additional medical problems or because the patient did not accept surgical treatment voluntarily. After the first evaluation of all patients in the conservative treatment group, closed reduction and shoulder arm slings were applied, and control evaluation was performed radiographically. The patients were evaluated with control radiographs once a week for the first three weeks and then at the sixth and twelfth weeks. After the second week, passive joint range-of-motion (ROM) exercises were started, and at the sixth week, the shoulder arm sling was removed and active ROM exercises were started. After the sixth week, the patients were directed to the physical therapy unit, and they were gradually oriented in terms of carrying loads and using them in daily activities at the twelfth week.

Arthroplasty treatment protocol

According to the preference of the surgeon, hemiarthroplasty (Biomet, USA) or reverse total shoulder arthroplasty (Zimmer-Biomet, USA) was applied to patients in Groups 2 and 3, depending on factors, such as the presence of an intact rotator cuff, absence of arthrosis in the glenoid, and the desire to keep the operation time short. The deltopectoral approach was used as a surgical opening in all patients. The humeral stem applied to the patients in both groups was cemented. The patients stayed at the hospital for an average of one night after the surgery and were given an arm sling for one

week after which passive joint ROM exercises were started. After three weeks, the arm slings were removed, and patients were directed to the physical therapy unit.

Clinical evaluation

When the records of the patients were evaluated, the CONSTANT (14) score and ROM data were available at six months. Evaluation results were determined as bad for > 30 points, poor for 21 to 30 points, good for 11 to 20 points, and excellent for < 10 points. After assessing the records, patients were called for their final controls. CONSTANT scores were applied, results were compared with those of the patients' intact shoulders, and the differences recorded. Also, at the final control, UCLA (15) and VAS scores were also evaluated for the patients. UCLA shoulder scores were evaluated as bad–poor in case of < 27 points and good–excellent for > 27 points. The results were compared between each group.

Radiological evaluation

All patients were evaluated with shoulder anterior–posterior (AP) radiography and shoulder computerized tomography (CT) at the first admission. The conservatively followed patients were checked with shoulder AP radiography at the first, second, third, and sixth week and again at three and six months and were evaluated with shoulder AP radiography at their last follow-up. Patients who underwent arthroplasty were checked with AP shoulder radiography on the first post-operative day, sixth week, and at three and six months (Figure 1), and were evaluated with AP shoulder radiography again at the last follow-up. In the evaluation of arthroplasty patients, a radiolucent area of more than 2 mm around the humeral stem on direct radiography was considered a loosening sign.

Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) 22 package program. Data were expressed as mean and standard deviation (SD). The homogeneity of the data was evaluated with the Levene test. The analysis of quantitative variables between groups was performed using an analysis of variance (ANOVA) test. The results were evaluated at the 95% confidence interval (CI), and the significance was evaluated at the $P < 0.05$ level. A post-hoc Benferroni test was performed in groups with significant results based on the ANOVA test.

Results

The patients included in the study were mostly female. Three males and eight females were in Group 1, two males and seven females in Group 2, and two males and eights females in Group 3. The mean age of all patients was 73.5 (5.7) years. The mean age of the patients was 76.4 (4.2) in Group 1, 75.8 (6.7) in Group 2, and 68.3 (1.2) in Group 3. The mean follow-up period of all patients was 33 (2.5) months. The mean follow-up periods in Groups 1–3 3 were 33.3 (2.9), 32.8 (2), and 32.2 (2.9) months, respectively. When the fracture formation mechanisms of the patients were evaluated, 60% were due to simple falls (n = 18), 23% in-vehicle traffic accidents (n = 7), 10% falls from height (n = 3), and 7% were due to motorcycle injuries (n = 2). The injured extremity was the dominant one in 66% of the patients (n = 20). According to the evaluation of the patients at the time of injury, 6% were determined as ASA 3 and 63.3% as ASA 4. When the fracture type, gender, follow-up period, and dominant extremity status of the patients were compared, no statistically significant difference was found ($P > 0.05$) as shown in Table 1.

Table 1: Distribution of patients according to gender, American Society of Anesthesiologists (ASA) scores, mechanism of fracture formation, and fracture type

		Group 1 (n = 11)	Group 2 (n = 9)	Group 3 (n = 10)
Gender	Male	3(27.3%)	2(22.2%)	2(20%)
	Female	8(72.7%)	7(77.8%)	8(80%)
Dominant extremity	Right	6(54.5%)	6(66.7%)	8(80%)
	Left	5(45.5%)	3(33.3%)	2(20%)
ASA	ASA 3	0(0%)	4(44.4%)	7(70%)
	ASA 4	11(100%)	5(55.6%)	3(30%)
Mechanism of formation	Simple fall	7(63.6%)	7(77.8%)	4(40%)
	Traffic accident	2(18.2%)	1(11.1%)	4(40%)
	Falling from high	1(9.1%)	1(11.1%)	1(10%)
	Motorcycle accident	1(9.1%)	0(0%)	1(10%)
Fracture Type	Neer 3	6(54.5%)	6(66.7%)	7(70%)
	Neer 4	5(45.5%)	3(33.3%)	3(30%)

* Patient numbers are given along with their percentage in groups in parenthesis.

At six months, the mean CONSTANT score of all patients was 11 (3.9). In Group 1, Group 2 and Group 3, these scores were 16.9 (1.7), 14.4 (2.9), and 7.5 (1.3), respectively. When the CONSTANT scores of the patients were compared, no significant difference between scores at six months between groups 1 and 2 were found ($P = 0.272$). Significant differences between Groups 2 and 3 and Groups 1 and 3 were noted. ($P = 0.001$). CONSTANT scores at six months were observed to be better in the reverse total shoulder arthroplasty group. The CONSTANT scores of the patients at the last control were 10.1 (2.7), 29.6 (6.4), and 10.3 (3.1) in Groups 1–3, respectively. When the CONSTANT scores of the groups at the last control were compared, significant differences between Groups 1 and 2 and between Groups 2 and 3 were found ($P = 0.001$). No significant difference between Groups 1 and 3 was found ($P = 1$). Final control CONSTANT scores in the hemiarthroplasty group were low compared to the other groups and similar in the reverse shoulder prosthesis and conservative treatment groups (Tables 2, 3).

Table 2: UCLA, CONSTANT, VAS, 6thmonth mean CONSTANT scores and range of motion of the patients

	Group 1	Group 2	Group 3	*P*-value
UCLA	28.6 (2.4)	16.7 (2.8)	30.6 (2.9)	0.001
CONSTANT	30.6 (2.9)	29.6 (6.4)	10.3 (3.1)	0.001
VAS	5.0 (1.2)	8.6 (0.8)	2.0 (1.0)	0.001
CONSTANT 6th month	16.0 (1.7)	14.4 (2.9)	7.5 (1.3)	0.001
Flexion	128.6 (7.1)	94.4 (9.8)	165.0 (6.2)	0.001
Abduction	121.8 (6.8)	86.6 (5.0)	165.5 (6.4)	0.001
Extension	24.5 (4.7)	16.6 (5.0)	33.5 (2.4)	0.001
Total	(n = 30)			

Analysis of variance (ANOVA) test, UCLA: University of California at Los Angeles; VAS: visual analog scale

Table 3: Comparison of UCLA, CONSTANT, VAS and joint range-of-motion (ROM) of the groups (post hoc Bonferroni test)

Compared Groups			*P*-value
UCLA	Group 1	Group 2	0.001
		Group 3	0.337
	Group 2	Group 3	0.001
CONSTANT	Group 1	Group 2	0.001
		Group 3	1.000
	Group 2	Group 3	0.001
VAS	Group 1	Group 2	0.001
		Group 3	0.001
	Group 2	Group 3	0.001
CONSTANT 6th month	Group 1	Group 2	0.272
		Group 3	0.001
	Group 2	Group 3	0.001
Flexion	Group 1	Group 2	0.001
		Group 3	0.001
	Group 2	Group 3	0.001
Abduction	Group 1	Group 2	0.001
		Group 3	0.001
	Group 2	Group 3	0.001
Extension	Group 1	Group 2	0.001
		Group 3	0.001
	Group 2	Group 3	0.001

The mean UCLA score of all patients was 25.7 (6.5). In Groups 1–3, 3, these scores were 28.2 (2.4), 16.7 (2.8), and 30.6 (2.95), respectively. When the UCLA scores of all groups were compared, statistically significant differences were found between Groups 1 and 2 and Groups 2 and 3 ($P = 0.001$), but no statistically significant difference was found between Groups 1 and 3 ($P = 0.337$). The mean UCLA scores were found to be significantly higher in Groups 1 and 3 compared to Group 2 (Tables 2, 3).

When the VAS scores of the patients were compared, significant differences between all groups were found ($P < 0.05$). The highest VAS scores were observed in group 2, the second highest in group 1, and the lowest in group 3 (Tables 2, 3).

While the mean flexion, abduction, and extension scores were 128 (7.1), 121 (6.8), and 24.5 (4.7) in group 1, they were 94.4 (9.4), 86.6 (5), and 16.6 (5) in group 2 and 165 (6), 165 (6.4) and 33.5 (2.4) in group 3, respectively. When the groups were compared in terms of joint ROM, statistically significant differences between all groups ($P = 0.001$) were found (Table 2, 3).

Superficial infection developed in two patients in groups 2 and 3. These patients have recovered with oral antibiotic therapy. The fractures of all patients in group 1 were healed. No patient was lost during the follow-up.

When the radiographs of the patients in groups 2 and 3 were examined at the last follow-up, loosening was detected in one patient in group 2. Surgery was recommended, but the patient refused. No loosening was detected in any patient in group 3.

Discussion

Proximal humeral fractures are seen as the most common fracture type after hip and wrist fractures, especially in elderly patients with osteoporosis. In the United States alone, more than 1.5 million osteoporotic fractures are reported annually, of which more than 400,000 are proximal humeral and pelvic fractures [16]. Proximal humeral fractures are more commonly defined according to the Neer classification system, and when evaluated according to this classification, most of the fractures are minimally displaced or displaced as 1-2-piece fractures. These fractures are mostly treated conservatively. No definitive scientific evidence regarding which treatment is the most appropriate treatment choice for complex fractures. Considering that proximal humeral fractures are more common in elderly osteoporotic patients, treatment costs and risk of morbidity constitute a social problem. Studies in the literature demonstrating different results have been published [8, 9, 17–19].

The development of implant technology used in surgery and the increase in the applicability of surgical techniques have brought the surgical option to the forefront in the treatment of proximal humeral three and four-part fractures [12, 13]. The results of open reduction and internal fixation are good and widely used in the treatment of patients with proximal humeral fractures who are less than 65 years of age. With the advances in locking plate technology, use of this technology in osteoporotic proximal humerus fractures has increased; however, the complications and revision surgery rates also seem to be high [20]. In a review published in 2012, it was reported that re-operation rates were unexpectedly high in complex proximal humerus fractures in which locking plate fixation treatments were applied [21].

In our daily practice, we apply locking plates to young patients in accordance with the literature. If our patients over 65 years of age will undergo surgery, arthroplasty is preferred over other options. The patient groups included in our study consisted of patients who underwent conservative treatment due to high ASA scores or patient rejection after the decision in favor of surgery or underwent arthroplasty after humeral fracture. For the patients who underwent arthroplasty, hemiarthroplasty and reverse total shoulder arthroplasty implantation decision was made as a result of intra-operative evaluations. It was observed that hemiarthroplasty was performed during the operation when the patient's rotator cuff was intact, no degenerative lesions on the glenoid were found, and a shorter operation time was desired. Also, reverse shoulder arthroplasty was performed in cases in which the rotator cuff was not intact, a degeneration existed on the glenoid, and tubercles were fragmented.

When the studies with hemiarthroplasty applications in proximal humerus fractures were evaluated, a study published in 2003 in which hemiarthroplasty was performed with the appropriate technique was found to affects shoulder functions positively and lead to a reduction in pain [17]. In the same study, it was determined that improper fixation of the tubercles in the hemiarthroplasty application caused poor clinical results, and the highest complication was nonunion in the greater tubercle. In another study in which comparing internal fixation and hemiarthroplasty applications were compared, patients were evaluated via the CONSTANT, Disabilities of the Arm, Shoulder and Hand (DASH) score, health-related quality of life (HRQoL) and EQ-5D (Euro QolGroup, Rotterdam, The Netherlands) scores [10]. While a significant increase in the HRQoL score in the hemiarthroplasty group was noted, no significant difference was observed in other results. In another review study, it was stated that when hemiarthroplasty applications are performed with the appropriate technique, clinical results are reported to be higher than internal fixation [22]. When the studies examining hemiarthroplasty applications were evaluated; while painless shoulder movements were expected, good clinical results were associated with tuberosity union, and that was observed at a higher rate in young patients with high bone stock in large tubercle [23, 24]. Complications of hemiarthroplasty include infection, wound problems, nerve injuries, intra-operative fractures, instability, nonunion and migration of tuberosities, rotator cuff tears, component malposition and loosening, joint stiffness, and heterotrophic ossification [25]. When the literature is evaluated, it seems difficult to perform successful hemiarthroplasty surgeries in proximal humeral fractures because a successful surgery depends on many factors. In our study, the postoperative results of the hemiarthroplasty group had lower clinical scores when compared with conservative treatment and reverse total shoulder arthroplasty.

When comparing reverse shoulder arthroplasty and hemiarthroplasty applications in complex proximal humerus fractures in the literature; reverse shoulder arthroplasty was found to provide better clinical results, ROM, and fewer

complications [26, 27]. The basic requirement in reverse shoulder prosthesis applications is sufficient deltoid muscle strength. The fact that the anatomical placement and tubercle healing are not needed and the reverse shoulder prosthesis is not affected by the absence of the rotator cuff can be factors leading to better clinical results compared to hemiarthroplasty [27–29]. In our study, clinical results and joint ROM were significantly better in reverse shoulder arthroplasty applications compared to hemiarthroplasty; our results are in line with the literature. We think that the comparison of CONSTANT scores with a healthy shoulder during clinical evaluation in our study creates a different result compared to the general literature, and this finding is valuable in showing joint function on an individual basis. In this way, we believe the age of a patient is not relevant to that clinical regression of the shoulder functions.

In our study, no significant difference between the patients who underwent reverse shoulder arthroplasty and the conservative follow-up in terms of clinical outcomes was found. Our results are also compatible with the literature. In a study conducted by Lopiz et al. [30] in 2019 in which 29 patients with reverse shoulder arthroplasty and 30 patients with proximal humerus fractures who had conservative treatment were compared, they found no significant difference between the two methods based on VAS scores and clinical scores during the 12-month follow-up period. They reported that in the group treated with reverse shoulder arthroplasty, they did not encounter any significant complications other than the suprascapular nerve injury. Complications developed in only two cases, and in these two cases, no problem other than the difficulty of pain control was reported. In the conservatively followed group, all cases resulted in radiological malunion, and osteonecrosis was observed in 58% of them. However, the development of osteonecrosis was not associated with low CONSTANT and DASH scores. In our study, all patients who were treated conservatively recovered in the presence of malunion. However, the presence of malunion did not affect clinical scores. In fact, the joint ROM and clinical scores were significantly higher than those of hemiarthroplasty group. This difference suggests that it may be related to the low expectation in the elderly patient group with a high morbidity rate. Additional surgical trauma in the hemiarthroplasty group may be associated with poor clinical outcomes. The same situation was not encountered in the reverse shoulder arthroplasty group, and this finding may be related to the fact that reverse shoulder arthroplasty applications are independent of tuberosity healing and rotator cuff strength. The patients who underwent surgery who were included in the study did not develop any complications other than superficial infections.

Our study has limitations, including a retrospective nature and a small number of patients. Longer patient follow-ups may yield more meaningful results. However, evaluating the advanced age group shortens the follow-up period. We think that the comparison of the three groups in our study is an advantage in terms of contributing to the literature.

Conclusions

As a result, conservative treatment applications in three and four-part proximal humerus fractures in elderly patients do not yield poor clinical results when compared with arthroplasty applications. Conservative treatment shows even better results than hemiarthroplasty. The most important finding of our study was that the results of conservative treatment and reverse shoulder prosthesis treatment were similarly good, and the clinical results of the patients who underwent hemiarthroplasty were worse than these two groups. This finding shows us that the choice of hemiarthroplasty treatment in three and four-part old proximal humerus fractures produces a poor quality of life for the patients.

In this study, we support the hypothesis that the results of the surgical option are not more effective than the conservative treatment and that surgery should not be rushed in elderly.

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Can intra-operative methylprednisolone application be effective for post-operative pain, nausea and vomiting in laparoscopic cholecystectomy operations?

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Ethics Committee Approval
This study was approved by the Uşak University Non-Interventional Clinical Research Ethics Committee, 135-08-11.
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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

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Abstract

Background/Aim: Post-operative nausea, vomiting (PONV), and pain are common symptoms after laparoscopic cholecystectomy (LC) that is performed under general anesthesia. These symptoms lead to prolongation of post-operative recovery and hospital stay. In this study, the efficacy of intra-operative methylprednisolone (MP) administration on post-operative pain and PONV was investigated in patients undergoing LC under general anesthesia.

Methods: This study was conducted at Uşak University Faculty of Medicine Hospital. Patients who underwent LC under general anesthesia between 01.11.2018 and 01.06.2019 were evaluated using the prospective cohort method. While intra-operative MP was administered to one group of patients who underwent LC (MP group), MP was not administered to the second group (non-MP). The pain was evaluated using the Visual Analog Scale (VAS) while PONV was evaluated with the Verbal Descriptive Scale (VDS in patients at post-operative hours 0, 1, 2, 6, 12, 18, and 24. On the first post-operative day, patient satisfaction was assessed.

Results: The study cohort consisted of 76 patients. The VAS was used to measure post-operative pain, and it was discovered that the MP group had significantly reduced VAS values at post-operative hours 0, 1, 2, 6, 12, 18, and 24 ($P < 0.001$). In the VDS evaluation, no difference between the two groups only at post-operative hour 12 ($P = 0.52$) was found, while the VDS value was found to be lower in the MP group than in the non-MP group at post-operative hours 0, 1, 2, 6, 18, and 24 ($P < 0.001$). The mean total analgesic use at post-operative hour 48 was 69.08 (26.91) mg in the MP group and 96.71 (42.38) mg in the non-MP group. The difference was statistically significant ($P < 0.001$).

Conclusion: PONV and discomfort incidence decreased after intra-operative MP administration. The decrease in these symptoms was positively reflected in post-operative patient satisfaction.

Keywords: Methylprednisolone, Postoperative pain, Postoperative nausea-vomiting

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Introduction

Nausea, pain, and vomiting are the main symptoms affecting post-operative patient comfort. These symptoms may lead to a prolongation of hospitalization and cause hospitalization again after discharge. Also, these symptoms are also included in the etiologies of complications, such as post-operative wound dehiscence, esophageal rupture, and pneumothorax [1]. While post-operative nausea and vomiting (PONV) occurs at a rate of around 30% in patients who do not receive prophylaxis, this rate may increase to 80% depending on a patient's individual predisposition [2, 3]. After gynecological and laparoscopic procedures, PONV is thought to occur more often. However, it is still controversial as to which operations trigger this situation more frequently [4]. If no prophylaxis is applied in laparoscopic cholecystectomy (LC) operations, the incidence of PONV is reported to be 53% to72% [5].

To achieve early mobilization, reduce the length of the hospital stay, and reduce complications, it is crucial to prevent post-operative pain. Opioids are frequently preferred for this purpose. Opioids, however, are also linked to PONV and other adverse effects. Therefore, minimizing the need for opioids and trying multimodal treatment methods are considered the most optimal strategy in post-operative pain management [6, 7].

It has been proposed that pre-operative glucocorticoids lead to a reduction in the incidence of PONV and may play a role in reducing post-operative pain [8, 9]. In this study, the efficacy of intra-operative methylprednisolone (MP) administration in relieving PONV and post-operative pain in patients undergoing LC was investigated.

Materials and methods

Patients who had elective LC surgery at Uşak University Medical Faculty Hospital were included in this study. The post-operative efficacy resulting from intra-operative MP administration was evaluated in the study. Participants in the study ranged in age from 18 to 70 and were American Society of Anesthesiologists (ASA) I-II patients. Those weighing < 50 kg or >120 kg, those with a body mass index (BMI) > 30, patients with ASA III-IV scores, those who had used any analgesic in the last 12 h, those with known gastritis and ulcer diagnosis or undergoing treatment, steroid users, those who were allergic to any of the study drugs to be used, those with aspartate transaminase (AST) >70 U/L, alanine aminotransferase (ALT) >110 U/L, and/or creatinine >2 mg/dl, and those with intellectual disability were excluded from the study. The study was conducted in accordance with the ethical standards specified in the Helsinki Declaration and was approved by the local ethics committee (Uşak University Non-Interventional Clinical Research Ethics Committee, 135-08-11). Written patient consent was obtained from the patient or the patient's spouse or legal guardian. Patient data were collected prospectively.

Selection of groups and collection of data

Intra-operative MP (2 mg/kg)was administered to one patient group (MP group), while intra-operative MP was not administered to the other group (non-MP group). Patients were assigned to the groups consecutively. Age, gender, BMI, duration of anesthesia, duration of surgery, and Apfel score (Table 1) of the patients were recorded.

Table 1: Apfel risk scoring

Risk factors	Score
Female gender	1
Not smoking	1
PONV story	1
Post-operative opioid use	1

PONV: Post-operative nausea and vomiting

Patients in both groups underwent surgery under general anesthesia. Each patient received 6 mg/kg thiopental, 0.6 mg/kg rocuronium bromide, and 0.5 mg/kg fentanyl in induction. For LC, 10 mm trocar entries were made 5 cm below the umbilicus and 5 cm below the xiphoid, and 5 mm trocar entry was from the right subcostal midclavicular and anterior axillary line. Intra-abdominal pressure (12 mmHg) was applied to the patients during LC. Anesthesia was maintained with 50% oxygen, 50% air mixture, and 2% sevoflurane. Inhalation anesthetics were stopped after placing the last skin suture. The patients were kept in the post-operative recovery unit for 1 h after which the patients with stable vital signs were transferred to the services. The patient's arrival in the post-operative recovery unit was considered as 0 min, and the patient's pain was evaluated and recorded with the VAS (Visual Analog Scale) at post-operative 0, 1, 2, 6, 12, 18, and 24 h. In the evaluation of the VAS, the patients were asked to score between 1 and 10 for their pain for which 0 indicated no pain and 10 reflected the worst possible pain. Also, whether the patients had nausea and vomiting at post-operative hours 0, 1, 2, 6, 12, 18, and 24 was evaluated with the Verbal Descriptive Scale (VDS) in this study. Severity of nausea and vomiting in the VDS assessment was classified into five groups: (1) None at all: 0; (2) Mild degree nausea: 1; (3) Moderate degree nausea: 2; (4) Frequently vomiting: 3; and (5) Severely vomiting: 4. No prophylactic antiemetic administration was given to the patients. The first post-operative analgesic requirement hours and total amount of analgesic consumption during the first post-operative 48 h were recorded. In case of post-analgesic requirements, 75 mg diclofenac sodium was administered intramuscularly to the patients. Patients were asked about their satisfaction on the first post-operative day. Scores were between 0 and 1 with 0: Not at all satisfied and 10: Very satisfied. Patients were asked to score accordingly.

Statistical analysis

According to how the groups were distributed, descriptive statistics for numerical variables were summarized as mean (standard deviation [SD]) or median with interquartile range (IQR). Mean parameter comparisons between groups with and without MP were obtained using with the independent-sample t test for normally distributed groups. For group comparisons in which no normal distribution was found, the Mann–Whitney U test was used. A 5% type 1 error level was considered statistically significant for all tests. Statistics Package for the Social Sciences 22.0 statistical package (IBM Corp.; Armonk, NY, USA) was used to conduct statistical analyses.

Results

G*power 3 software was used to determine the sample size. With a margin of error of 5% and a power of 0.80, 38 cases in each group were found to be sufficient to compare the

statistical significance of numerical variables between the MP and non-MP groups using the Mann–Whitney U test in the study. A total of 76 patients was included with 38 patients in each group.

A total of 76 patients in the study with 34 men and 42 women were assessed. The patients' average age was 44.6 (8.4) years. No differences between MP group and non-MP group were found in terms of age (P = 0.78), gender (P = 0.49), BMI (P = 0.33), duration of anesthesia (P = 0.22), surgery time (P = 0.80), and Apfel score (P = 0.37) as shown in Table 2. In the evaluation of the groups in terms of VAS, statistical significance in favor of the MP group (P < 0.001, Table 3) was observed. In terms of VDS, statistical significance was also found in favor of the MP group at other times, except at the 12th hour (P = 0.52, Table 3). The mean time for the need for the first post-operative analgesic administration was found to be 204.21 (37.02) min and 128.03 (11.71) min in the MP and non-MP groups, respectively (P < 0.001). Again, the amount of analgesic consumption in the first 48 h was significantly lower in the MP group (P < 0.001). The mean value of analgesic consumption was 69.08 (26.91) mg in MP group , whereas it was 96.71 (42.38) mg in the non-MP group. Patient satisfaction was evaluated on the first post-operative day. The median satisfaction score was found to be 8 (min: 5–max: 9) in patients belonging to the MP group and 6 (min: 4–max: 8) in the non-MP group (P < 0.001).

Table 2: Demographic distribution of groups

	MP group	Non-MP group	*P*-value
Gender (male/female)	16/22	18/20	0.49
Age (years) Mean (SD)	44.6 (8.4)	44.9 (7.1)	0.78
Anesthesia time (minutes) Mean (SD)	115.2 (8.8)	117.3 (9.2)	0.22
Surgery time (minutes) Mean (SD)	98.2 (9.4)	98.8 (9.5)	0.80
Apfel score median (min-max)	1 (0–3)	1(0–2)	0.37
BMI (kg/m^2) Mean (SD)	29.13 (3.9)	28.3 (4.7)	0.33

MP: Methyl prednisolone, BMI: Body mass index

Table 3: VAS and VDS evaluation of groups

	VAS 0	VAS 1	VAS 2	VAS 6	VAS 12	VAS 18	VAS 24
MP group (min–max)	5 (4–6)	5 (4–5)	4 (3–5)	4 (3–4)	3 (3–4)	2 (1–2)	1 (0–1)
Non-MP group (min–max)	6 (5–7)	6 (5–7)	6 (5–6)	5 (5–6)	5 (4–5)	4 (2–4)	3 (1-–3)
P-value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
	VDS 0	VDS 1	VDS 2	VDS 6	VDS 12	VDS 18	VDS 24
MP group (min–max)	0 (0–1)	0 (0–1)	0 (0–2)	0 (0–2)	0 (0–3)	0 (0–2)	0 (0–2)
Non-MP group (min–max)	0 (0–1)	0 (0–1)	0 (0–3)	0 (0–4)	0 (0–4)	0 (0–4)	0 (0–4)
P-value	0.01	0.025	0.044	0.042	0.052	0.039	0.031

VAS: Visual Analog scale, VDS: Verbal Descriptive Scala

Discussion

Post-operative pain, nausea, and vomiting are frequent adverse effects of anesthesia. The incidence of PONV in all surgical procedures can be observed at a rate of 20 - 77%. Post-operative pain is closely associated with a prolonged recovery period [2, 10, 11]. Prevention of nausea, vomiting, and pain is very important for post-operative patient comfort. In this study, PONV and pain were observed less in patients who received intra-operative MP, and a higher level of patient satisfaction was achieved.

Wang et al. [12] reported that PONV was reduced after pre-operative administration of dexamethasone in LC patients. Also, Henzi et al. [13] obtained similar results in their study and found that 8 mg dexamethasone administered pre-operatively led to a reduction in PONV in LC patients. In a meta-analysis evaluation, it was stated that peri-operative single-dose glucocorticoid administration reduced the incidence of PONV in large abdominal operations [8]. Despite the results of this study, in a similar study conducted in laparoscopic appendectomy patients, it was suggested that pre-operative administration of8 mg dexamethasone did not produce a reduction in the incidence of PONV, and pre-operative dexamethasone administration was not recommended for the prevention of PONV [14]. In another study conducted in orthopedic surgery patients, 125 mg MP was administered pre-operatively, and it was found to lead to a reduction in PONV. However, inconsistent results were reported in several studies involving patients undergoing intra-abdominal surgery [15–17]. Nausea and vomiting are mediated by muscarinic M1, dopamine D2, histamine H1, 5-hydroxytryptamine (HT)-3, and neurokinin 1 neurotransmitter receptors [18]. It is stated that a single dose of glucocorticoids prevents nausea and vomiting by inhibiting the synthesis of prostaglandins and endogenous opioids [13]. In this study, 125 mg of MP was administered intra-operatively to patients with LC, and nausea and vomiting were evaluated using the VDS. PONV was found to be significantly less in the MP administered group compared to the non-administered patient group.

Pain is one of the most important causes of the prolonged recovery period after LC [11]. Opioids have been the mainstay in reducing post-operative pain. However, opioids may not be used in effective doses due to their side effects (constipation, sedation, neuroexcitation, delirium, and others). These side effects can lead to a worsening of the already impaired quality of life in patients. Therefore, a multimodal approach and new researches for postoperative pain control is being used. For this purpose, pre-operative use of glucocorticoids for post-operative pain control has become a current issue. Aabakke et al. [17] administered 125 mg MP pre-operatively to the patients who had undergone open hysterectomy and stated that it had no benefit in post-operative pain control. Consistent with this study, in a meta-analysis investigating the efficacy of glucocorticoids, nine studies conducted on major abdominal surgery reported that glucocorticoids did not lead to a reduction in post-operative pain [8]. On the other hand, Lunn et al. [15] reported that pre-operative administration of 125 mg MP reduced pain after orthopedic surgery. In another study evaluating post-operative pain in patients who underwent LC, it was revealed that 125 mg MP administered pre-operatively was effective in producing a reduction in post-operative pain [10]. Contradictory study results for pre-operatively administered glucocorticoids for postoperative pain control have been published. It has been suggested that the analgesic effect of glucocorticoids is related to edema reduction [8]. It has also been demonstrated that systemic corticosteroid administration leads to a reduction in tissue levels of bradykinin and reduction in pain by suppressing the release of neuropeptides from nerve endings [19, 20]. In parallel with this literature information, in this study, post-operative VAS values were significantly lower in the MP-administered group compared to the non-administered group. Also, it was determined that the total amount of post-operative analgesic consumption was less in the MP-administered group.

In a meta-analysis conducted by Waldron et al. [21], it was stated that pre-operative administration of 8 mg dexamethasone led to an increase in post-operative patient satisfaction. In another study addressing this issue, the post-operative comfort in patients who had received pre-operative MP was evaluated using a questionnaire. Parameters, such as sleep quality, PONV, unaided mobilization, and fatigue were questioned in the questionnaire, and it was revealed that preoperative MP administration did not increase patient satisfaction [14]. Preventing nausea and vomiting after surgery and reducing pain are important for patient comfort. In this study, a reduction in both PONV and post-operative pain was observed in MP administered patients. These results were also reflected in patient satisfaction for which post-operative patient satisfaction in the MP-administered group was significantly better than in the non-administered group.

Limitations

The study was conducted in a limited patient group, and our patients consisted of only LC patients. Therefore, our results do not cover all operations. In addition, this study was performed in a single center and was not compared with the placebo group.

Conclusion

In this study, it was demonstrated that intra-operative administration of 125 mg MP led to a decrease in PONV and post-operative pain. An increase in post-operative patient comfort associated with the reduction of these symptoms was detected.

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Health sciences students' viewpoint on innovative approaches in histology courses

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Ethics Committee Approval
The study was carried out with the approval of Siirt University Ethics Committee dated 23.08.2022 and numbered 3198.
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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

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Abstract

Background/Aim: It is crucial to improve histology education quality and train competent individuals in the fields of health and medical sciences. Feedback received from students can provide guidance to achieve these goals. This study aims to reveal the opinions of Faculty of Health Sciences students about histology education and to identify their need for innovative approaches to improve this course.

Methods: This study was conducted with 174 students who were enrolled in their first year of the general histology course at the Faculty of Health Sciences, Siirt University, during the 2021–2022 academic year. The students answered survey questions electronically at the end of the semester, and the students' opinions about the general histology course were obtained. A content analysis technique was used to evaluate the collected data.

Results: Half of the students thought that the histology course was difficult. More than half of the students (57.5%) stated that the length of the theoretical course was sufficient and that practical courses should be supported by various applications. Most of the students (63.8%) stated that the histology course was important for their profession and that it would be more efficient to teach this course by integrating it with clinical sciences. Most of the students (81%) reported that the histology course integrated with technological tools would contribute to their education. Nearly half of the students (49.4%) had a negative response to teaching this course via the distance education method.

Conclusion: Histology education is considered by students to be a difficult course to learn. For students to overcome these difficulties, it can be helpful to provide both theoretical and practical courses at close intervals in a holistic manner. Additionally, integrating this course with clinical sciences can also increase student performance. It is believed that for student success, it is important to integrate educational models with traditional methods supported by technological educational materials and distance education systems.

Keywords: Histology, Health and medical education, Technology-mediated learning

Introduction

Instructors need to use effective techniques to obtain health-related information [1]. To minimize the problems in health and medical education, correct educational practices that include adequate human resources and technology need to be adopted [2]. One of the important factors that directly affect health and medical education practices today is the Covid-19 pandemic. The role of distance education and practices, which includes various teaching technologies, has increased due to the impact of the pandemic [3].

Basic medical education is one of the practices that play an important role in health and medical education. The science of histology also plays a key role in this education [4]. Histology can be defined as the scientific study of the microscopic structure of cells and tissues [5]. This branch of science is considered by students to contain difficult-to-understand and abstract subjects, and students often have difficulty associating this theory with the practice thereof. Additionally, health sciences students often do not know the importance of learning the normal structures of the body for future clinical activities [6].

In histology education, which first started in the 13th century, innovative approaches emerged with the development of new techniques and methods [4]. In today's world, where classical optical microscopes are widely used in histology education, digital images containing histology preparation images are also now used [7]. Furthermore, the 'Digital microscope slide to improve microscope usage skills' [8], 'Educational set comprising smart preparations' [9], and the 'Education set consisting of smart interactive models and a software program' [10] are also among the innovations recently introduced in histology education.

It is crucial to improve histology education quality and train competent individuals in the fields of health and medical sciences. The education system can be further improved by obtaining feedback from students in these fields. Additionally, the number of studies addressing histology education with innovative approaches is increasing in the current literature. This study aims to reveal the opinions of the Faculty of Health Sciences students about histology education and to identify the needs for innovative approaches to improve this course.

Materials and methods

Study design and procedure

This study is qualitative research conducted to determine the views of students taking histology courses in health sciences. Because quite different analysis methods and techniques are used in qualitative research, it is not possible to talk about a common language in qualitative data analysis. Because the aim was to determine the existing opinions, the survey method, one of the qualitative research techniques, was used. The study, conducted as an online survey, began in September 2022 and lasted four weeks. In line with the collected data, the evaluation of the interviews was made by content analysis.

Sampling strategy and participants

This study was conducted with 174 students who were enrolled in their first year of the general histology course (two hours of theoretical lessons per week) at the Faculty of Health Sciences, Siirt University, during the 2021–2022 academic year. Demographic data like gender was assessed. The random sampling method was used among the students of the Faculty of Health Sciences to form the sample of the study.

Data collection tools and methods

The data in the study were obtained by using a survey prepared by the researcher using Google Forms. The first introductory paragraph of the survey provided information to the participants about the aims of the study, its confidentiality, and that they may not participate in the study if requested. The study consisted of seven closed-ended questions under five categories based on students' views on histology education. The questions in the second and third categories were formed by combining two separate questions. Each question included examples of Likert-type questions with three or five possible answer options, considering the literature [11, 12]. The students answered the survey questions electronically at the end of the semester, and the students' opinions about the general histology course were obtained. Within the scope of this course, the structure and functions of epithelial, connective, muscle, and nerve tissues were taught to the students. The students were not asked to provide identifying information such as first name, last name, or student number to ensure the reliability of the responses.

The opinions of the students about the 'difficulty of the histology course, its importance and utility in professional life, its integration with clinical sciences and technological tools and equipment, and the feasibility of distance education for the course were obtained using the survey form. Additionally, the opinions of the students about the adequacy of the length of the theoretical histology course and the necessity of practical courses were also captured. The questions in the survey form were multiple-choice. The time required to complete the online survey was approximately five to seven minutes.

Data analysis

Descriptive statistics, including percentages, were calculated to summarize student response patterns for survey items. In addition, the content analysis technique was used to evaluate the data collected in this study. Content analysis is a systematic and repeatable technique in which the words that make up a text are coded within the framework of certain rules and converted into smaller categories, and the text is summarized. Content analysis is used to provide convenience to both researchers and readers in summarizing and making different research data meaningful for a specific purpose [13]. The data were analyzed under five categories within the scope of the study's research questions. The main factor in evaluating the research data using content analysis is the transformation of the collected data into concepts and associations that can be explained. In content analysis, similar data are brought together within the framework of certain concepts and themes. In this way, the data can be interpreted at a level that the reader can understand [14].

Ethical aspect

The study was conducted with the approval of the Ethics Committee of Siirt University dated 23 August 2022 and numbered 3198. A Research Permit Form was obtained from the institution where the study was conducted. Additionally, the

Survey Informed Consent Form was completed by the participants.

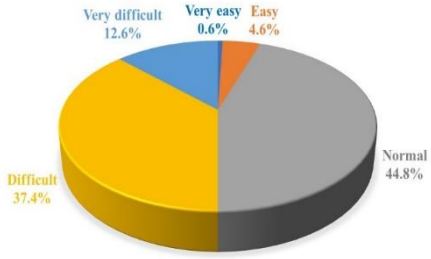
Results

Of the 174 students who participated in the study, 70 were male (40.2%), and 104 were female (59.8%). The content analysis results of the data collected from the students through the survey form are given below.

1. What are the opinions of the students about the 'difficulty of the histology course'?

Within the scope of the study, the students were asked to rate the difficulty of the histology course from 1 to 5 (1: very easy, 2: easy, 3: normal, 4: difficult, and 5: very difficult). Among the participants, one student (0.6%) answered this question as very easy, eight students (4.6%) answered the question as easy, 78 students (44.8%) answered the question as normal, 65 students (37.4%) answered the question as difficult, and 22 students (12.6%) answered the question as very difficult (Figure 1).

Figure 1: The degree of difficulty of the histology course



2. What are the opinions of the students about the 'adequacy of the theoretical histology course hours and the necessity of practical courses (microscope applications)'?

Within the scope of the study, the students were asked whether the length of the theoretical histology course was adequate. The length of the course was found to be sufficient by 100 students (57.5%) and insufficient by 42 students (24.1%). Thirty-two students (18.4%) reported that they were undecided (Figure 2).

Within the scope of the study, the students were asked about their opinions on the necessity of practical histology courses. Practical histology education was considered necessary by 127 students (73%), while it was stated that it was not necessary by 34 students (19.5%). Thirteen students (7.5%) reported that they were undecided (Figure 3).

Figure 2: The level of adequacy of the length of the theoretical histology course

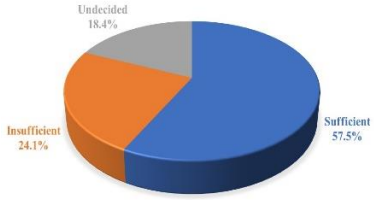
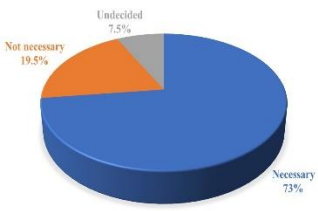


Figure 3: The necessity of practical histology education



3. What are the opinions of the students about 'the importance of the histology course in professional life and its integration with clinical sciences education'?

Within the scope of the study, the students were asked about their opinions on the importance of the histology course in their profession. Among the participants, 111 students (63.8%) answered this question as important, while 26 students (14.9%) stated that it was not important. Thirty-seven students (21.3%) reported that they were undecided (Figure 4).

Figure 4: The importance of the histology course in professional life

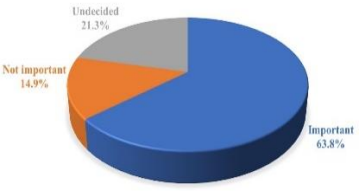
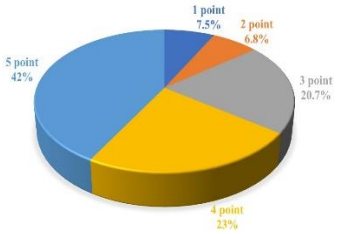


Figure 5: The necessity and utility of the histology course for clinical applications

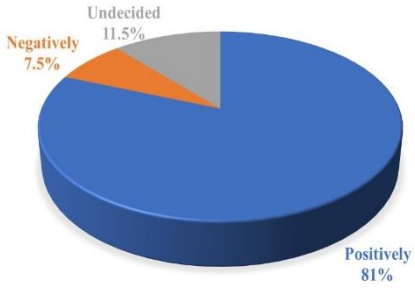


Within the scope of the study, the students were asked about the necessity and utility of the histology course for clinical applications. The students were asked to rate a statement from 1 to 5 (1 = somewhat necessary; 5 = very necessary). Among the participants, 13 students (7.5%) rated the statement as 1, 12 students (6.8%) rated the statement as 2, 36 students (20.7%) rated the statement as 3, 40 students (23%) rated the statement as 4, and 73 students (42%) rated the statement as 5 (Figure 5).

4. What are the opinions of the students about 'the contribution of histology courses integrated with technological tools to this branch of science'?

Within the scope of the study, the students were asked about their opinions on using innovative approaches such as technology-adapted auxiliary course tools (education sets, mobile applications, and smart and digital devices) in histology education. Among the participants, 141 students (81%) answered this question positively, while 13 students (7.5%) answered negatively. Twenty students (11.5%) reported that they were undecided (Figure 6).

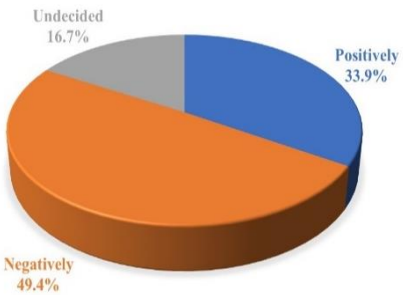
Figure 6: The contribution of technology-adapted auxiliary course tools to histology education



5. What are the opinions of the students about 'the histology course delivered by distance education'?

Within the scope of the study, the students were asked about their opinions on delivering the histology course using 'distance education.' Among the participants, 59 students (33.9%) answered this question positively, while 86 students (49.4%) answered negatively. Twenty-nine students (16.7%) reported that they were undecided (Figure 7).

Figure 7: Delivering the histology course by the distance education method



Discussion

The purpose of histology education is to train individuals who know the vital processes taking place from the molecular and cellular level to the whole organism and can use this health information effectively. Today, advances in technology have enabled this branch of science to gain momentum in its development [12]. In this study, the opinions of the students about histology education and their ideas about the necessity of integrating clinical sciences with technological tools were discussed. In this regard, the feedback received from the students contained information about the current education system and provided guidance on how this education should be implemented in the future.

Histology education, which is an important part of health and medical education, is considered by students to be a difficult course to learn. The reason for the difficulty of this course is that theoretical and practical courses are not delivered with a holistic approach [10]. In this study, half of the students (37.4% difficult; 12.6% very difficult) thought that the histology course was difficult, which is consistent with the literature. Additionally, more than half of the students stated that the length of the theoretical course was sufficient (57.5%) and that practical courses should be supported by various technology applications (73%). Therefore, delivering theoretical and practical courses in an integrated manner may help students overcome the difficulties experienced in histology education. In another study, it was found that the success rate was higher in a group in which theoretical knowledge and practical applications were delivered in an integrated manner in the respective histology course [15], which supports this idea.

In terms of clinical applications in professional life, it is crucial to have good basic medical science education [16]. In studies conducted on anatomy education, which is one of the important components of basic medical sciences, it was reported that this course would contribute to professional life, and that it was necessary for clinical applications [14]. Similarly, most of the students in this study (63.8%) described the histology course to be important for their profession. Additionally, the students also stated that it would be more efficient (42%, 5 points; 23%, 4 points) to facilitate the course by integrating it with clinical sciences. Today, educational systems that integrate basic and clinical sciences are becoming increasingly widespread [17]. For example, in a study in which histology was integrated with clinical applications, students stated that the histology course contributed to their learning, their approach to patients, and their general medical practices [18]. Therefore, given the students' approaches to these issues, it can be concluded that it would be better to integrate basic medical sciences courses such as anatomy, histology, and embryology with clinical sciences, and that it should not be limited to the first years of education.

It has become inevitable for the technological developments of our century to be implemented in education. These developments facilitate new applications in the field of education. The use of technology also increases the delivery of basic medical sciences courses such as histology, physiology, and anatomy [19, 20]. Studies show that applications integrating technology increase academic achievement, histological identification skills, interactive learning, and student satisfaction in histology education [21, 22]. In this study, most of the students (81%) stated that the histology course integrated with technological tools would positively affect the quality of education they received. Innovative technological educational models may provide more alternatives in histology education. Thus, technological educational materials supported by traditional methods can enable students to learn more quickly and efficiently.

Today, there are innovative educational materials that are believed to have potential benefits for histology education. The use of virtual microscopic images created by transferring real microscopic images to digital media is one of the most common examples of this in histology education [23, 24]. Such methods enable students to continue their education in a comprehensive and effective way outside the laboratory, while also being an effective solution to today's problems [24, 25]. Additionally, the fact that digital content can also be shared with other anatomical, radiological, and clinical sciences courses allows for multidisciplinary learning in medicine and health education [26]. Similarly, models that deliver histology education through gamification have also been introduced recently [27], and it is believed that such methods will provide benefits in the preparation of a holistic curriculum between health disciplines [28].

An example of innovative products that can be used in histology education is technology such as 'Digital microscope slide to improve microscope usage skills.' This device is related to a digital slide system, which eliminates the need for the physical use of a slide and the need for sample tissue to be examined together with the slide to provide convenience for those who need to receive microscope usage training. Thanks to the device being integrated into the table part of the microscope, students will be able to examine the images of these preparations, which have been digitized and uploaded to the device, under the microscope, instead of the classical preparations. Thus, there will be no need to prepare thousands of ready-made preparations and use them in laboratories. Only the presence of this device with a microscope will suffice [8].

The 'Educational set comprising smart preparations,' in which the QR code technology is integrated into conventional preparations that have been examined in detail in this study, can also be useful in histology education. This education set basically consists of 'preparations with QR codes' and a 'display device' that allows for scanning the QR code on a preparation. When the QR code on the preparation is scanned, all written and audiovisual information about the stained tissue on the preparation (e.g., the lung, heart, or kidney) can be accessed easily via the display device. Thus, students can learn independently without any instructor or histology atlas book [9].

Health and medical education is one of the fields still affected by the ongoing Covid-19 pandemic [29], which necessitates the need for alternative methods of education rather than face-to-face and practical applications for this education [30, 31]. The most significant alternative methods of education include 'distance or online education' practices [31]. In this study, students were asked about their opinions on the delivery of the histology course using distance education, and 49.4% of the students answered this question negatively. This finding is

important in that it shows that distance or online education methods alone are not considered sufficient in health and medical education. As a matter of fact, in a study in which a neuroanatomy course was given by distance education method, it was stated that students had difficulty understanding the course with this method, and preferred face-to-face education [32]. Additionally, the fact that approximately one-third of the students had a positive opinion of such methods suggests that these methods can be used to support face-to-face education and practical applications. In a study conducted with pharmacy students, the histology course that was delivered remotely was found to be successful despite the lack of face-to-face interaction with instructors, which supports this idea [33].

Technology-supported materials have significant contributions to the implementation of distance or online education [34]. It is stated that technology-enhanced learning is more widely used than other methods in distance education practices in health and medical education, especially today, when the Covid-19 pandemic continues to impact our lives. These new technologies used in education have advantages such as being more cost-effective, more easily accessible, and more flexible compared to traditional methods [31]. In another study, an 'Education set consisting of smart interactive models and a software program' was applied to measure the success of the histology and anatomy course, which was found to be more successful than the traditional method. In this education set, each tissue or organ is designed as a separate smart model. Additionally, in this education set, students had the opportunity to observe histological and anatomical formations on the same model. The touch and voice keys on the formations allowed the students to learn the formations independently. Moreover, when students scanned the QR code on the smart model using their smart devices, they were able to access all the information about the relevant tissue or organ online. The accessed information enabled the students to receive distance education outside of the laboratory [10]. In light of this information, it is believed that remote and technology-enhanced methods will provide more effective results when integrated with traditional methods.

Limitations

The study was conducted with students of the Faculty of Health Sciences, Siirt University. The study can be expanded by adding students from different universities and faculties (such as medical and veterinary) to the research. Additionally, the survey questions prepared for the students can be further diversified to analyze the expectations of the students more comprehensively. Moreover, by conducting prototype studies for innovative technological tools mentioned in the study, their direct impact on student success can be revealed.

Conclusion

It is believed that it would be more effective to combine theoretical knowledge and practical applications in histology education, which is considered by students to be a difficult course. Additionally, it can be concluded that these courses are important for the future profession of these students, and it would be more beneficial to deliver them integrated with clinical sciences. Today, as technology is developing in all areas of life, there is a need for educational materials integrated with technological tools. Therefore, it is believed that adequately designed technological educational materials can change students' perspectives on this subject in histology education in cases where distance education alone is not considered sufficient.

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Evaluation of clinical features and risk factors related to late recurrence (>5 years) in patients with breast cancer

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Ethics Committee Approval
The study was carried out with the approval of the Ethics Committee of Istanbul University Istanbul Faculty of Medicine (File no: 2021/2173) on 24.12.2021.
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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

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Abstract

Background/Aim: Over the years, disease-free survival (DFS) has been considerably prolonged with effective treatments in resectable breast cancer patients. However, a limited number of studies evaluating the predictive and prognostic factors of the disease in breast cancer patients who develop late recurrence are available. In this respect, we investigated clinicopathological features and risk factors affecting the survival of patients who developed breast cancer recurrence (BCR) after 60 months (late BCR).

Methods: In this retrospective cohort study, clinicopathological features and survival outcomes of 45 late BCR patients were evaluated. The demographic and medical data of the patients were obtained from the retrospective registry system of our center. Statistical analyses were performed to determine the risk factors affecting DFS.

Results: The median age of the cohort was 49 (24–78) years. Twenty-three postmenopausal patients were included in the study, and the mean age of menopause was 50 (43–55) years. Fourteen (31.1%) patients were stage 3 at diagnosis. In the adjuvant period, 80% of the patients underwent radiotherapy, and 79.5% underwent chemotherapy. The mean duration of adjuvant hormone therapy was 64 (69–129) months. Adjuvant ovarian suppression therapy was applied to 14 patients. The three most common sites of recurrence were bones (57.8%), locoregional (26.7%), and distant lymph nodes (26.7%). The median DFS of the cohort was 116.9 (3.7) months (109.6–124.1 months). Disease-related deaths occurred in only five patients, and the median overall survival (OS) could not be achieved. Based on a log-rank analysis, the median DFS was longer in patients whose adjuvant hormone therapy duration was 5–10 years and in those with bone or lymph node recurrence (P = 0.025 and P = 0.001, respectively). DFS was significantly shorter in patients with liver metastases (P = 0.005). Based on a chi-squared analysis, bone and lymph node metastases were higher in luminal A-like group (P = 0.030), and liver metastasis was lower (P = 0.039). Luminal biology did not affect late BCR (P = 0.075).

Conclusions: Prolonged adjuvant hormone therapy (5–10 years) delays breast cancer recurrence. However, luminal features are insufficient to predict recurrence as the recurrence period increases. In addition, different sites of metastases are associated with long-term survival and luminal subgroups.

Keywords: Breast cancer, Recurrence, Survival, Hormonotherapy

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Introduction

Breast cancer is the most common cancer type worldwide with approximately one million cases annually. The 2018 data of the United States (US) reflect similar statistics, and it is the second most common cause of cancer-related death among women [1]. Although it is the leading cancer for both developed and developing countries, the 5-year survival rate in high-income countries, such as the US and Japan, is around 80%, it is 60% in middle-income countries, and 40% in low-income countries [2]. The sociocultural level, a significant risk factor, may result from country-associated differences, such as patient awareness of breast cancer, access to health services, screening programs, and estrogen exposure. Risk factors for the development of breast cancer can be listed as estrogen-related factors, such as early menarche, late menopause, low parity, having children at a late age, advanced age, excessive alcohol consumption, family history, exogenous and endogenous estrogen exposure, and BReast CAncer (BRCA) 1/2 mutation [3–6]. The average onset for breast cancer is 62 years [7]. Breast cancer-related mortality is higher under the age of 40 and again at over 80 years [8]. The majority of breast cancers originate from ductal or lobular epithelial components. The most common subtype is infiltrative ductal carcinoma (76%) followed by invasive lobular carcinoma (8%) and other rarer types [9, 10]. Lobular carcinoma tends to be multicentric, bilateral, and often hormone receptor-positive when compared with ductal carcinoma and is more common in older women as metastases are more likely to occur later in life and in atypical sites [11, 12].

The primary goal for operable tumors without distant metastases is to delay recurrence. In addition to pathological features, patient-related factors, such as age, menopausal status, hormone receptor positivity, human epidermal growth receptor factor 2 (Her2) expression status, and tumor genomic features are traditional indicators for prognostic and predictive evaluation. In the post-operative setting, the duration of disease-free survival (DFS) was significantly prolonged after undergoing chemotherapy, radiotherapy, and hormonal therapies. In hormone receptor positive patients, results of phase 3 randomized studies demonstrated that prolonging tamoxifen to 10 years in the adjuvant period significantly delays recurrence [13–15]. In addition, several clinical trials have demonstrated the efficacy of extended aromatase inhibitor therapy (>5 years) in post-menopausal women [16, 17]. Nonetheless, recurrence can be seen in patients even in the third decade after diagnosis [18]. In subsequent years, the rate of patients with late relapse will increase thanks to effective treatment methods. Therefore, we found it appropriate to contribute to the literature by identifying clinicopathological features and risk factors affecting survival in this patient group.

Materials and methods

Patients' selection criteria

Medical information from patients whose ICD 10 diagnoses between C50.0 and C50.9 at the Medical Oncology Department at Istanbul University Institute of Oncology was retrospectively scanned from the medical registry system between June 2005 and June 2016. Male patients, patients <18 years, patients who received chemotherapy before primary tumor surgery, de novo metastatic disease, bilateral breast cancer, recurrence <60 months after primary surgery, and unconfirmed pathological diagnosis were not excluded from the study. The demographic and clinical data of the remaining 45 patients with a histologically confirmed diagnosis of breast cancer based on surgical specimens were determined.

Demographic and histological variables

Demographic information of the patients, including age and menopause information, was recorded. Patients who did not have a menstrual cycle for at least one year before adjuvant therapy or who had bilateral oophorectomy were considered postmenopausal.

T and N stages were determined based on the American Joint Committee on Cancer (AJCC) tumor/node/metastasis (TNM) 8 in surgical specimens. World Health Organization 2012 pathological calcification was used for histopathological types [19]. Primary tumor grade, estrogen receptor (ER), progesterone receptor [PR], and human epidermal growth factor receptor 2 (cERB2) status were recorded according to the modified Scarff–Bloom–Richardson system [20]. The tumors with <1% of hormone receptor (ER or PR) expression were considered as hormone receptor negative [21]. Immunohistochemically, 3+ staining of the cerbB2 receptor or Her2/centromeric region of chromosome 17 (CEP 17) Ratio ≥2.0 with *in situ* hybridization (in case of 2+ positive staining) were accepted as cerbB2 receptor positivity. The surrogate definition of intrinsic subtypes was based on Sn Gallen 2015 consensus [22].

Treatment Features and Survival

Patients were scheduled to receive adjuvant treatment 1–4 weeks after primary tumor surgery. Radiotherapy was administered to all patients with BCS and mastectomized patients with a high risk of local recurrence. Patients with positive hormone receptors were treated with tamoxifen, anastrozole, or letrozole for at least five years after radiotherapy. Ovarian suppression with luteinizing hormone–releasing hormone (LHRH) agonist was continued for 3 to 5 years in premenopausal patients who presented a high risk of relapse.

The patients with high risk for recurrence had received adjuvant chemotherapy protocol:

i. FAC/FEC: 600 mg/m^2 fluorouracil on day 1, 60 mg/m^2 doxorubicin (epirubicin 90 mg/m^2) on day 1, 600 mg/m^2 cyclophosphamide on day 1, every 21 days for six cycles.
ii. 4AC + 4T: 60 mg/m^2 doxorubicin and 600 mg/m^2 cyclophosphamide on day 1, respectively, every 21 days for four cycles, followed by 100 mg/m^2 docetaxel on day 1, every 21 days for 4 cycles.
iii. 4AC + 12P: 60 mg/m^2 doxorubicin and 600 mg/m^2 cyclophosphamide on day 1, respectively, every 21 days for four cycles, followed by 80 mg/m^2 paclitaxel on day 1, every 7 days for 12 cycles.
iv. 4TC: 600 mg/m^2 cyclophosphamide on day and 100 mg/m^2 docetaxel on day 1, every 21 days for four cycles.
v. Trastuzumab: after an 8 mg/kg loading dose, 6 mg/kg trastuzumab were given on day 1, every 21 days for one year.

The sites of metastasis at recurrence of all patients were recorded. OS and DFS were the primary targets in the survival analysis. Time-to-event endpoint for OS was considered disease-related death or the last follow-up date. Since all patients relapsed, DFS was calculated based on disease relapse date.

Statistical analysis

IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp. was used for statistical analysis. For

descriptive statistics, numbers and percentages are given for categorical variables. Proportions in pathological response groups were analyzed using the chi-squared or Fisher's exact test. Survival rates were calculated using the Kaplan–Meier analysis. Risk factors were analyzed using univariate and multivariate Cox regression analysis. The statistical significance level was accepted as $P < 0.05$

Results

Clinicopathologic and treatment features

Detailed demographic and clinical findings at diagnosis are summarized in Table 1. The median age at diagnosis was 49 (24–78) years, and most of the patients were never smokers (77.8%). Fourteen of the patients reported a history of cancer in their first-degree relatives. The mean age of menarche was 14 (11–22) years. The mean of the first delivery was 22 (17–32), and the mean parity was 2 (1–6). Twenty-three postmenopausal patients were included in the study, and the mean age of menopause was 50 (43–55). Fifteen patients were stage I, 16 were stage II, and 14 were stage III at the time of diagnosis. The most common histological type was invasive ductal carcinoma (IDC; 91.1%). When luminal biology was evaluated, the most common subtype was luminal A-like (73.2%), which was followed by luminal B-like Her2 negative (12.2%), triple negative (9.8%), luminal B-like Her2 positive (2.4%) and Her2 positive only (2.4%), respectively. Grades 2 and 3 histology were 84.4% and 15.6%, respectively. Lymphovascular invasion was observed in 42.9% of the tumors. The incidence of necrosis was 19%.

Table 1: General demographic characteristics and disease findings at diagnosis

Baseline characteristics	n	%
Age at diagnosis*	49 (10)	24–78
Age at onset of menarche*	14 (2)	11–22
Age at onset of menopause*	50 (2)	43–55
Age of first pregnancy*	22 (3)	17–32
Number of parity*	2 (1)	1–6
Smoking status		
Active-smoker	3	6.7%
Ex-smoker	7	15.6%
Never-smoker	35	77.8%
Comorbidity	18	40.0%
Family history for cancer	14	31.1%
Menopause		
Premenopausal	22	48.9%
Postmenopausal	23	51.1%
Stage at diagnosis		
1	15	33.3%
2	16	35.6%
3	14	31.1%
Histology		
Invasive ductal carcinoma	41	91.1%
Invasive lobular carcinoma	3	6.7%
Other subtypes	1	2.2%
Luminal biology		
Luminal A like	33	73.3%
Luminal B like Her2 negative	6	13.4%
Luminal B like Her2 positive	1	2.2%
Her2 positive only	1	2.2%
Triple negative	4	8.9%
Histological grade		
1	0	0.0%
2	38	84.4%
3	7	15.6%
Nuclear grade†		
1	1	2.3%
2	37	84.1%
3	6	13.6%
Lymphovascular invasion	18	42.9%
Necrosis	8	19.0%

* "median and standard deviation" is used instead of "N", and "minimum–maximum" is used instead of "%".
†The tumor grade is missing in one patient.

All patients underwent surgery before systemic treatments. The mean pathological tumor diameter was 2.7 cm (0.9–3.5 cm), and the mean number of metastatic positive lymph nodes was 3 (0–20). In the adjuvant period, 80% of the patients underwent radiotherapy, and 79.5% underwent chemotherapy. When we examine the hormonotherapy profile, tamoxifen was the most common treatment (57.7%) followed by anastrozole (28.9%) and letrozole (2.2%). The mean duration of adjuvant hormone therapy was 64 (69–129) months. Adjuvant ovarian suppression therapy was administered to 14 patients. The three most common sites of recurrence were bone (57.8%), locoregional (26.7%), and distant lymph nodes (26.7%). Treatment features and metastasis sites at first relapse of patients are summarized in Table 2.

Table 2: Treatment features and metastasis sites at first relapse

Detailed clinical features	n	%
Primary tumor diameter	2,7 (1.8)	0.9–5.5
Number of dissected lymph nodes*	11 (8)	1–30
Number of metastatic lymph nodes*	3 (5)	0–20
Adjuvant radiotherapy	36	80.0%
Adjuvant chemotherapy	35	79.5%
Adjuvant trastuzumab therapy	2	4.5%
Adjuvant hormonotherapy		
None	5	11.2%
Tamoxifen	26	57.7%
Anastrozole	13	28.9%
Letrozole	1	2.2%
Adjuvant hormonotherapy duration	64 (27)	69–129
Recurrence under hormonotherapy treatment	10	22.2%
Adjuvant ovarian suppression	14	31.1%
Recurrence sites		
Bone	26	57.8%
Locoregional	12	26.7%
Lymph nodes	12	26.7%
Liver	7	15.6%
Lung	5	11.1%
Ovary	2	4.4%
Brain	1	2.2%
Peritoneum	1	2.2%

* "median and standard deviation" is used instead of "N", and "minimum–maximum" is used instead of "%".

Survival and risk factors

The median DFS of the cohort was 116.9 (3.7) months (109.6–124.1 months). Disease-related death occurred in only eight patients, and the median OS could not be reached. Fifteen and twenty-year survival rates were 81.7% and 72.6%, respectively (Figure 1).

In the log-rank analysis, risk factors affecting DFS were duration of adjuvant hormone therapy (<5 versus 5–10 years; $P = 0.025$), liver metastasis ($P = 0.005$), and presence of bone or lymph node metastasis ($P = 0.001$) as shown in Figure 2. The median DFS was significantly shorter in patients with liver metastases than without (87.2 versus 121.3 months). Bone or lymph node metastases were associated with a longer DFS than visceral metastases (132.8 versus 91.8 months). Since only two patients with luminal B like-Her2 positive and Her2 positive only were included in the study, these subgroups were excluded from the log-rank analysis. The median DFS was 130.5 (9.3) months (112.2–148.8 months) in those with luminal A-like, 89.1 (12.5) months (64.4–113.7 months) in those with luminal B-like Her2 negative, and 69.8 (28.6) months (13.7–126.0 months) in those with triple negative ($P = 0.075$). Those who received adjuvant hormone therapy <5 years had a shorter median DFS than those who received 5–10 years (104.6 versus 130.8 months). Comorbidity, menopausal status, disease stage, histological grade, nuclear grade, lymphovascular invasion, necrosis, ducal *in situ* carcinoma (DCIS) component, type of surgery, adjuvant radiotherapy, and adjuvant chemotherapy had no statistically significant effect on DFS ($P > 0.05$). The multivariate Cox regression analysis found no significant prognostic risk factor on DFS (Table 3).

Figure 1: Kaplan-Meier survival curves in patients with breast cancer relapse

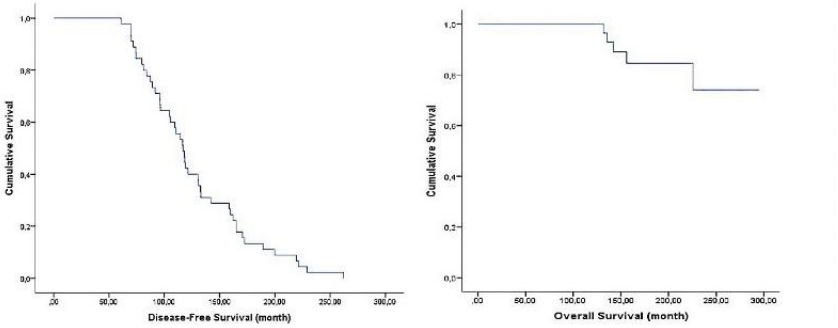

Figure 2: Survival analysis of risk groups affecting on disease-free survival

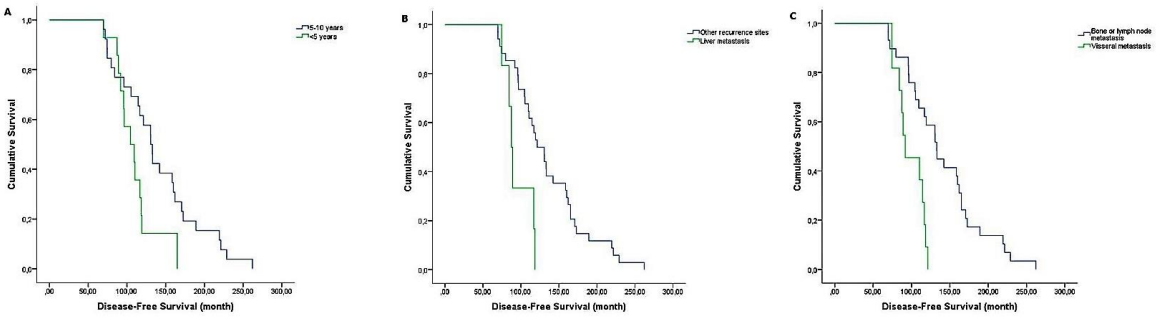

Table 3: Multivariate analysis for disease-related death

Risk Factors	n (%)	*P*-value	HR	95% CI
Adjuvant hormone therapy duration <5 years	28 (62.2)		1	
Adjuvant hormone therapy duration 5–10 years	17 (37.8)	0.115	0.54	0.25–1.16
Luminal A like	33 (73.4)		1	
Luminal B like Her2 negative	6 (13.5)	0.858	1.17	0.33–1.75
Triple negative	4 (9.1)	0.745	1.477	0.14–15.5
Other metastasis	38 (84.4)		1	
Liver metastasis	7 (15.6)	0.761	.812	0.21–3.11
Other metastasis	16 (35.6)		1	
Bone or lymph node metastasis	29 (64.4)	0.79	0.33	0.09–1.13

CI: Confidence interval; ECOG–PS: Eastern Cooperative Oncology Group–Performance status; HR: Hazard ratio

Discussion

In this study, we aimed to determine the clinical characteristics and risk factors of patients with late BCR five years after primary treatment. The mean age of our study patients was 45 years, and the rate of stage III disease was 31.1%. In a Danish study evaluating breast cancer patients with recurrence after ten years, the mean age at diagnosis was 55, and stage III breast cancer was 8.8% [23]. Likewise, in randomized prospective TEAM and IDEAL studies evaluating extended hormone therapies, the mean age was 55–63 years, and the rate of stage 3 patients was approximately 15% [17, 24]. It is noteworthy that our cohort was younger and had more stage 3 diseases compared to other population-based studies.

Pedersen et al. [23] found that tumor size larger than 20 mm, lymph node-positive disease, and ER positivity was associated with an increased risk of recurrence in 36,924 breast cancer patients who developed recurrence between 10 and 32 years. In a study evaluating late BCR in patients with ER positive breast cancer, nodal involvement and tumor diameter were found to be two crucial prognostic risk factors. In this study, none of the immuno-histochemical (IHC) markers (ER, PgR, cerbB2, and Ki67 score) were significant for late BCR [25]. In our study, no relationship was observed between the initial stage, which includes T and N, and late BCR. This difference may be related to the limited number of our cohorts compared to other studies. In our study, DFS was longest in patients with luminal A-like followed by luminal B-like Her2-negative and triple-negative patients, but statistical significance was not reached. Those who received prolonged hormone therapy (5–10 years) had a significantly longer DFS than those who did not. The essential factors in the emergence of late BCR seem to be the initial T and N stages. It has also been demonstrated in our study in line with phase 3 randomized studies, that hormone therapy is protective for the first ten years in hormone receptor positive tumors. Conflicting results about whether ER positivity is protective in late BCR have been published. In our cohort, liver metastases predicted an earlier recurrence, whereas lymph or bone metastasis was associated with longer DFS. In a study by Abha et al. [26] that investigated the relationship between luminal biology and distant metastasis in 531 breast cancer patients, they revealed that luminal tumors were present with bone metastasis. In another retrospective study evaluating site-specific metastasis in breast cancer, it was shown that liver, brain, and lung metastases were higher in non-luminal subtypes [27]. In addition, many studies have revealed that liver metastasis is associated with poor survival [28, 29]. In a chi-squared analysis, bone and lymph node metastases were higher in luminal A-like group, whereas liver metastasis was found to be lower. The results of our study are compatible with studies in the literature.

Limitations

Our major study limitations are the low number of patients and the single-center design of the study. In addition, we could not compare our findings with an earlier relapsed control arm. Therefore, our statistical results may have revealed different results compared to other clinical studies. Multicenter studies with larger samples are needed to confirm this finding.

Conclusions

Late BCR has unique clinical features compared to early BCR. Although prolonged hormone therapy appears effective, risk assessments based on luminal biology appear insufficient in late BCR. In addition, our results show that different sites of metastasis are associated with survival and luminal subgroups.

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The relationship between initial lactate levels and outcomes in patients diagnosed with diabetic ketoacidosis in the emergency department

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Ethics Committee Approval
Ethical approval was obtained from Şişli Hamidiye Etfal local ethics committee (ethics committee ruling number: 2193, date: 29.11.2022).
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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

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Abstract

Background/Aim: Diabetic ketoacidosis (DKA) is an endocrinological emergency frequently seen in emergency departments (ED). It can result in mortality if not treated appropriately. The aim of this study was to examine the relationship between baseline lactate levels and patient outcomes in DKA patients admitted to the emergency department (ED).

Methods: This retrospective cohort study was carried out in the ED of a tertiary hospital between May 2022 and November 2022 using the data of patients diagnosed with DKA. Patients with missing data, patients transferred from another hospital, patients with a diagnosis other than DKA, and patients who could not be followed up were excluded from the study. The primary outcome of the study was admission to the intensive care unit (ICU) and/or in-hospital mortality. The area under the curve (AUC) was calculated by receiver operating characteristic (ROC) regression analyses to predict critically ill patients with lactate levels.

Results: The study was completed with 95 patients. The mean age of the patients was 53.1 years and n = 46 were female. Twenty (21.1%) of these patients were admitted to the intensive care unit and 22 (23.2%) died. The statistical analysis showed that lactate levels were statistically significant in predicting critically ill patients ($P < 0.001$). ROC analysis showed that a lactate level of 2.6 mmol/dL could predict critically ill patients. The area under the curve was 0.823 (95% confidence interval: 0.731-0.894, sensitivity: 71.4, Specificity: 69.8), the Youden index was 0.476, and the *P*-value was 0.001.

Conclusion: According to the results of this study, there is a significant relationship between the initial lactate levels in DKA patients and patients who will require critical care. Therefore, lactate can be used as an appropriate follow-up tool in the management of DKA patients.

Keywords: Diabetic ketoacidosis, Lactate, Mortality

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Introduction

Diabetic ketoacidosis (DKA) is one of the acute complications of diabetes mellitus (DM) and can even result in death if not treated appropriately [1, 2]. The incidence of DKA has been shown to be 4.6-8 episodes per 1000 diabetic patients per year. It is thought to be responsible for 500.000 hospitalizations per year in the United States (USA) with a total cost of approximately $2.4 billion [3].

The incidence of DKA among diabetic patients is reported to be 0.4-0.8%. In 2009, 140,000 DKA patients were hospitalized in the United States of America (USA), and one-year hospital expenses were reported to be approximately 1 billion dollars [4]. DKA is more common in younger children and those with lower socioeconomic status. Low socioeconomic status and low educational levels reduce the compliance of the patients with the treatment and can cause DKA to recur more frequently. Mortality rates of DKA are found to be 1-5% [5].

Lactate is produced from most tissues in the body (kidney, erythrocyte, skeletal muscle, brain). Most lactate is metabolized by the liver followed by kidney and skeletal muscle [6]. The prognosis of patients can be evaluated with lactate follow-up via blood gases [7]. The increased blood level of lactate may be due to overproduction or lack of use. Both of these situations indicate a malfunction in the systems. Common causes of increased lactate include hypotension, hypoperfusion, infections, lack of oxygenation, circulation, pulmonary and hemoglobin transfer problems, malignancy, kidney failures, liver failures, diabetes, malnutrition, hypothermia, hyperthermia, dehydration, epilepsy, toxins, various operations, alcohol, drugs, sepsis, and excessive exercise [8]. The aim of this study was to examine the relationship between initial lactate levels and patient outcomes in DKA patients admitted to the emergency department (ED).

Materials and methods

This retrospective, observational study was conducted in the ED of the Şişli Hamidiye Etfal Training and Research Hospital in Istanbul, Turkey. The Şişli Hamidiye Etfal Training and Research Hospital Clinical Research Ethics committee approved the analysis and issued a waiver of consent (ethics committee ruling number: 2193; date: 29.11.2022). All patients over the age of 18 and diagnosed with DKA who applied to the ED of a tertiary hospital between May 2022 and November 2022 were included. Patients with missing data, patients transferred from another hospital, patients with a diagnosis other than DKA, and patients who could not be followed up were excluded. The diagnosis of DKA was made via blood glucose at presentation >250 mg/dL with ketonemia or blood acidemia (pH <7.3 or serum bicarbonate concentration <15 mEq/L) [5]. After a study form was created, the patients' age, gender, laboratory data at the time of application, and patient outcomes were recorded. Blood gas lactate levels were measured using a Hitachi 917 automated analyzer (Roche Diagnostics, Mannheim, Germany).

The primary outcome was admission to the intensive care unit (ICU) and/or in-hospital mortality. The sample size was based on the number of patients in studies with a 95% confidence interval [9]. At least 74 patients were needed considering a 0.5% margin of error.

Statistical analysis

Statistical analysis used SPSS v. 25.0 software package (SPSS Inc., Chicago, IL, USA) and MedCalc ver. 12.5 (MedCalc Software Ltd, Ostend, Belgium). Descriptive criteria included mean and standard deviation as a percentage distribution. The conformity of the data to the normal distribution was checked with the Kolmogorov-Smirnov test. The area under the curve (AUC) was calculated by receiver operating characteristic (ROC) regression analyses. We calculated the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of lactate levels to assess performance; *P*-values below 0.05 were considered statistically significant.

Results

The study contained 95 patients after excluding three patients transferred from another hospital, 11 patients diagnosed other than DKA, and eight patients who could not be followed up during the period. The mean age of the patients was 53.1 years, and 46 were female. Of these patients, 15 (15.7%) were discharged, 38 (40%) were hospitalized, 20 (21.1%) were hospitalized in the intensive care unit, and 22 (23.2%) died. The patients included in the study were divided into two groups as critical (ICU admission and/or deceased) and non-critical (outpatient and/or inpatient unit). Some of their features were then compared. The blood gas data (HCO_3 and pH) of the patients in the critically ill group were lower than those in the non-critical group, but the lactate levels were higher ($P = 0.003$, $P = 0.001$ and $P = 0.001$, respectively) (Table 1).

Table 1: Comparison of various characteristics of patients with critical and non-critical diabetic ketoacidosis.

	Outpatient and/or Inpatient unit n = 53		ICU admission and/or Deceased n = 42		*P*-value
	Mean n	SD %	Mean N	SD %	
Age	49.2	18.8	57.1	21.3	0.58
Gender					0.019
Woman	20	37.7	26	61.9	
Man	33	62.3	16	38.1	
Systolic blood pressure (mmHg)	123.2	18.6	119.1	31.8	0.448
Diastolic blood pressure (mmHg)	74.9	11.9	71.1	20.9	0.289
Pulse rate (bpm)	93.4	15.7	107.1	21.9	0.001
Respiratory rate	17.2	4.9	25.9	8.9	0.001
SPO_2 (%)	97.0	2.7	93.8	8.4	0.010
Glucose (mg/dL)	553.9	193.1	634.9	210.3	0.054
Creatinine (mg/dL)	1.38	0.87	1.79	1.17	0.051
Blood urea nitrogen (mg/dL)	30.1	24.8	41.5	28.6	0.043
AST (IU/L)	23.0	20.9	54.8	123.4	0.068
ALT (IU/L)	21.2	18.6	36.7	75.8	0.153
Albumin (g/L)	35.3	16.2	29.9	15.4	0.117
Sodium (mEq/dL)	129.8	5.6	131.3	9.3	0.334
Potassium(mEq/dL)	4.8	0.8	5.0	0.9	0.304
Chloride (mg/dL)	91.9	7.0	92.9	10.1	0.560
pH	7.22	0.10	7.14	0.14	0.001*
PCO_2 (mmHg)	33.9	10.3	30.6	10.3	0.126
HCO_3 (mEq/dL)	14.7	4.4	11.8	4.9	0.003*
Lactate (mmol/dL)	2.26	0.74	3.91	1.68	0.001*

* Student's t-test

The statistical analysis showed that lactate levels were significantly higher in predicting critically ill patients ($P < 0.001$, Table 1). ROC analysis of lactate level could help predict critically ill patients: The area under the curve was 0.823 (95% CI 0.731-0.894), the Youden index was 0.476, and the *P*-value was 0.001. A lactate level cut-off of >2.6 mmol/L could identify critically ill patients with a sensitivity of 71.4% and a specificity of 69.8%. The positive predictive value was 65.2, and the negative predictive value was 75.5 (Figure 1, Table 2).

Figure 1: Receiver operating characteristic curve of lactate level predicting critically ill patients.

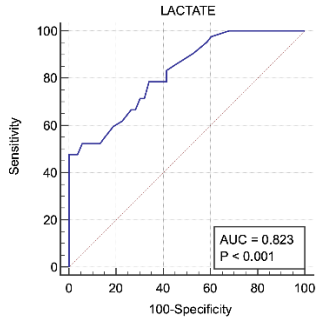

Table 2: Values of lactate level in predicting critical patients in diabetic ketoacidosis patients.

	AUC	Cut-Off	Sensitivity	Specificity	PPV	NPV	Youden Index
Lactate	0.823 (0.731-0.894)	>2.6	71.4	69.8	65.2	75.5	0.476

AUC: area under curve, PPV: positive predictive value, NPV: negative predictive value

Discussion

This study evaluated the relationship between initial lactate levels and outcomes in DKA patients admitted to the ED. We concluded that elevated lactate levels at the time of admission may predict in-hospital mortality and ICU admission.

Although DKA is mostly seen in type 1 DM, it can also occur in type 2 DM patients with precipitating conditions such as infection, trauma, or surgery that create a catabolic process [10]. The classic triad of DKA occurs as a result of hyperglycemia, ketonemia-ketonuria, metabolic acidosis, insufficient insulin activity, and the effect of contra-insulinary hormones. This hormonal disorder causes the substances transported from the tissues (muscle amino acids, lactate, and pyruvate as well as free fatty acids and glycerol from adipose tissue) to be actively converted into glucose and ketone bodies (beta hydroxy butyrate, acetoacetate, acetone). This in turn increases release into circulation. Osmotic diuresis develops, thus resulting in hyperglycemia (>250 mg/dl), ketoacidosis (pH <7.30), dehydration, and electrolyte loss [11].

DKA severity can be graded as mild, moderate, and severe depending on the level of metabolic acidosis and changes in mental status. As the disease worsens, mental status changes may progress from wakefulness to lethargy or even coma. Therefore, DKA is an important cause of mortality and morbidity in DM patients. Although overall mortality is less than 5% in experienced centers, it is the main cause of mortality in patients with type 1 DM and is responsible for 50% of deaths in patients with DM under 24 years of age [11].

Lactate is used as a prognostic tool in many diseases [12-14]. Normal blood lactate levels are 1 mEq/l. Even lactate levels >1.5 mEq/l have been shown to be associated with mortality [15]. It is thus desirable to keep lactate levels below 2 mEq/l in patient follow-ups. A lactate level of 4 mEq/l and above means a very high-risk mortality [16].

There are various studies in the literature examining lactate levels and outcomes of DKA patients. Taskin et al. [17] analyzed the data of 43 patients admitted to the ICU for DKA. They emphasized that low or high lactate levels at the time of admission would not make a difference in terms of mortality or ICU stay, but they emphasized that lactate kinetics should be used during patient follow-up. In another study conducted in Turkey, they concluded that lactate levels were significantly correlated with DKA severity (mild-moderate-severe) in 230 DKA patients presenting with ED [18]. Suwarto et al. [9] found that a lactate level >4 mmol/L could be used as an independent risk factor in estimating 5-day mortality from hospital admission. Our study was thus found to be compatible with the prior literature.

Limitations

This study does have some limitations. This single-center, retrospective design study was conducted in a relatively small population. The comorbidities of the patients were not examined and this may be an important factor in evaluating patient outcomes. Finally, only the lactate level at the time of application was examined—not dynamic changes in lactate levels.

Conclusion

DKA is a common endocrinological disease in EDs. It can result in mortality if not treated appropriately. Our results show that there is a significant relationship between the initial lactate levels in DKA patients and patients who will require critical care. Therefore, lactate can be used as an appropriate follow-up tool in the management of DKA patients.

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Association between diverticular disease and prevalence of colorectal adenomatous polyps or adenocarcinomas

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Ethics Committee Approval
This study was approved by the clinical research ethics committee of Erzurum Regional Training and Research Hospital (IRB approval number: 2022/11-131).
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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Abstract

Background/Aim: Although the link between diverticular disease (DD) of the colon and colon polyp is known, the relationship between colon adenocarcinoma is not clear. This study evaluated the association between DD and adenomatous polyp or colon adenocarcinoma.

Methods: Patients who underwent colonoscopy for the first time in 2020-2021 were evaluated and included in this retrospective cohort study. Patients with a previous history of cancer diagnosis, colon surgery, DD, and inflammatory bowel disease were excluded from the study. Age, gender, colonoscopy indications, colonoscopy diagnoses, presence of DD, characteristics of polyps (pathology, diameter, number, localization), and presence of adenocarcinoma were recorded. Obtained data were analyzed between DD and non-DD groups.

Results: A total of 2633 patients were included in the study. The prevalence of DD was 16.4%. Colon adenocarcinoma was detected in 4.7%. The adenomatous polyp rate was 14.1%. A significantly higher rate of adenomatous polyps was detected in the DD group compared to the non-DD group (19.7% vs. 12.9%; $P = 0.001$). Higher rates of high-grade dysplasia (3.0 vs. 1.1%; $P = 0.002$) and colon adenocarcinoma (7.2% vs. 4.2%; $P = 0.008$) were observed in the DD group also. In logistic regression analyses, it was observed that the presence of concomitant DD increases the risk of adenomatous polyps (OR: 1.469, 95% CI: 1.158–1.865), the risk of adenomatous polyps with positive villous component (OR: 2.378, 95% CI: 1.437–3.934), the risk of adenomatous polyps with high-grade dysplasia (OR: 2.822, 95% CI: 1.426–5.582), and the risk of colon adenocarcinoma (OR: 2.953, 95% CI: 1.445–6.533).

Conclusion: DD is associated with precancerous lesions of the colon (adenomatous polyp, villous adenoma, high-grade dysplasia) and colon adenocarcinoma. Further studies are needed to investigate its association with colon carcinogenesis and its role and value in cancer screening.

Keywords: Adenomatous polyp, Colon adenocarcinoma, Diverticular disease, High-grade dysplasia, Villous adenomatous polyp

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Introduction

Colon adenocarcinoma ranks third among all cancers in men and second in women. Its incidence among all cancers is 12%. Colon adenocarcinoma has significant mortality and is the third deadliest among all cancers [1, 2]. Adenomatous polyps are benign tumors of the colon with low-grade dysplasia. Sporadic colon adenocarcinoma progress from adenomatous polyps due to an increase in dysplasia with the effects of genetic and epigenetic changes [3]. Advanced age, increased number of polyps, larger polyp size (>10 mm), and high-grade dysplasia are risk factors for the development of malignancy from the polyp. In the presence of a diameter greater than 10 mm, the presence of a villous component, and severe dysplasia, conversion to adenocarcinoma is observed at an annual rate of 3%, 17%, and 37%, respectively [4, 5].

In the presence of typical symptoms and findings (including rectal bleeding, anemia, weight loss, defecation irregularity, and pain) for colon adenocarcinoma, further examination with colonoscopy is already required. The most important preventive method for colon adenocarcinoma is to include the cases in the colonoscopy screening program according to the risk factors. In cases with risk factors, such as a family history of polyps or colon adenocarcinoma, and the presence of ulcerative colitis, early screening can detect polyps before dysplasia progresses and adenocarcinoma transformation develops. Cure can be provided in cases with simple polypectomies. As a result of the increase in colonoscopies for screening purposes, the prevalence and incidence of colon adenocarcinoma have decreased in the last 20 years compared to previous years. [2, 6, 7].

Colonic diverticula are outpouchings of the intestinal wall that occur due to defects in the muscle layer of the colon wall. The prevalence of diverticular disease has increased in recent years. Advanced age, obesity, low fiber diet, and low exercise are risk factors for the development of colon adenocarcinoma as well as for the development of diverticular disease. Studies are showing an increased incidence of colon polyps in diverticular disease. However, when the literature is examined, the relationships between colon adenocarcinoma and diverticular disease are conflicted. Although some studies did not find a significant relationship between diverticular disease and colon adenocarcinoma, some studies reported an increased incidence of colon adenocarcinoma in diverticular disease [8–10]. In the present study, we evaluated the association between diverticular disease and adenomatous polyps or colon adenocarcinoma.

Materials and methods

This study was carried out after the approval of Erzurum Training and Research Hospital's Clinical and Research Ethics Committee with decision number 2022/11-131. Patients who underwent colonoscopy for the first time in the gastroenterology department of Erzurum Training and Research Hospital in 2020–2021 were evaluated retrospectively and included in the study.

Colonoscopy indications were determined as abdominal pain, constipation, chronic diarrhea, rectal bleeding, weight loss, family history of colon adenocarcinoma, family history of colorectal polyps, iron deficiency anemia, and occult blood positivity in stool. Patients with a previous history of cancer diagnosis, a history of colon surgery, and a history of inflammatory bowel disease were excluded from the study.

In all cases, intestinal preparation was provided with sennoside oral laxative and sodium dihydrogen phosphate + disodium hydrogen phosphate enema. Patients with adequate bowel preparation and colonic visibility were included in the study. The patients were sedated by the anesthesiologist with propofol, midazolam, and fentanyl throughout the procedure. In patients, full colonoscopy was accepted as ileum intubation and clear evaluation of all colon areas from the cecum backward. Patients who could not be evaluated clearly and whose all areas could not be evaluated due to early termination of the procedure for any reason were excluded from the study.

Age, gender, colonoscopy indications, and colonoscopy diagnoses were evaluated and recorded. The presence of diverticula in any location of the colon in the colonoscopy procedure was considered a diverticular disease. The diagnosis of polyp detected on the colonoscopy was confirmed by histopathological evaluation. All polyps were evaluated by a gastrointestinal pathologist. As a result of the histopathological evaluation, polyps were recorded as hyperplastic, serrated, and adenomatous. The degree of dysplasia (low-grade, high-grade) and pathological subtypes according to the villous component (tubular, tubulovillous, and villous) in adenomas were determined and recorded. Location, number, and size of polyps were recorded.

According to the presence of diverticula, the cases were divided into two groups: diverticular disease (DD) and non-diverticular disease (non-DD). Both groups were compared in terms of the variables mentioned above.

Statistical analysis

Parameters were analyzed by using the "SPSS 22 for Windows" statistics program. Categorical (nominal) values are expressed as a percentage (%) and compared with the chi-square test (2). Continuous numerical (quantitative) values were expressed as mean (SD). Quantitative variables were compared with the "Student t-test." Univariate analyses were conducted using Fisher's exact test to identify candidate risk factors for colon polyps and colon adenocarcinoma. All factors which were significant in univariate analyses were entered into multivariate logistic regression models. Logistic regression analysis was used to evaluate the association of DD with the prevalence of adenomatous polyps and colon adenocarcinoma. The results are expressed as odds ratios (OR) with 95% CI. $P < 0.05$ was determined as statistically significant.

Results

A total of 2,633 patients were included in the study. The mean age of the patients was 53.66 (16.33). 55.8% (n = 1468) of the patients were male. The prevalence of DD was 16.4% (n = 431). Colon adenocarcinoma was detected in 4.7% (n = 124). The prevalence of polyps was 20.8% (n = 547). Among all patients, the rate of hyperplastic polyps was 6.2% (n = 164), the serrated polyp rate was 0.5% (n = 12), and the adenomatous polyp rate was 14.1% (n = 371). 11.3% (n = 298) tubular

adenoma, 1.4% (n = 38) tubulovillous adenoma, and 1.4% (n = 36) villous adenoma were detected in all patients. The prevalence of adenomatous polyps with high-grade dysplasia was 1.4% (n = 37) of all patients. The rate of patients with more than one polyp was 11.7% (n = 64). The rate of patients diagnosed with polyps with a diameter ≥ 10 mm was 27.5% (n = 150). Polyps were located 36.1% (n = 197) in the rectum, 21.5% (n = 18) in the sigmoid colon, 16.5% (n = 91) in the descending colon, 11.4% (n = 62) in the transverse colon, 9.7% (n = 53) in the ascending colon, and 4.8% (n = 26) in the cecum.

Table 1 and Table 2 summarize the comparison of the variables between the DD and non-DD groups. The DD and non-DD groups were similar in age, gender, colonoscopy indications, and family history of polyps or colon adenocarcinoma. It was observed that polyps were detected at a significantly higher rate in the DD group than in the non-DD group (26.5% vs. 19.7%, [*P* = 0.002]). There was no difference between the DD group and non-DD groups in terms of serrated polyp and hyperplastic polyp rates. A significantly higher rate of adenomatous polyps (tubular adenoma, tubulovillous adenoma, villous adenoma) was detected in the DD group compared to the non-DD group (19.7% vs. 12.9%, [*P* = 0.001]). The high-grade dysplasia rate was found to be higher in the DD group than in the non-DD group (3.0% vs. 1.1%, [*P* = 0.002]). Colon adenocarcinoma was observed at a significantly higher rate in the DD group than in the non-DD group (7.2% vs. 4.2%, [*P* = 0.008]). There was no difference between DD and non-DD groups in terms of the distribution of polyps, polyp diameters, and multiple polyp ratio.

Table 1: Demographic characteristics of patients

	Non-DD	DD	*P*-value
Age	53.54 (16.35)	54.26 (16.25)	0.403
Gender (Male)	55.6%	56.4%	0.791
Colonoscopy indication			
Iron deficiency anemia	13.9%	14.8%	0.651
Occult blood positivity in stool	18.8%	19.3%	0.841
Chronic diarrhea	10.8%	10.7%	0.981
Constipation	16.9%	17.2%	0.871
Rectal bleeding	9.4%	9.0%	0.934
Abdominal pain	14.7%	14.6%	0.882
Weight loss	2.9%	2.8%	0.971
Polyp history in family	8.1%	7.7%	0.712
Colon cancer history in family	4.5%	3.9%	0.693

Table 2: Comparison of polyp and colon cancer rates between Non-DD and DD groups

	Non-DD	DD	*P*-value
Colon cancer	4.2%	7.2%	0.008
Polyp	19.7%	26.5%	0.002
Hyperplastic polyp	6.4%	6.0%	0.798
Serrated polyp	0.4%	0.7%	0.445
Adenoma	12.9%	19.7%	0.001
Tubular adenoma	10.5%	14.6%	0.018
Tubulovillous adenoma	1.2%	2.7%	0.011
Villous adenoma	1.2%	2.5%	0.021
Polyp with high-grade dysplasia	1.1%	3.0%	0.002
Number of polyps ≥ 1	11.4%	13.2%	0.625
Polyp diameter ≥ 10 mm	27.8%	26.3%	0.814

In logistic regression analyses, it was observed that the risk of adenomatous polyps increased with age (OR: 1010, 95% CI: 1.004–1.016), the presence of a family history of polyp (OR: 3.899, 95% CI: 2.653–5.730), the presence of a family history of colon adenocarcinoma (OR: 2.681, 95% CI: 1.993–3.606), and concomitant DD in the case (OR: 1.469, 95% CI: 1.158–1.865). Gender did not increase the risk of adenomatous polyp (Table 3).

In logistic regression analyses, it was observed that the risk of adenomatous polyps with a positive villous component increased with age (OR: 1.027, 95% CI: 1.011–1.042) and concomitant DD in the case (OR: 2.378, 95% CI: 1.437–3.934). Gender and a family history of polyps or colon adenocarcinoma did not increase the risk of adenomatous polyps with a positive villous component (Table 4).

Table 3: Risk factors for adenomatous polyp

	Adenomatous Polyp (+/-) 95% CI			
	OR	Lower	Upper	*P*-value
Age	1.010	1.004	1.016	0.002
Gender (Male)	0.808	0.669	0.975	0.058
Polyp history in family	3.899	2.653	5.730	0.001
Colon cancer history in family	2.681	1.993	3.606	0.001
Diverticular disease	1.469	1.158	1.865	0.001

CI: Confidence Interval, OR: Odds Ratio

Table 4: Risk factors for polyp with villous component (+)

	Villous component (+/-) 95% CI			
	OR	Lower	Upper	*P*-value
Age	1.027	1.011	1.042	0.001
Gender	0.667	0.419	1.060	0.096
Polyp history in family	1.583	0.626	4.002	0.380
Colon cancer history in family	1.833	0.927	3.625	0.082
Diverticular disease	2.378	1.437	3.934	0.001

In logistic regression analyses, it was observed that the risk of adenomatous polyps with high-grade dysplasia increased with age (OR: 1035, 95% CI: 1.013–1.058), a family history of colon adenocarcinoma (OR: 3.809, 95% CI: 1.783–8.183), and concomitant DD in the case (OR: 2.822, 95% CI: 1.426–5.582). Gender and a family history of polyps did not increase the risk of adenomatous polyps with high-grade dysplasia (Table 5).

Table 5: Risk factors for polyp with high-grade dysplasia

	High-Grade Dysplasia (+/-) 95% CI			
	OR	Lower	Upper	*P*-value
Age	1.035	1.013	1.058	0.002
Gender	0.836	0.437	1.599	0.620
Polyp history in family	1.921	0.581	6.348	0.225
Colon cancer history in family	3.809	1.773	8.183	0.002
Diverticular disease	2.822	1.426	5.588	0.005

In logistic regression analyses, it was observed that the risk of colon adenocarcinoma increased with age (OR: 1.017, 95% CI: 1.005–1.028), a family history of polyps (OR: 1.168, 95% CI: 1.083–1.259), a family history of colon adenocarcinoma (OR: 2.967, 95% CI: 1.553–5.597), and concomitant DD in the case (OR: 2.953, 95% CI: 1.445–6.533). Gender did not increase the risk of colon adenocarcinoma (Table 6).

Table 6: Risk factors for colon adenocarcinoma

	Colon Adenocarcinoma (+/-) 95% CI			
	OR	Lower	Upper	*P*-value
Age	1.017	1.005	1.028	0.004
Gender	1.367	0.730	2.560	0.329
Polyp history in family	1.168	1.083	1.259	0.001
Colon cancer history in family	2.967	1.537	5.597	0.001
Diverticular disease	2.953	1.445	6.033	0.004

Discussion

Colonic pre-neoplastic/neoplastic lesions and diverticular disease have similar risk factors, including age, obesity, low physical activity, low fiber diet, excess red meat, and carbohydrate consumption [11, 12]. It is thought that the frequency of co-occurrence will increase because they have similar risk factors. Many studies have investigated the association of DD with colonic adenomatous polyps or adenocarcinoma. In most of these studies, it has been reported that adenomatous colon polyps are clearly significantly increased in the presence of diverticular disease [8, 13–15]. There are still conflicting results regarding the relationship between DD and colon adenocarcinoma. A few studies have reported a significant relationship between DD and colon adenocarcinoma. These studies even suggest a vigilant follow-up procedure for preventing colon adenocarcinoma for patients with DD [9, 15–

18]. On the other side, some studies determined that there is no relationship between DD and colon adenocarcinoma. These mentioned studies indicate that there is a bias between DD and increased incidence or prevalence of colon adenocarcinoma. These studies stated that it is not a correct decision to include patients with DD in the surveillance program for polyps or colon adenocarcinoma according to these data [10, 19–23].

We aimed to investigate the relationship between diverticular disease and both premalignant (adenomatous polyp) and malignant (adenocarcinoma) lesions of the colon by retrospectively evaluating the data in our clinic. In the present study, the incidence of DD, adenomatous polyps, and colon adenocarcinoma was 16.4%, 20.8%, and 4.7%, respectively. In the present study, colon adenomatous polyps and adenocarcinomas were found at a significantly higher rate in the DD group, similar to some cross-sectional, case-control, and cohort studies in the literature (19.7% vs. 12.9% and 7.2% vs. 4.2%). In logistic regression analysis, it was determined that the presence of DD increased the relative risk for both adenomatous polyps and colon adenocarcinoma (OR: 1.469 and OR: 2.953). Colonoscopy indications of the DD and non-DD groups were similar. There was also no difference in risk factors such as advanced age, family history of colon cancer or polyps between DD and non-DD groups. According to the higher incidence of both premalignant and malignant lesions of the same carcinogenesis pathway in DD, we can strongly suggest that DD is associated with colon adenocarcinoma.

Most colon adenocarcinomas (sporadic 90%) develop from adenomatous polyps. It is known that the risk of malignant transformation is low in hyperplastic polyps. The risk of malignant transformation of serrated polyps and tubular adenoma is similar. There are certain risk factors for the transformation of adenomatous polyp into adenocarcinoma. The risk of cancer development from adenomatous polyps increases in the presence of polyps larger than 10 mm, multiple polyps, high villous components, and a high degree of dysplasia [3–5, 24]. It was observed that DD especially increased the frequency of adenomatous polyps, similar to the studies in literature, while no change was observed in the incidence of serrated and hyperplastic polyps with DD. Studies in the literature mostly investigated the relationship between DD and colon adenomatous polyps or adenocarcinoma [8, 9, 13-18]. Different from the studies in the literature investigating the relationship between DD and adenomatous polyps, it was observed in our study that the presence of DD increased the risk of adenomatous polyps with a villous component or high degree of dysplasia. We think that DD is associated with all phases of the adenoma-cancer sequence.

It is unclear what kind of pathogenesis the relationship between the presence of DD and pre-neoplastic or neoplastic lesions of the colon can be based on. In fact, the pathogenesis of the two diseases is actually completely different. In diverticular disease, diverticula are formed due to the weakening of the muscular layer of the colon [25]. In colorectal carcinogenesis, malignant transformation is observed in polyps as a result of the adenomatous polyp/adenocarcinoma sequence triggered by genetic and epigenetic changes in cells of the mucosal area [26]. It is accepted that both genetic and environmental factors have a role in diverticular disease and adenomatous colon polyps or adenocarcinoma development [25, 26]. The fact that age and similar environmental factors increase the risk for both diseases suggests that there is a possible involvement in similar genetic pathways. A positive family history is a risk factor for both DD and adenomatous colon polyps or adenocarcinoma. When all these findings are evaluated, it would be appropriate to support the relationship between DD and adenomatous polyp/colon adenocarcinoma, especially with genetic and epigenetic studies.

Limitations

The disadvantage of the study is that it is a retrospective study like the studies in the literature. In particular, it may be insufficient to show the cause-effect relationship between DD and adenomatous polyps or colon adenocarcinoma. However, the analyses showed that more adenomatous polyps and colon adenocarcinomas were encountered in patients with DD, which have risk factors similar to patients with non-DD. The high rate of adenomatous polyps with villous components or high degree of dysplasia, similar to adenocarcinoma, may suggest that there is a relationship between DD and both precancerous (adenomatous polyp) and cancerous (adenocarcinoma) lesions of the colon.

Conclusion

Age and family history of colon polyps or adenocarcinoma increase the risk of both adenomatous colon polyps and colon adenocarcinoma. DD is associated with adenomatous polyps and colon adenocarcinoma. A significant association is observed between DD and adenomatous polyps with villous component or high-grade dysplasia, which have malignant potential. Our results suggest that DD increases both pre-neoplastic (adenomatous polyp) and neoplastic lesions (adenocarcinoma) of the colon. There is a need for further genetic/epigenetic and prospective studies to investigate the relationship between DD and adenomatous polyps and adenocarcinoma.

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Anxiety-depression levels and coping strategies among renal transplant waitlisted and non-waitlisted hemodialysis patients

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Ethics Committee Approval
This study was approved by the Ethics Committee of Baskent University Faculty of Medicine (09/03/2016 date, App Nb: 9460333-604.01.02/8605, Project No: KA16/39). All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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

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Abstract

Background/Aim: Although hemodialysis provides prolonged survival, patients face major challenges, including conflicts about life decisions, limited physical, mental, and lifestyle activities, psychological distress, high prevalence of anxiety and depression symptoms, and compromised health-related quality of life. This study aimed to compare anxiety-depression levels and coping strategies between renal transplant waitlisted and non-waitlisted hemodialysis patients.

Methods: A total of 75 hemodialysis patients were enrolled in this case-control analytical study, including renal transplant waitlisted (n = 35) and non-waitlisted (n = 40) patients. Patients were asked to fill out a questionnaire including socio-demographic characteristics, duration of hemodialysis, the Hospital Anxiety and Depression Scale, and the Ways of Coping Questionnaire. Comparative analyses were performed via Student's t-test and Pearson Correlation test.

Results: The mean age was 57.1 (15.3) years; 56% were males, and being under hemodialysis treatment was 7.3 (5.9) years. Waitlisted and non-waitlisted patients were matched. Overall, 28 patients (37.3%) had anxiety, and 34 patients (45.3%) had depression. There was no significant difference between the two groups regarding depression scores. Anxiety was more common among non-waitlisted than waitlisted patients (P = 0.043). The fatalistic approach was the most common coping strategy applied, and there was a significant negative correlation between anxiety scores and the problem-solving approach (P = 0.034) and a significant positive correlation between depression scores and the helplessness approach (r = 0.234, P = 0.043).

Conclusion: Both waitlisted and non-waitlisted hemodialysis patients have significantly higher levels of depression, with anxiety being higher in non-waitlisted patients. Since coping strategies differed concerning anxiety and depression but not transplant candidacy, psychiatric evaluation and counseling would be essential in hemodialysis patients to control the stressors.

Keywords: Depression, Anxiety, Coping strategies, Hemodialysis, Renal transplantation

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Introduction

Chronic kidney disease (CKD) is a global public health problem that leads to end-stage renal disease (ESRD) [1], necessitating renal replacement therapy via dialysis or renal transplantation [2-4]. Data from the CREDIT (A population-based survey of Chronic REnal Disease In Turkey) study revealed the overall prevalence of CKD to be 15.7% (in adults) in this population-based study [5]. The Turkish Society of Nephrology 2016 annual report indicated that the point prevalence of ESRD needing renal replacement therapy was 933 per million population, with a marked increase in ESRD prevalence compared to previous years. In Turkey, the total number of renal transplants from cadaveric donors was only 779 in 2016 [6].

ESRD is associated with dramatic and stressful life changes, which require major coping efforts among patients and their families [7]. Although hemodialysis provides prolonged survival, patients face major challenges, including conflicts about life decisions, limited physical, mental, and lifestyle activities, psychological distress, high prevalence of anxiety and depression symptoms, and compromised health-related quality of life [3, 8-13]. As renal transplantation is the only cure, the transplant program's waitlist with a limited donor pool only serves the renal transplant candidacy [4]. Hence not every patient is lucky enough to receive a kidney transplant or eligible to be referred for the waitlist, presumably because of co-morbid diseases or lower life expectancy [4, 13]. Besides, renal transplant candidates on a waitlist experience hope and uncertainty regarding the timely availability of a renal transplant [13, 14].

Given that hemodialysis is inevitable in ESRD [15], a wide range of coping mechanisms has been suggested to be used by patients with ESRD to manage the illness and daily life, depending on the personality, religion, culture, moral and socio-economic support [4, 16-18]. Dietary restrictions, financial and transportation problems, changes in the family, social and work-life practices, duration of treatment, uncertainty about the future, and sleep disturbance, muscle cramps, fatigue, pruritus are considered among the stressors specific to a disease or hemodialysis treatment in patients with ESRD [19-21]. Accordingly, awareness of the coping strategies used by hemodialysis patients and identifying patients who could benefit from psychological counseling was associated with better control of stressors, more appropriate definition of therapeutic targets, increased adherence to the therapeutic regimen, and, therefore, a higher therapeutic effect [7, 21, 22].

This study was designed to evaluate coping strategies, anxiety, and depression levels among renal transplant waitlisted and non-waitlisted hemodialysis patients.

Materials and methods

Study population

A total of 75 hemodialysis patients with chronic renal failure but with no previous renal transplant history were enrolled in this case-control analytical study at the university hospital dialysis center.

Sample size calculation was done in accordance with previous similar studies using G*power 3.1.9.7. The sample size is calculated as there should be a total of 32 people, including at least 16 people in each group, with 95% confidence (1-alpha), 95.5% test power, d = 1.337 (large) effect size [7].

The study group included renal transplant waitlisted (n = 35) and non-waitlisted (n = 40) patients, with 56% male gender. The exclusion criteria were the presence of malignancy, having another organ failure, functional or not functional renal transplant, any psychiatric disorder diagnosis, and psychiatric treatment for psychiatric disease.

Written informed consent was obtained from each patient following a detailed explanation of the objectives and protocol of the study under the ethical principles stated in the "Declaration of Helsinki" and approved by the Ethics Committee.

Assessments

The patients were asked to fill in questionnaires that included socio-demographic characteristics, age, gender, marital status, educational status, duration of hemodialysis, the Hospital Anxiety and Depression Scale (HADS), and the Ways of Coping Questionnaire (WCQ).

Ways of Coping Questionnaire (WCQ)

WCQ, developed and later revised by Folkman and Lazarus, addresses a broad range of cognitive and behavioral strategies that individuals use when encountering an internal or external stressful situation [23]. In this study, a 42-item version of the Turkish adaptation of the questionnaire by Karancı et al. was used, including eight additional items about fatalism and superstition that are considered relevant to Turkish culture [24]. Based on the WCQ used in our study, four different approaches, including problem-solving, optimism, fatalism, helplessness, and avoidance, were evaluated.

Hospital Anxiety and Depression Scale (HADS)

Anxiety and depression levels were determined using the Turkish version of HADS, which was initially developed by Zigmond and Snaith (1983) [25] and adapted to Turkish by Aydemir (1997) [26]. The HADS is a fourteen-item scale with seven items related to anxiety (HADS-A) and seven related to depression (HADS-D). Each item on the questionnaire is scored from 0 - 3, leading to an overall score ranging from 0 to 21 and categorized as normal (scores 0 - 7) and borderline abnormal/abnormal (scores 8 - 21) status for anxiety or depression.

Statistical analysis

Statistical analysis was performed using SPSS version 18.0, SPSS Inc. Chicago, IL, USA. Student's t-test and Chi-square test (Fisher exact test) were used to compare parametric and categorical variables, respectively. Correlation analysis was performed using the Pearson Correlation test, and $P < 0.05$ was considered statistically significant.

Results

Socio-demographic characteristics

There was no significant difference between waitlisted and non-waitlisted patients in terms of marital status, educational status, and duration of hemodialysis. A higher percentage of

males than females was noted among waitlisted patients (68.6% vs. 31.4%, *P* = 0.034) (Table 1).

Table 1: Socio-demographic characteristics

		Total (n = 75)	Waitlisted (n = 35)	Non waitlisted (n = 40)	*P*-value
Age (years), mean (SD)		57.1 (15.3)	47.9 (13.2)	65.2 (12.3)	<0.001
Gender n (%)	Female	33 (44.0)	11 (31.4)	22 (55.0)	0.034
	Male	42 (56.0)	24 (68.6)*	18 (45.0)	
Marital status n (%)	Married	48 (64)	21 (60)	27 (67.5)	0.678
	Single	15 (20)	11 (31.4)	4 (10)	
	Widow(er)	12 (16)	3 (8.6)	9 (22.5)	
	Divorced	0 (0)	0 (0)	0 (0)	
Educational status n (%)	Illiterate	6 (8)	1 (2.9)	5 (12.5)	0.487
	Primary school	36 (48)	14 (40)	22 (55)	
	High school	22 (29.3)	15 (42.8)	7 (17.5)	
	University	11 (14.7)	5 (14.3)	6 (15)	
Duration of hemodialysis (years), mean (SD)		7.3 (5.9)	8.2 (5.9)	6.5 (5.7)	0.221

*$P < 0.05$; compared to females. Fisher exact test, SD: standard deviation, Student's t-test

In the non-waitlisted group of hemodialysis patients, the reasons for not being referred to the wait list were older age (24 patients aged > 65 years, nine patients aged > 75 years) and co-morbid conditions such as complicated diabetes mellitus (n = 17), severe coronary artery disease (n = 9), cerebrovascular disease (n = 1), or severe peripheral artery disease (n = 26). In the waitlisted group (n = 35), only three patients were older than 65, and four had hypertension.

Coping strategy scores

The overall fatalistic approach was the most commonly used strategy (mean score: 2.36 [0.4]), followed by the problem-solving approach (mean score: 2.22 [0.4]). There was no significant difference in coping strategy scores between waitlisted and non-waitlisted patient groups (Table 2).

Table 2: Coping strategy scores

	Total (n = 75)	Waitlisted (n = 35)	Non-waitlisted (n = 40)	*P*-value
Coping strategy scores, mean (SD)				
Problem solving-optimistic approach	2.22 (0.4)	2.27 (0.4)	2.18 (0.4)	0.384
Fatalistic approach	2.36 (0.4)	2.33 (0.4)	2.38 (0.5)	0.638
Helplessness approach	1.93 (0.4)	1.89 (0.4)	1.95 (0.5)	0.535
Avoidance approach	1.90 (0.4)	1.83 (0.3)	1.96 (0.4)	0.153

SD: standard deviation, Student's t-test

HADS scores

Based on HADS-A and HADS-D scores, 37.3% of patients had anxiety, and 45.3% had depression. Mean HADS-A scores were significantly higher (9.25 [5.5] vs. 6.85 [5.1], *P* = 0.05), and anxiety was more common among non-waitlisted than waitlisted patient groups (47.5% vs. 25.7%, *P* = 0.043). There was no significant difference between waitlisted and non-waitlisted patient groups concerning depression scores (Table 3).

Table 3: Hospital anxiety and depression scale scores

HADS Questionnaire scores	Total (n = 75)	Waitlisted (n = 35)	Non-waitlisted (n = 40)	*P*-value
HADS-A				
Overall score, mean (SD)	8.13 (5.4)	6.85 (5.1)	9.25 (5.5)	0.050[a]
Category, n (%)				
Normal (scores 0-10)	47 (62.7)	26 (74.3)	21 (52.5)	0.043[b]
Anxiety (scores >10)	28 (37.3)	9 (25.7)	19 (47.5)	
HADS-D				
Overall score, mean (SD)	6.33 (4.1)	5.94 (3.7)	6.67 (4.6)	0.447[a]
Category, n (%)	n (%)	n (%)	n (%)	
Normal (scores 0 - 7)	41 (54.7)	20 (57.1)	21 (52.5)	0.433[b]
Depression (scores >7)	34 (45.3)	15 (42.9)	19 (47.5)	

SD: standard deviation, HADS: Hospital Anxiety and Depression Scale, HADS-A: Anxiety scale, HADS-D: Depression scale, [a] Student's t-test, [b] Fisher Exact Test

Gender influence

There was no significant difference between overall females and males for mean HADS-A scores (9.09 [6.2] vs. 7.38 [4.7], *P* = 0.179), HADS-D scores (5.88 [3.7] vs. 6.69 [4.6], *P* = 0.408), and coping strategies including problem solving-optimistic approach (2.25 [0.4] vs. 2.21 [0.5], *P* = 0.693), fatalistic approach (2.41 [0.4] vs. 2.31 [0.5], *P* = 0.371), helplessness approach (1.90 [0.4] vs. 1.95 [0.4], *P* = 0.655), and avoidance approach (1.89 [0.4] vs. 1.92 [0.4], *P* = 0.733).

Correlation between HADS and coping strategy scores

There was a significant negative correlation between HADS-A scores and the use of the problem-solving approach (r = -0.245, *P* = 0.034) and a significant correlation between HADS-D scores and the use of the helplessness approach (r = 0.234, *P* = 0.043) (Table 4).

Table 4: Correlation between HADS and coping strategy scores

Coping strategy		HADS-A	HADS-D
Problem solving-optimistic approach	r	-0.245	-0.124
	P-value	0.034	0.288
Fatalistic approach	r	-0.025	-0.025
	P-value	0.834	0.833
Helplessness approach	r	0.186	0.234
	P-value	0.111	0.043
Avoidance approach	r	0.070	0.146
	P-value	0.562	0.213

HADS: Hospital Anxiety and Depression Scale, HADS-A: Anxiety scale, HADS-D: Depression scale, r: correlation coefficient, Pearson Correlation test

Age, duration of dialysis, and coping strategy scores with respect to HADS categories

The use of the problem-solving-optimistic approach was more common among patients without anxiety than those with anxiety (2.30 [0.4] vs. 2.09 [0.4], *P* = 0.039). In contrast, the use of the helplessness approach (2.08 [0.3] vs. 1.80 [0.4], *P* = 0.002) and the avoidance approach (2.01 [0.4] vs. 1.81 [0.4], *P* = 0.030) were more common in patients with depression. The mean age and mean duration of hemodialysis did not differ significantly between patients with and without anxiety or depression (Table 5).

Table 5: Age, duration of dialysis, and coping strategy scores with respect to HADS categories

Mean (SD)	HADS-A Category			HADS-D Category		
	Normal (n = 47)	Anxiety (n = 28)	*P*-value	Normal (n = 41)	Depression (n = 34)	*P*-value
Age (years)	55.4 (15.7)	60.0 (14.5)	0.214	55.8 (16.3)	58.8 (14.0)	0.405
Duration of HD (years)	7.10 (5.9)	7.57 (5.9)	0.742	7.43 (6.1)	7.08 (5.6)	0.799
Coping strategy scores						
Problem solving-optimistic approach	2.30 (0.4)	2.09 (0.4)	0.039	2.21 (0.4)	2.24 (0.4)	0.786
Fatalistic approach	2.38 (0.5)	2.32 (0.4)	0.601	2.32 (0.5)	2.40 (0.4)	0.418
Helplessness approach	1.88 (0.5)	2.0 (0.3)	0.226	1.80 (0.4)	2.08 (0.3)	0.002
Avoidance approach	1.89 (0.4)	1.92 (0.3)	0.714	1.81 (0.4)	2.01 (0.4)	0.030

SD: standard deviation, HADS: Hospital Anxiety and Depression Scale, HADS-A: Anxiety scale, HADS-D: Depression scale, Student's t-test

Discussion

Our findings revealed anxiety in 37.3% and depression in 45.3% of hemodialysis patients, using the fatalistic approach as the most common coping strategy. There was no significant difference between transplant waitlisted and non-waitlisted patient groups regarding coping strategies and HADS-D depression scores. Higher HADS-A scores that imply a higher rate of anxiety were also recorded among the non-waitlisted than the waitlisted patient groups. However, even though hemodialysis enables prolonged survival, hemodialysis patients face unique struggles related to physical, mental, and lifestyle limitations [3, 8, 13, 14].

Anxiety, depression, and a feeling of inadequacy have been reported to be the most frequent psychosocial stressors [27-29], leading to a high prevalence of depression and anxiety symptoms among hemodialysis patients [9, 10]. Accordingly,

depression was evident in 45.3% of our patients, similarly in waitlisted and non-waitlisted patient groups. In comparison, a two-fold higher rate of anxiety (47.5%) was noted among the non-waitlisted patient group. Our results are consistent with previous studies showing a high rate of depression in ESRD patients [30-32]. As depression has been reported to be the most frequent co-morbidity in patients with ESRD, with a higher mortality rate, psychiatric counseling is essential in hemodialysis patients to enable early diagnosis and treatment of depression [7, 30, 33-35]. Confirming this, we found depression in almost half of our patients regardless of the renal transplant candidacy.

A positive relationship between the female gender and higher anxiety levels has been reported in some studies [36, 37]. However, no gender influence was evident on anxiety or depression scores in our cohort.

After renal transplantation, improvement in anxiety or depression as compared with hemodialysis patients was reported in some studies [7, 38, 39]. In contrast, in other studies, a similar rate of depression was reported among renal transplant recipients versus hemodialysis patients [40, 41]. Our findings suggest that being on a waitlist is likely to reduce anxiety but not depression.

The fatalistic approach was our cohort's most commonly used coping strategy, followed by a problem-solving approach regardless of the renal transplant candidacy. This seems consistent with the increased likelihood of hemodialysis patients using religion-based strategies to cope with their illness, as reported in previous studies [7, 22, 42-44]. In contrast, problem-focused coping was common in studies reported from other countries [19, 20, 45, 46]. Indeed, the use of religion-based coping strategies has been associated with finding hope, the meaning of life, and strength in severely ill patients [7, 13, 47-50].

Problem-focused coping strategies have been suggested to be more commonly used in changeable and controllable situations, while emotion-focused coping has been associated with unchangeable and uncontrollable situations [51]. Accordingly, the use of emotion-oriented coping strategies like the fatalistic approach rather than a more effective strategy in our cohort may suggest the role of cultural factors and patient perception of disease control.

Notably, while adopting religious coping methods has been associated with reduced psychological distress in hemodialysis patients [13, 52], depression was evident in almost half of the patients in our cohort regardless of renal transplant candidacy.

Besides, the more common use of helplessness and avoidance strategies in our patients with depression agrees with the reported association between the use of less active and more passive coping strategies with increasing symptoms of depression in patients living with chronic illnesses [53]. Along with the less common use of the problem-solving approach in patients with anxiety, our findings support that more active coping, planning, and social support-seeking coping strategies are more adaptive in the early periods of disease or high expectancy of recovery [7].

Our study showed no association between age, gender, duration of hemodialysis, or transplant candidacy with coping strategies. Besides, depression and anxiety were associated with the more common use of specific coping strategies. This should be considered in assessing therapeutic goals and efficacy and encouraging positive coping methods [7, 21].

Limitations

Our study, being a single-center instead of a multi-center study, could be considered a limitation. However, a broader evaluation would be suitable for further research, including the economic situation and support mechanisms.

Conclusion

Our findings revealed similarly high rates of depression in waitlisted and non-waitlisted hemodialysis patients and a higher rate of anxiety among non-waitlisted patients. Since coping strategies differ concerning anxiety and depression but not transplant candidacy, psychiatric evaluation and counseling are essential in hemodialysis patients to control the stressors.

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Preeclampsia development and neonatal outcomes in pregnant women who were anemic in the first trimester

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Ethics Committee Approval
Ethical approval was obtained from Gazi University Ethics Committee (Decision no: 2022-1237, 8 November 2022).
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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Abstract

Background/Aim: Anemia is a disease that can be easily treated, but it is still widespread worldwide. Anemia can affect nearly 40% of women. Anemia has been extensively studied and related to a variety of pregnancy complications. The primary purpose of our study was to discover the relationship between preeclampsia and anemia in the first trimester, and the secondary goal was to analyze the outcomes of newborns born to these mothers.

Methods: This study was compiled as a retrospective cohort study. Age, gravida, parity, and thyroid stimulating hormone (TSH) levels were recorded in a patient's first visit file. Hemoglobin counts in the first trimester were analyzed as hemogram values. Those with a hemoglobin value <11 g/dl during pregnancy were classified as anemic. The patients' file records were reviewed to determine mode of delivery, birth weight, and Appearance, Pulse, Grimace, Activity, and Respiration (APGAR) scores. To confirm a diagnosis of preeclampsia, the American Congress of Obstetricians and Gynecologists (ACOG) criteria were used.

Results: After the exclusion of 186 women due to comorbidities and multiple pregnancies, 364 women were evaluated. The number of anemic pregnant women in the first trimester was 87 (23.9%), and 277 non-anemic women were matched with the anemic group. No statistical difference between the groups in terms of demographic characteristics, such as age, gravida, body mass index (BMI), and TSH were found. No statistical difference between the groups in terms of delivery type, infant birth weight, and APGAR scores were found ($P > 0.05$). Preeclampsia frequency was statistically higher in pregnant women who were anemic in the first trimester ($P = 0.032$).

Conclusion: Preeclampsia was found to be more common in pregnant women who were anemic in the first trimester. Although it would seem that neonatal outcomes are unaffected, we believe that the unaffected outcomes are due to iron replacement. To avoid pregnancy complications, it is crucial for women not to be anemic prior to becoming pregnant.

Keywords: Preeclampsia, Anemia, Pregnancy problems

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Introduction

The World Health Organization defines maternal anemia during pregnancy as hemoglobin (Hb) concentrations of ≤11.0 g/dL. Anemia is dangerous for both the mother and the fetus [1]. Anemia is a disease that can be easily treated, but it is still widespread worldwide. Recent observational data from a multi-centered study on 2103 women in the United Kingdom show that the prevalence is higher than 24.4% [2]. Another global study revealed that anemia affects nearly 40% of women [3].

Anemia has been extensively researched and is believed to be related to a number of pregnancy problems. Greater risks of maternal and perinatal mortality have been associated with preterm birth, hypertension, low birth weight, small-for-gestational-age (SGA) live births, anemia during pregnancy, and cesarean delivery [4–9]. Additionally, iron deficiency anemia causes an increases in the risk for needing a blood transfusion and/or cesarean section and was linked to a lower Appearance, Pulse, Grimace, Activity, and Respiration (APGAR) scores in a different study involving anemia's effects on mothers and newborns [10].

A number of infiltrating trophoblast cells were discovered in anemia in a study evaluating trophoblast invasion and apoptosis in pregnant anemic women in contrast to the diminished invasion of preeclampsia spiral arteries [11]. These data contradict studies that support unfavorable outcomes.

Studies on the relationship between adverse neonatal outcomes, such as preeclampsia and anemia, have yielded conflicting results. Our study's goal was to discover the link between preeclampsia and anemia in the first trimester and secondarily, to evaluate outcomes of the newborns born to these mothers.

Materials and methods

Patients who gave birth in our hospital between January and June 2022 were screened retrospectively. The study was conducted with the approval of the Gazi University Ethics Committee (Decision no: 2022-1237). All procedures were carried out in accordance with the Helsinki Declaration's ethical rules and principles. Because of the retrospective nature of the study and the use of anonymous research findings, informed consent was waived.

Data from a total of 550 women who gave birth were scanned. Multiple pregnancies and the mother having comorbid conditions, such as diabetes mellitus, hypertension, kidney diseases, hematological diseases, thyroid hormone dysfunction, and connective tissue diseases, were among the study's exclusion criteria. Because of multiple pregnancies and additional maternal disease, 186 women were excluded from the study. In total, 364 pregnant women were included in the study.

Age, gravida, parity, and thyroid stimulating hormone (TSH) levels were recorded in the patients' first visit file. Hemoglobin counts in the first trimester were analyzed as hemogram values. Those with a hemoglobin value <11 g/dl during pregnancy were classified as anemic (n = 87), while those with hemoglobin values ≥11 g/dl were classified as non-anemic (n = 277). Patients' hemoglobin levels at the time of delivery were re-evaluated. In the second trimester, all pregnant women were given iron supplements. The patients' file records were reviewed to determine mode of delivery, birth weight, and APGAR scores (first and fifth minutes).

To confirm a diagnosis of preeclampsia, the American Congress of Obstetricians and Gynecologists (ACOG) criteria were used. After the 20th gestational week, preeclampsia is diagnosed when the arterial blood pressure measurement is 140/90 mmHg or higher, accompanied by proteinuria or thrombocytopenia, pulmonary edema, renal failure, abnormal liver function, and vision problems.

Statistical analysis

The data was analyzed using the IBM SPSS Statistics 22 program. Descriptive statistics (mean, standard deviation) for numerical variables are provided when analyzing the study data. To compare the two groups, the independent sample t- and the chi-squared tests were used. Pearson's correlation analysis was used to investigate the relationship between numerical variables. Statistics were deemed significant at $P < 0.05$.

Results

After exclusion of 186 women due to comorbidities and multiple pregnancies, 364 women were evaluated out of 550 women who gave birth. The number of anemic pregnant women in the first trimester was 87 (23.9%) and the number of non-anemic pregnant women was 277 (76.1%).

Anemic and non-anemic pregnant women's demographic data were compared. In terms of age, gravida, parity, body mass index (BMI), and TSH levels, no statistical differences between the groups were found (Table 1).

Table 1: Comparison of demographic features and birth characteristics

	Anemic group (n = 87)	Control group (n = 277)	*P*-value
Age*	33.3 (5.4)	32.5 (5.6)	0.18
Gravida (median [min-max]) ¶	2 (1–7)	2 (1–6)	0.06
Parity ¶	1 (0–3)	0 (0–3)	0.06
BMI*	22 (1.4)	23.3 (2.0)	0.22
TSH*	2.1 (1.3)	2.0 (1.3)	0.59

*Data are given as mean (standard deviation [SD]) ¶ Data is given as median (minimum–maximum), BMI: Body mass index, TSH: Thyroid stimulating hormone

The groups were compared in terms of mode of delivery, baby weight, and first and fifth minute Apgar scores. No statistically significant differences between groups were found (Table 2). Sixty (16.5%) pregnant women were diagnosed with preeclampsia in the study. Preeclampsia frequency was statistically higher in pregnant women who were anemic in the first trimester ($P = 0.032$) as shown in Table 3.

Table 2: Comparison of maternal and neonatal outcomes

	Anemic group (n = 87)	Control group (n = 277)	*P*-value
Baby weight *	2677.87 (622)	2966 (818)	0.47
First minute Apgar *	8.3 (1.6)	7.8 (2.3)	0.31
Fifth minute APGAR score*	9.5 (1.2)	9.1 (2.0)	0.30

APGAR: Appearance, Pulse, Grimace, Activity, and Respiration, *Data are given as mean (SD)

Table 3: Comparison of the presence of preeclampsia in pregnancy based on anemia status

	Anemic group (n = 87)	Control group (n = 277)	*P*-value
Preeclampsia Group (n = 60)	21 (24.1%)	39 (14.1%)	0.032
Non-Preeclampsia Group (n = 364)	66 (75.9%)	239 (85.9%)	

Chi-squared test

At the time of delivery, the patients' hemoglobin levels were re-evaluated. The number of pregnant women who were anemic had decreased to 48. This value represented 13.2% of the population.

Discussion

Anemia, a serious public health issue, is becoming increasingly important in terms of causing fetal and maternal mortality during pregnancy. After excluding pregnant women with comorbidities and multiple pregnancies from our study, we found anemia in about a quarter (23.9%) of the pregnant women. Preeclampsia occurs at a statistically significant higher rate in anemic pregnant women.

Anemia and hypertensive diseases could not be linked in an old meta-analysis conducted by Xiong et al. [12] regardless of pregnancy stage. According to the review by Pea-Rosas et al. [13], no association between preeclampsia and anemia exists. Lin et al. [14] found no link between hypertensive diseases of pregnancy and anemia in a multicenter study of the Chinese population. Although this study included a large number of patients, the fact that it was multicenter raises concerns about data bias and inconsistency. Ali et al. [8] discovered that the risk of severe anemia and preeclampsia increased by 3.6 times in their study. However, a correlation was found in this study with patients who were diagnosed with anemia at the time of admission. It is difficult to conclude from this study that anemia detected at any stage of pregnancy causes preeclampsia. Because our study included pregnant women who were anemic in the first trimester, we believe that using our methodology would be more accurate in evaluating anemia as an increase in the risk of preeclampsia. The unadjusted odds ratios (ORs) for preeclampsia and mild, moderate, and severe anemia increased with anemia severity and were highest in women with unspecified anemia, according to a study by Smith et al. [15].

We also reviewed neonatal outcomes in our study. No difference in baby weights and APGAR scores of pregnant women who were anemic in the first trimester according to these evaluations were found. Bora et al. [16] studied 580 newborn babies and discovered that the baby birth weight decreased significantly with the decrease in hemoglobin. In 2018, a systematic review and meta-analysis found a relationship between maternal anemia and low birth weight, and 7243 articles were discussed in this study [17]. Iron replacement in these pregnant women may explain the lack of difference in neonatal outcomes in our study. Iron replacement is performed as a national health policy in the follow-up of pregnant women regardless of whether or not anemia is found. Anemic pregnant women recover with iron replacement in the subsequent trimesters. As a result, no statistically significant difference in neonatal outcomes was found. According to Haider et al.'s review [18], daily iron supplementation improves birth weight in a dose-response manner and may lead to a reduction in the risk of low birth weight.

Limitations

The study's most significant limitation is its retrospective design.

Conclusion

According to our findings, anemia plays a significant role in our society’s health. Preeclampsia was found to be more common in pregnant women who were anemic in the first trimester. Although it would seem that neonatal outcomes are unaffected, we believe that this finding is due to iron replacement. In order to avoid pregnancy complications, it's crucial for women not to be anemic prior to becoming pregnant.

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Case report

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Thoracic surgery with erector spinae plane block in a patient with Duchenne muscular dystrophy

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Informed Consent
The authors stated that the written consent was obtained from the patient presented with images in the study.

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Abstract

The management of general anesthesia is very difficult in patients with Duchenne muscular dystrophy (DMD) due to the potential for difficult airway problems, malignant hyperthermia, and cardiorespiratory complications. Therefore, peripheral nerve and plane blocks may be a good choice in DMD patients. In this case, we aimed to show the anesthetic efficiency of erector spinae plane (ESP) block in an 18-year-old male patient with DMD scheduled for video-assisted thoracoscopy surgery (VATS) exploration due to prolonged air leak. On surgery day, ultrasound (US)-guided one-sided ESP block (ESPB) was performed under sedation. Decortication surgery was performed in 3 hours. The patient's intraoperative hemodynamic parameters were stable, and no pain or complications were recorded. The patients' visual analog scale (VAS) scores were recorded at postoperative hour 0, 2, 6, and 12 as 0, 0, 2, and 2, respectively. In conclusion, safe and effective anesthesia can be provided by ESPB with US guidance in thoracic surgery.

Keywords: Erector spinae plane block, Duchenne muscular dystrophy, Regional anesthesia, Ultrasound, Dexmedetomidine, Postoperative analgesia

Introduction

Duchenne muscular dystrophy (DMD) is an X-linked recessive disease characterized by progressive muscular degeneration and muscle weakness. Cardiomyopathy and respiratory failure may develop as the disease progresses. Because of the potential for difficult airway problems, malignant hyperthermia, and cardiorespiratory complications, general anesthesia management is highly challenging. To minimize these risks, regional anesthetic techniques may be attractive options [1, 2]. In our patient's case, we evaluated the anesthetic efficacy of a single-sided erector spinae plane (ESP) block (ESPB) under ultrasound (US) guidance in a patient with DMD who was scheduled for decortication with video-assisted thoracoscopy surgery (VATS).

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Case presentation

An 18-year-old male patient with DMD for 16 years was scheduled to undergo VATS exploration due to prolonged air leakage in his past tracheostomy. Thorax computerized tomography and chest X-ray images of the patient are shown in Figures 1 and 2.

Figure 1: Thorax computerized tomography of the patient.

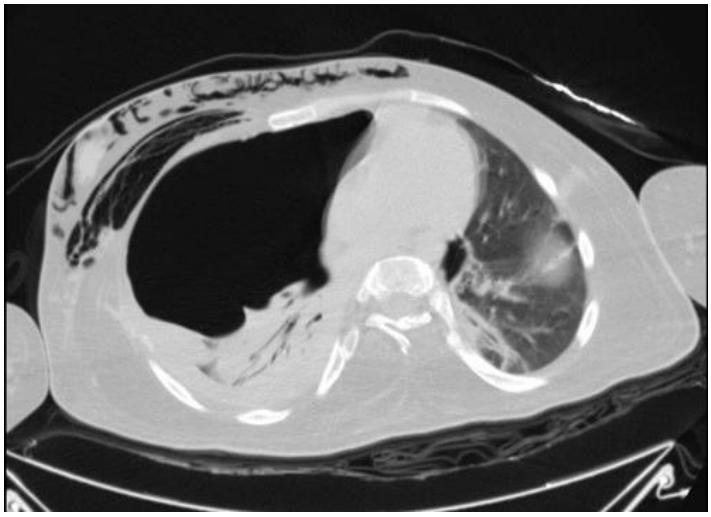
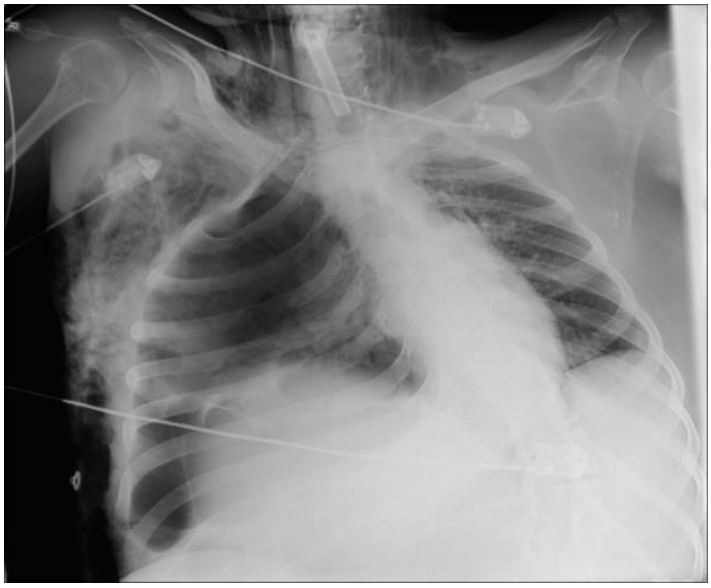


Figure 2: Chest X-ray of the patient.



The patient's anesthesia management was planned in the form of a one-sided ESPB with US guidance accompanied by sedation. After sedation with 0.03–0.05 mg/kg intravenous midazolam (Dormicum®, Roche), proper skin sterilization was performed while the patient was sitting, and a linear 10–18 MHz US probe (Esaote MyLab 30®, Geneva, Italy) was placed between two transverse processes in the right paramedian plane. Transverse processes and erector spinae muscle were determined at the T5 level. After the skin and subcutaneous tissue were anesthetized with 2% lidocaine, the 18-gauge, 50-mm needle (Pajunk®, Geisingen, Germany) hit the transverse processes in an in-plane position, the needle was withdrawn by 1–2 mm, and the fascia under the erector spinae muscle was reached (Figures 3, 4). Following needle-tip aspiration to control the presence of blood and/or air, 20 mL of 0.5% bupivacaine hydrochloride (Marcaine®, AstraZeneca) was administered intravenously. Pain and sensory block were evaluated at 20 minutes post-block, after which surgery was allowed. After beginning surgery, 1 µg/kg of dexmedetomidine IV bolus was followed by an infusion of 0.2 µg/kg/h and continued during the surgery. Decortication surgery was performed in 3 hours. In the intraoperative period, the patient's hemodynamic parameters were stable, and he had no pain or any complications. The patient's visual analog score (VAS, pain score: 0–10) recorded at postoperative hours 0, 2, 6, and 12 were 0, 0, 2, and 2, respectively. Informed consent for publication was obtained from the patient's guardian.

Figure 3: Patient position before injection.

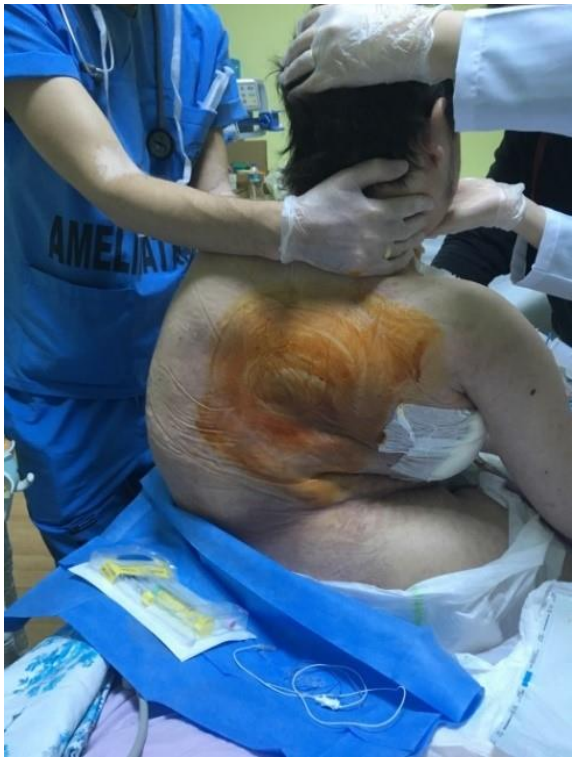
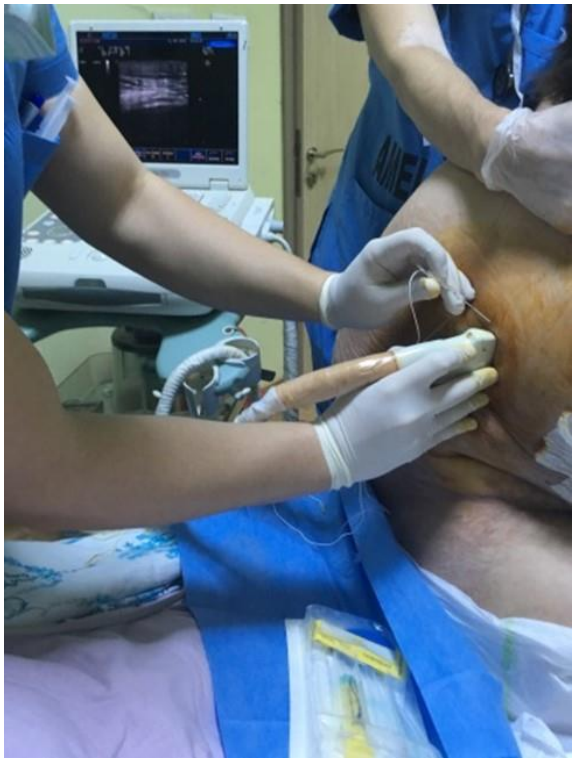


Figure 4: Patient and probe position during injection.



Discussion

DMD is a progressive muscle degeneration that characteristically holds proximal muscles. Respiratory failure and cardiomyopathy are common, because DMD affects the cardiac and respiratory muscles. Since the complications parallel the severity of the disease, this should be kept in mind in the management of anesthesia. The most undesirable complication associated with general anesthesia is malignant hyperthermia and anesthesia-induced rhabdomyolysis, which has a mortality rate of 30%. While critical risks are involved, such as prolonged effect and residual block in the use of neuromuscular blockers, hemodynamic instability and hypotension due to overdose in total intravenous anesthesia (TIVA) can complicate the management of anesthesia. Because of all these risks, peripheral nerve blocks may be a preferred choice [3, 4].

One of the widely used plane block is ESPB. It is a safe, effective technique that ensures adequate analgesia for acute or chronic pain and can be performed as a single injection or via catheter inserted into the ESP [5]. The target of injection is the musculofascial plane between the erector spinae muscle groups and the transverse process of vertebrae. The injection can be made sagittally or transversely. After the spinal nerves pass through the intervertebral foramen, these nerves travel through the erector spinae musculofascial plane. The purpose of the ESPB is to provide visceral and parietal analgesia by affecting the ventral and dorsal rami of the spinal nerves [6]. Although it is a relatively novel technique, its beneficial properties are shown in various operations; in parallel, the number of publications about ESPB is growing [5].

It has been assumed that ESPB has lower complication rates than central neuraxial blocks, as the target of blockage is located more distally from important structures like pleura or main vessels. ESPB is easy to perform via US. In our patient, neuroaxial structures were not easy to examine, and central blocks or general anesthesia were not utilized because of the risk of malign hyperthermia. This block is not the first-line anesthetic technique in many centers; however, it is a good second option, and it can be utilized when another type of anesthesia can cause major complications or is contraindicated [7]. Accordingly, thoracic ESPB was selected in our case.

Thoracic operations are not only painful, but they also impair lung function and cause atelectasis [8]. Therefore, general anesthesia contributes lung disorientation because of single lung ventilation, airway injuries, and lung injuries. Alagoz et al. [9] performed ESPB and thoracic paravertebral block in combination for VATS and added intravenous sedoanalgesia. They concluded that the combination of these methods provided optimal surgical conditions and effective postoperative analgesia.

In a patient with DMD and severe respiratory failure, a case report was reported by Vandepitte et al. [10] in which the intercostal nerve block, accompanied by US, was administered for anesthetic purposes in a thoracic surgery. What made our case unique was the provision of anesthetic efficacy with ESPB alone in VATS.

Conclusion

Safe and effective anesthesia can be provided with ESPB performed with US in thoracic surgery, and the patient's comfort can be increased by providing a high level of analgesia during the postoperative period; accordingly, we contend that severe complications that may occur as a result of general anesthesia can be avoided.

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What is the impact of a large cyst size on the radiological diagnosis of pulmonary hydatid cyst in children?

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Informed Consent
The authors stated that the written consent was obtained from the parents of the patient presented with images in the study.

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

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Abstract

Anamnesis, physical examination, and laboratory investigation of patients admitted to the clinic provide non-specific findings for pulmonary hydatid cysts. Obtaining an accurate diagnosis of this cystic lesion is only possible by radiological examination. An uncomplicated intact simple cyst in an early phase could be easily and precisely diagnosed by chest roentgenogram and computed tomography scan of the thorax. Complicated late cases may have confusing and challenging atypical radiological signs. In this case report, we report a giant pulmonary hydatid cyst (13 x 8 x 12 cm) with atypical radiological findings in a 4-year-old girl who was hospitalized with fever and cough and was treated with oral antibiotics for pneumonia.

Keywords: Pulmonary hydatid cyst, Radiology, Children

Introduction

Echinococcus granulosis causes hydatid disease. The lungs are the most common sites of this parasitosis in children [1, 2]. A pulmonary hydatid cyst (PHC) with a diameter of more than 10 cm is called a giant cyst [3–5]. A well-circumscribed spherical or oval homogenous opacity in the pulmonary field is the typical radiological image determined in the most of the hydatid cysts even in the giant ones. Lung tissue elasticity allows the hydatid cyst to reach a very large size that may result in an atypical radiological presentation in delayed cases. We report an unusually large PHC in a 4-year-old girl.

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Case presentation

A 4-year-old girl had been admitted five months ago to an outside facility with fever and cough and was treated for pneumonia with an oral antibiotic. Her symptoms ceased after the treatment; however, progressive shortness of breath with fatigue, lethargy, anorexia, and weight loss developed, and she presented to our hospital with a 10-day history of cough, fever, diaphoresis, dyspnea, and left-sided chest pain. During physical examination, she was febrile, pale, cachectic, and in moderate respiratory distress with an oxygen saturation of 92%. She had decreased breath sounds on the left. She had significant iron deficiency anemia with a hemoglobin concentration of 7.2 g/dl. The white blood count was 15,500/mm^3, and eosinophilia was present. On chest roentgenogram, a homogenous opacification occupying the whole left lung field and shift of the mediastinum to the right were determined (Figure 1). A computerized tomography (CT) scan of the thorax showed a thick-walled intact giant cyst (13 x 8 x 12 cm) that completely filled the left hemithorax (Figure 2). The upper left lobe from which the cyst had originated was totally collapsed. The main body part of the cyst was located on the left upper hemithorax, and the basal part of the cyst settled on and squeezed the atelectatic lower lobe (Figure 3).

Figure 1: Homogenous opacification occupying the left hemithorax and shift of the mediastinum to the right caused by the giant hydatid cyst as viewed on chest roentgenogram

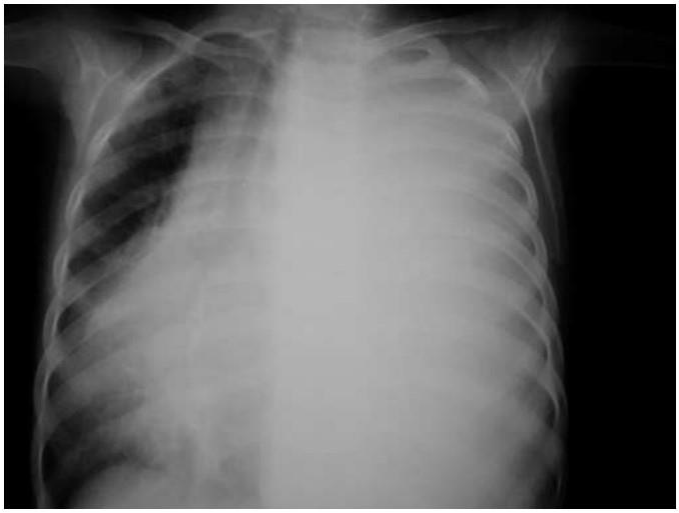


Figure 2: Computed tomography (CT) scan of the thorax showing a thick-walled intact giant hydatid cyst in the left hemithorax

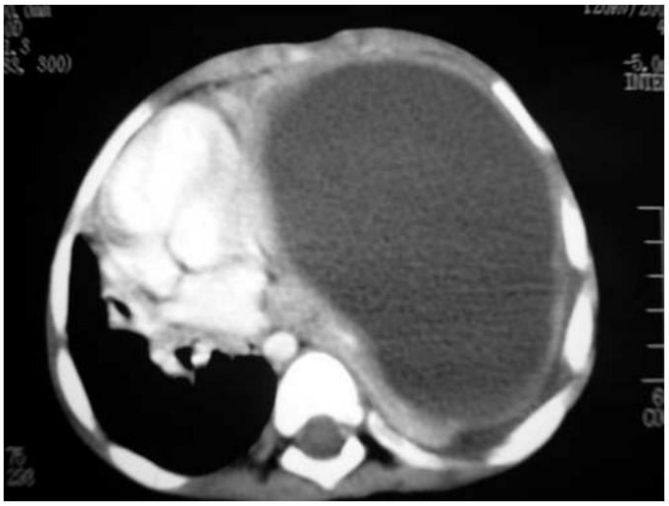
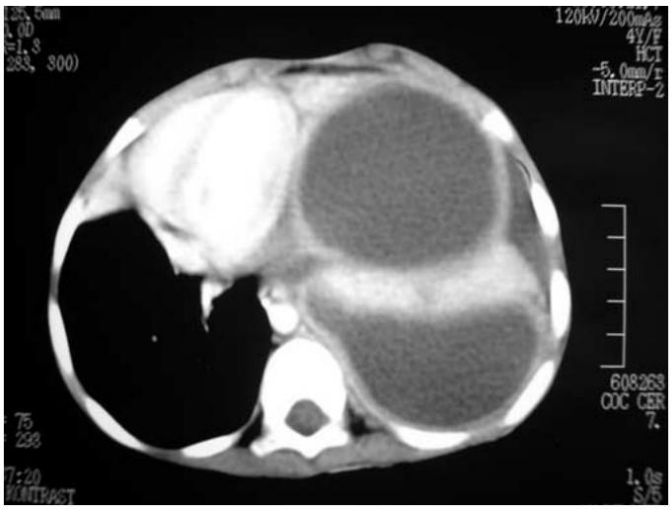
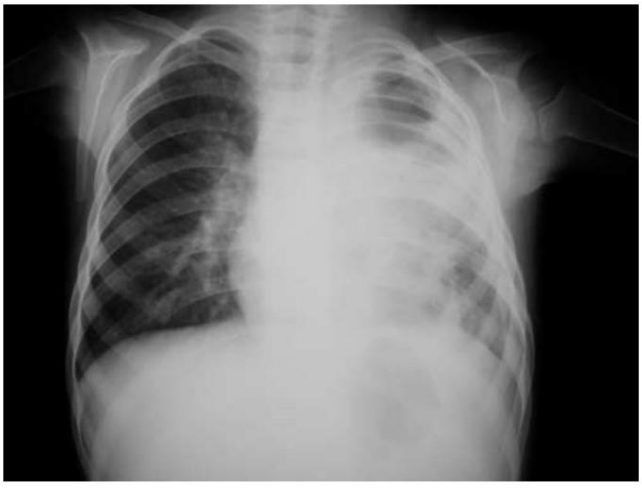


Figure 3: CT scan of the thorax showing the basal part of the cyst squeezing the atelectatic lower lobe



Indirect hemagglutination test for *Echinococcosis* was positive. Since the patient was living in a region endemic for hydatid disease, the clinical, laboratory, and radiological findings suggested the diagnosis of PHC. A classical posterolateral thoracotomy was performed. After the cyst was aspirated, it was irrigated with saline solution to prevent contamination after which cystectomy with left upper lobectomy was performed. The operative findings and histopathological examination confirmed the diagnosis. An early post-operative chest roentgenogram showed a fully expanded left lower lobe and post-lobectomy space at the upper hemithorax (Figure 4). The patient recovered uneventfully and was discharged with albendazole treatment on the 12th post-operative day. Written informed consent to use study information was obtained from the patient's family.

Figure 4: Post-operative chest roentgenogram showing the fully expanded left lower lobe and postlobectomy space at the left upper hemithorax



Discussion

In children, PHC may remain asymptomatic for a long time so that the elasticity of the lung tissue and the immune system allow the cyst to reach a very large size [3, 6, 7]. The findings in the history and the physical examination of the patient with PHC are often nonspecific. A chest roentgenogram is needed in suspected cases, especially in endemic regions. While chest roentgenogram of an early phase PHC showing a well-circumscribed spherical or oval cyst with homogenous opacity provides significant clues to make a plan for the further diagnostic work up, late presentation with atypical findings on

the chest roentgenogram may cause confusion in the differential diagnosis. In our case, the clinical findings most probably were overlooked, and a chest roentgenogram was not required at her first visit to the primary care physician. At her second visit, the delay in presentation caused enlargement of the cyst and atypical findings on the chest roentgenogram. In our patient, the combination of the history of previous pulmonary infection, current clinical presentation, and chest roentgenogram showing a homogenous density occupying the whole left lung field and shift of the mediastinum to the right strongly suggested a diagnosis of diffuse empyema secondary to underlying pneumonia. The attending clinician may decide to perform thoracentesis in such cases; however, this procedure can lead to an increase in PHC-associated morbidity and mortality. Thoracentesis may cause the cyst to rupture thus resulting in intense inflammation, pleural and bronchial seeding, acute anaphylaxis, and severe hypotensive shock [8–10]. Therefore, before any therapeutic intervention, a CT scan of the thorax should be considered to clarify this type of opacity based on the chest roentgenogram findings. CT can distinguish cystic from solid lesions and shows the morphological features of the hydatid cyst, including its size, location, and extension in the thoracic cavity and its relationship to the lung parenchyma and neighboring organs. CT examination also demonstrates multiple cysts, secondary bacterial infection, cyst wall calcification, and the state of the affected lobes [4, 11, 12]. It may identify the pathognomonic features in complicated or ruptured hydatid cysts. If communication develops between the cysts and the bronchial tree, air may enter between the pericyst and exocyst, producing crescent or inverse crescent signs. If communication occurs directly with the endocyst, the air–fluid level can be observed or a totally air-filled cyst termed as dry cyst sign can be identified [4, 8, 13]. CT also shows the daughter cysts and endocyst membranes and water lily sign caused by floating membrane on the fluid within the cyst after rupture. CT is especially crucial for making a correct diagnosis and for pre-operative evaluation of large and atypical cysts. In our case, although the cyst had an unusual size, shape, and location, the demonstration of a thick-walled intact cyst with the water density based on the CT examination provided an accurate pre-operative PHC diagnosis. The state of the affected pulmonary parenchyma and possibility of lobectomy of the left upper lobe were also determined by pre-operative CT examination.

Conclusion

In conclusion, it is recommended that patients with giant lung hydatid cysts be evaluated pre-operatively using CT both to determine the characteristics of the cyst, such as the size and location, and to allow the surgeon to decide more easily what kind of surgical procedure should be performed during the operation.

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