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Original Article

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Predictive Value of Initial ^{18}F-FDG PET/CT for Identifying EGFR and KRAS Mutations in Patients with Non-small-cell Lung Cancer

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Abstract

Aim: Since the importance of epidermal growth factor receptor (EGFR) and Kirsten rat sarcoma viral oncogene homolog gene (KRAS) mutation status in predicting treatment response in non-small cell lung cancer (NSCLC) patients is well known, we aimed to evaluate whether initial fluorine-18-fluorodeoxyglucose (^{18}F-FDG) positron emission tomography-computed tomography (PET/CT) imaging could non-invasively predict EGFR or KRAS mutation states in this patient group.

Methods: This retrospective observational study examined patients with NSCLC who underwent ^{18}F-FDG PET/CT for staging from August 2021 to January 2024. Age, sex, smoking status, pathological data, EGFR and KRAS mutation status, and metabolic and volumetric PET parameters were recorded. Groups were based on gene mutation status as follows: EGFR-mutations (mt) vs. EGFR wild-type (EGFR-wt) and KRAS-mt vs. KRAS-wt.

Results: Ninety-nine patients with a mean age of 62.96±9.66 (range: 37-87) were included. The EGFR-mt group had lower metabolic tumor volume (MTV) (p=0.015) and total lesion glycolysis (TLG) (p=0.017) values. MTV had an area under the receiver operating characteristic curve (AUC) of 0.667 [95% confidence interval (CI): 0.547-0.788, p=0.015], and with a ≤24.9 cut-off, yielded 60.87% sensitivity, 68.42% specificity, and 66.67% accuracy to detect EGFR-mt. For TLG, the AUC was 0.664 (95% CI: 0.540-0.788, p=0.017) and a ≤408.1 cut-off yielded 86.96% sensitivity, 43.42% specificity, 53.54% accuracy, and 91.67% NPV. KRAS-mt was detected in 34 (34.34%) patients, and there were no significant differences between the KRAS-mt and KRAS-wt groups in terms of PET parameters.

Conclusion: Primary tumor parameters derived from initial ^{18}F-FDG PET/CT can predict EGFR mutation status but not KRAS mutation status. The high negative predictive value of TLG can be used to rule out EGFR-mt status, possibly preventing unnecessary treatments in patients without favorable genetic properties, especially when genetic analyses are not possible.

Keywords: Lung cancer, non-small-cell lung cancer, ^{18}F-FDG PET/CT, EGFR, KRAS

Introduction

Lung cancer is one of the leading causes of cancer-related deaths worldwide, with approximately 85% of patients suffering from non-small-cell lung cancer (NSCLC) (1). Tyrosine kinase inhibitors (TKIs) have been shown to improve NSCLC outcomes (2), and it has been established that a kinase domain mutation of the epidermal growth factor receptor (EGFR) is associated with a favorable response to TKIs. In fact, progression-free survival (PFS) is longer with the use of TKIs compared with chemotherapy in patients with EGFR mutations (EGFR-mt) (3). The Kirsten rat sarcoma viral oncogene homolog gene (KRAS) encodes a member of the small GTPase superfamily that has an impact on the EGFR pathway. Mutations in this gene (KRAS-mt) are associated with an unfavorable response to TKIs (4).

Fluorine-18-fluorodeoxyglucose (^{18}F-FDG) positron emission tomography-computed tomography (PET/CT)

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a non-invasive molecular imaging method that is widely used for staging, response assessment, and recurrence detection in NSCLC (5). The EGFR mutation status plays an important role in the management of patients with advanced NSCLC; regardless of other factors, the molecular profiling of EGFR is essential to guide clinical treatment (6). However, obtaining high-quality tumor tissue for EGFR-mt testing is difficult in many cases, given the shortage of biopsy samples and the physical condition of patients. Therefore, a non-invasive and simple method to identify EGFR-mt is necessary to inform treatment decisions. Recent studies, the results of which are still controversial, have focused on whether EGFR-mt and KRAS-mt states can be associated with metabolic parameters obtained via ^{18}F-FDG PET/CT, especially the maximum standardized uptake value (SUV_{max}) (5,7). Few reports have explored the utility of metabolic tumor volume (MTV) and total lesion glycolysis (TLG) values in this context, and these studies have revealed inconsistent findings (8).

We hypothesized that initial ^{18}F-FDG PET/CT parameters obtained from the primary lesion could be utilized to predict the presence or absence of EGFR-mt or KRAS-mt in patients with NSCLC.

Methods

Study Design and Patients

This study was approved by the Clinical Research Ethics Committee of University of Health Sciences Turkey, Basaksehir Cam and Sakura City Hospital (date: July 2023, approval no.: 2023.07.296). Informed consent was obtained from all participants included in the study. Diagnostic and therapeutic procedures were performed in accordance with national guidelines and the principles of the Declaration of Helsinki (1964). All patients received appropriate information and provided written informed consent.

This retrospective observational cohort study examined patients with NSCLC who underwent ^{18}F-FDG PET/CT imaging for staging purposes at baseline between August 2021 and January 2024. The inclusion criteria were as follows: pathologically confirmed diagnosis of NSCLC, being treatment-naïve before ^{18}F-FDG PET/CT imaging, confirmation of EGFR-mt and KRAS-mt status within 1 month from the time of imaging, and availability of all relevant clinical and imaging data. The exclusion criteria were having received any antitumor therapy before ^{18}F-FDG PET/CT, having a history of other malignancies, having comorbidities affecting the metabolic parameters examined by ^{18}F-FDG PET/CT (such as diabetes mellitus), and having pneumonia or other infections that might confound the analyses (Figure 1).

Age, sex, smoking characteristics (pack years), histopathology, clinical stage, primary tumor site, nodal involvement, metastasis, operation history, molecular analysis results concerning EGFR and KRAS (mutations), and metabolic and volumetric parameters derived from ^{18}F-FDG PET/CT were recorded. The cancer stage was determined according to the 8th TNM classification for lung and pleural tumors (9).

Patients were classified according to mutation status as EGFR-mt and EGFR wild type (EGFR-wt) or KRAS-mt and KRAS wild type (EGFR-wt). The specimens were defined as EGFR-mt if mutations were identified in exons 18, 19, 20, and 21. The presence of KRAS-mt was determined by detecting mutations in KRAS codons 12, 13, and 61.

^{18}F-FDG PET/CT Procedure

Imaging was performed after at least 6 h of fasting and the presence of a glucose level of <150 mg/dL. After ^{18}F-FDG injection, patients were left to rest for 50 min before imaging with the Ingenuity TF 64 scanner (Philips Medical Systems, OH, USA). Low-dose CT was performed with the following settings: 113 mAs, 120 kV, and 4-mm section thickness. The PET images were recorded in the caudocranial axis on the identical transverse field of view for a duration of 3 min per bed, and corrections for attenuation were based on the initial CT images. All obtained images (PET, CT, corrected, and uncorrected) were assessed on maximum intensity projection images as well as transaxial, coronal, and sagittal cross-sectional images. Reconstructions were performed according to the EANM procedure guidelines for tumor imaging (version 2.015) (10).

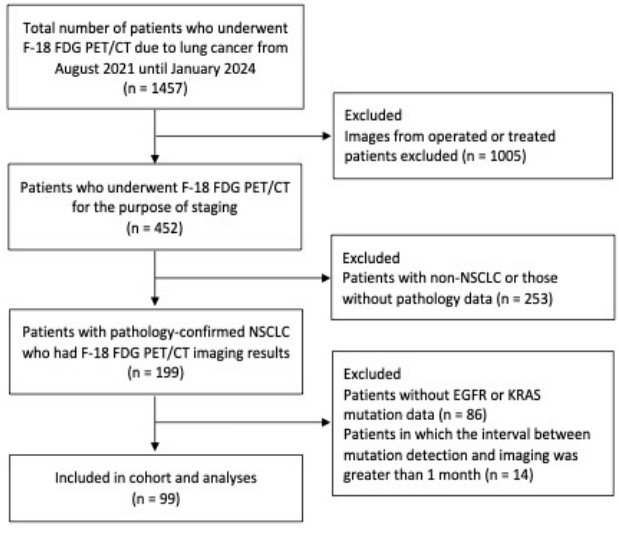

Figure 1. Flowchart of the study

Imaging Assessment

For the evaluation of images, a volume of interest (VOI) including all relevant tissue was delineated on attenuation-corrected ^{18}F-FDG PET/CT images of the primary tumor (automated contouring and manual correction) in the axial, sagittal, and coronal planes (Figure 2).

SUV_{max} was defined as the highest SUV measured from any voxel within the VOI. The mean SUV (SUV_{mean}) was defined as the SUV inside the VOI. The MTV was recorded with a threshold of 40% (of the SUV_{max}) within the VOI, and TLG was calculated by multiplying the MTV by the SUV_{mean}. To normalize FDG uptake, three spherical regions of interest (ROIs) with a diameter of 3 cm were placed in sites with homogenous FDG uptake inside the right lobe of the liver, and the mean value was calculated (liver-SUV_{mean}). The lung-to-liver ratio (SUV_{max}/liver-SUV_{mean}) was then calculated to generate normalized SUV data. All evaluations and calculations were performed by two nuclear medicine physicians who were blinded to the study data.

Pathologic Evaluation

Genomic DNA was extracted from formalin-fixed, paraffin-embedded non-small-cell lung carcinoma tissues, and EGFR-mt were detected using real-time PCR. Tissues were sectioned to 5 µm, deparaffinized, and then subjected to genomic DNA analysis using a DNA sample preparation kit according to the manufacturer's instructions. Following quantification of genomic DNA, real-time PCR was carried out to amplify the target area and detect the targeted mutations in exon 18 (G719A, G719C, and G719S), 19 (deletions and complex mutations), 20 (S768I, T790M, and insertions), and 21 (L858R and L861Q) using the EGFR mutation test (v2) on the Cobas® z480 analyzer, which were automatically analyzed and output by Cobas® 4800 software. For KRAS, we also used real-time PCR to detect targeted mutations. Additionally, we detected mutations in codons 12 and 13 of exon 2 and 61 of exon 3 in the KRAS gene using the KRAS mutation test on Cobas® z480 analyzer, which were again automatically analyzed and collected using the same software.

Statistical Analysis

All analyses were performed on IBM SPSS Statistics for Windows, Version 25.0 (IBM Corp., Armonk, NY, USA). For the normality check, the Kolmogorov-Smirnov test was used. Descriptive statistics were presented by using mean ± standard deviation for normally distributed continuous variables, median (25^{th} percentile-75^{th} percentile) for non-normally distributed continuous variables, and frequency (percentage) for categorical variables. Normally distributed variables were analyzed with the Student's t-test. Non-normally distributed variables were analyzed using the Mann-Whitney U test. Categorical variables were analyzed using the chi-square tests, the Fisher-Freeman-Halton test, or Fisher's exact test. Prediction performances were assessed using receiver operating characteristic (ROC) curve analysis. The optimal cut-off points were determined using the Youden index. Logistic regression analyses were performed to identify factors independently associated with EGFR and KRAS mutations. Variables were initially analyzed by univariate regression analysis, and those showing significance were included in the multivariate model. Detection of $p<0.05$ values was accepted to show statistically significant.

Results

We included 99 patients (77 males and 22 females) in our study; the mean age was 62.96±9.66 (range 37-87) years. Seventy-six (84.44%) patients had a smoking history. Most cases (71.72%) involved adenocarcinoma. Fifty-one (51.52%) patients were stage T4, 45 (45.45%) were stage N3, 54 (54.55%) had metastasis, and 54 (54.55%) were clinical stage IV. The right lung (63.64%) and upper lobe (53.54%) were the most common sites of primary lesions. The bone was the most common site of metastasis (31.31%). Ten (10.10%) patients underwent surgical treatment. EGFR and KRAS mutations (EGFR-mt and KRAS-mt) were detected in 23 (23.23%) and 34 (34.34%) patients, respectively (Table 1).

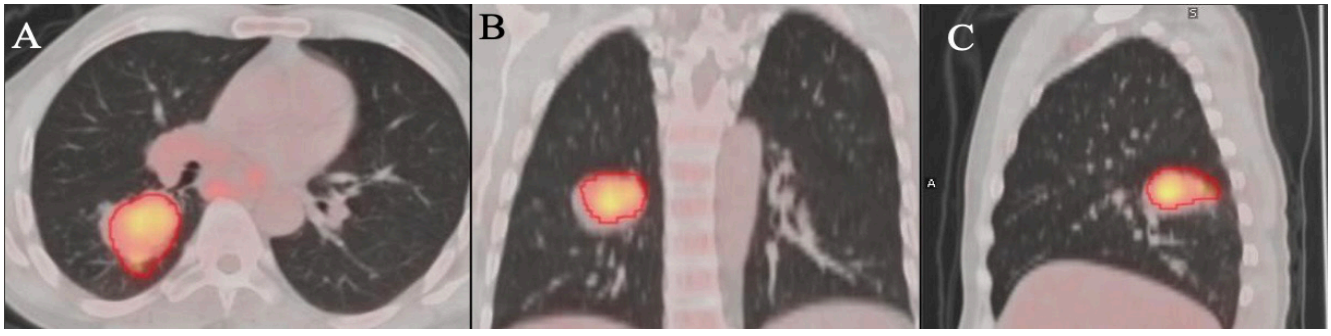

Figure 2. A VOI of the primary tumor in the axial (a), coronal (b), and sagittal (c) planes
VOI: Volume of interest

Table 1. Summary of variables	
Age	62.96±9.66
Sex	
Male	77 (77.78%)
Female	22 (22.22%)
Smoking	76 (84.44%)
Pack year	30 (20-48)
Histopathology	
Adenocarcinoma	71 (71.72%)
SCC	28 (28.28%)
T stage	
T1	5 (5.05%)
T2	25 (25.25%)
T3	18 (18.18%)
T4	51 (51.52%)
N stage	
N0	14 (14.14%)
N1	15 (15.15%)
N2	25 (25.25%)
N3	45 (45.45%)
M stage	
M0	45 (45.45%)
M1	54 (54.55%)
Clinical stage	
Stage I	4 (4.04%)
Stage II	5 (5.05%)
Stage III	36 (36.36%)
Stage IV	54 (54.55%)
Side	
Right	63 (63.64%)
Left	36 (36.36%)
Lobe	
Upper	53 (53.54%)
Middle	6 (6.06%)
Lower	40 (40.40%)
Metastasis location*	54 (54.55%)
Distant lymph node	16 (16.16%)
Contralateral lung	4 (4.04%)
Brain	19 (19.19%)
Liver	9 (9.09%)
Bone	31 (31.31%)
Adrenal gland	13 (13.13%)
Other	3 (3.03%)
Operation	10 (10.10%)
EGFR mutation	23 (23.23%)
Exon 18 G719X:	2 (2.02%)
Exon 19 deletion	17 (17.17%)
Exon 20 insertion	1 (1.01%)
Exon 21 L858R:	3 (3.03%)
KRAS mutation	34 (34.34%)
Codon 12	19 (19.19%)
Codon 13	1 (1.01%)
Codons 12 and 13	9 (9.09%)
Codon 61	5 (5.05%)
SUV_{mean}	6.4 (4.8-9.9)
SUV_{max}	11.4 (8.3-16.9)
MTV	36.0 (14.6-74.3)
TLG	281.88 (81.00-592.9)
Liver-SUV_{mean}	2.00±0.43
Normalized SUV	5.93 (4.29-8.10)

Descriptive statistics were presented by using mean ± standard deviation for normally distributed continuous variables, median (25^{th}-75^{th} percentiles) for non-normally distributed continuous variables, and frequency (percentage) for categorical variables. *Patients may have more than one of the following
SCC: Squamous cell carcinoma, MTV: Metabolic tumor volume, TLG: Total lesion glycolysis

Association between EGFR status, clinical features, and PET/CT results

Female frequency (p<0.001) was significantly higher in the EGFR-mt group than in the EGFR-wt group. Smoking frequency (p<0.001) and pack years (p=0.003) were significantly lower in the EGFR-mt group than in the EGFR-wt group. We found no significant differences between the EGFR mutation groups in terms of age, histopathology, T stage, N stage, M stage, clinical stage, side, lobe, and surgery percentages (Table 2).

MTV (p=0.015) and TLG (p=0.017) were significantly lower in the EGFR-mt group than in the EGFR-wt group. We found no significant differences between the EGFR mutation groups in terms of SUV_{mean}, SUV_{max}, liver-SUV_{mean}, and normalized SUV (Table 2).

When we evaluated the EGFR-mt prediction performance of the PET parameters, MTV had 60.87% sensitivity, 68.42% specificity, 66.67% accuracy, 36.84% positive predictive value (PPV), and 85.25% negative predictive value (NPV) for a cut-off value of 24.9 (equal or lower values represent the presence of EGFR mutation).

Table 2. Summary of variables associated with EGFR mutation			
	EGFR mutation		
	Negative (n=76)	**Positive (n=23)**	**p-value**
Age	62.83±9.33	63.39±10.91	0.808†
Sex			
Male	69 (90.79%)	8 (34.78%)	<0.001§
Female	7 (9.21%)	15 (65.22%)	
Smoking	65 (92.86%)	11 (55.00%)	<0.001§
Pack year	35 (20-50)	17.5 (0-30)	0.003‡
Histopathology			
Adenocarcinoma	52 (68.42%)	19 (82.61%)	0.289§
SCC	24 (31.58%)	4 (17.39%)	
T stage			
T1	2 (2.63%)	3 (13.04%)	0.080#
T2	17 (22.37%)	8 (34.78%)	
T3	16 (21.05%)	2 (8.70%)	
T4	41 (53.95%)	10 (43.48%)	
N stage			
N0	11 (14.47%)	3 (13.04%)	0.868#
N1	11 (14.47%)	4 (17.39%)	
N2	18 (23.68%)	7 (30.43%)	
N3	36 (47.37%)	9 (39.13%)	
M stage			
M0	36 (47.37%)	9 (39.13%)	0.648§
M1	40 (52.63%)	14 (60.87%)	
Clinical stage			
Stage I	2 (2.63%)	2 (8.70%)	0.305#
Stage II	5 (6.58%)	0 (0.00%)	
Stage III	29 (38.16%)	7 (30.43%)	
Stage IV	40 (52.63%)	14 (60.87%)	
Side			
Right	48 (63.16%)	15 (65.22%)	1.000§
Left	28 (36.84%)	8 (34.78%)	
Lobe			
Upper	41 (53.95%)	12 (52.17%)	0.928#
Middle	5 (6.58%)	1 (4.35%)	
Lower	30 (39.47%)	10 (43.48%)	
Operation	7 (9.21%)	3 (13.04%)	0.694¶
SUV_{mean}	6.7 (4.9-10.0)	5.8 (4.4-8.6)	0.272‡
SUV_{max}	11.75 (8.35-17.15)	9.8 (7.3-14.3)	0.208‡
MTV	42.6 (19.1-83.1)	18.1 (8.7-43.6)	0.015‡
TLG	355.77 (86.82-677)	138.06 (33.11-384.56)	0.017‡
Liver-SUV_{mean}	1.97±0.43	2.09±0.44	0.239†
Normalized SUV	6.32 (4.51-8.32)	4.62 (4.19-6.93)	0.063‡

Descriptive statistics were presented by using mean ± standard deviation for normally distributed continuous variables, median (25^{th}-75^{th} percentile) for non-normally distributed continuous variables, and frequency (percentage) for categorical variables.
†Student's t-test, ‡Mann-Whitney U test, chi-square test, #Fisher-Freeman-Halton test, Fisher's exact test

The area under the ROC curve (AUC) was 0.667 [95% confidence interval (CI): 0.547-0.788, p=0.015]. TLG had 86.96% sensitivity, 43.42% specificity, 53.54% accuracy, 31.75% PPV, and 91.67% NPV for a cut-off value of 408.1 (equal or lower values represent the presence of EGFR mutation). The AUC was 0.664 (95% CI: 0.540-0.788, p=0.017) (Figure 3).

The SUV_{mean}, SUV_{max}, and normalized SUV were non-significant in distinguishing patients with or without EGFR-mt (Table 3).

According to the multivariable logistic regression analysis, female sex was the only factor independently associated with EGFR-mt (OR: 12.882, 95% CI: 2.922-56.796, p=0.001) (Table 4).

The baseline PET/CT images of a patient with EGFR-mt infection are presented in Figure 4, and those of a patient with EGFR-wt infection are presented in Figure 5.

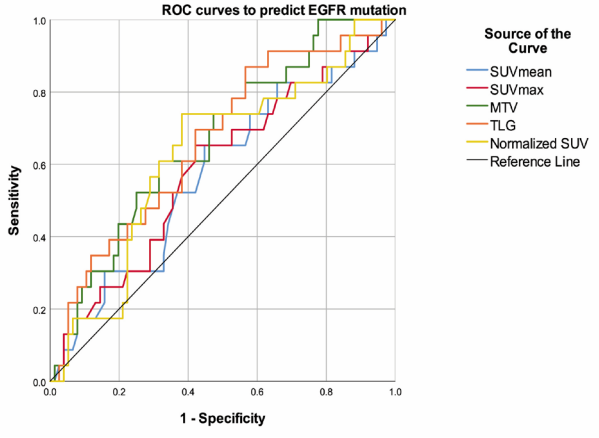

Figure 3. ROC curves of PET findings for EGFR mutation prediction

ROC: Receiver operating characteristic, PET: Positron emission tomography, EGFR: Epidermal growth factor receptor

Table 3. Performance of PET in predicting EGFR mutation, ROC curve analysis

	Cut-off	Sensitivity	Specificity	Accuracy	PPV	NPV	AUC (95% CI)	p-value
SUV_{mean}	**≤6.3**	**65.22%**	**55.26%**	**57.58%**	**30.61%**	**84.00%**	**0.576 (0.441-0.710)**	**0.272**
SUV_{max}	≤10.9	65.22%	57.89%	59.60%	31.91%	84.62%	0.587 (0.453-0.720)	0.208
MTV	≤24.9	60.87%	68.42%	66.67%	36.84%	85.25%	0.667 (0.547-0.788)	0.015
TLG	≤408.1	86.96%	43.42%	53.54%	31.75%	91.67%	0.664 (0.540-0.788)	0.017
Normalized SUV	≤5.59	73.91%	61.84%	64.65%	36.96%	88.68%	0.628 (0.499-0.758)	0.063

PPV: Positive predictive value, NPV: Negative predictive value, AUC: Area under the curve, ROC: Receiver operating characteristic, CI: Confidence interval, PET: Positron emission tomography, EGFR: Epidermal growth factor receptor

Table 4. Odds ratios for the EGFR mutation and logistic regression analysis

	Univariable		Multivariable	
	OR (95% CI)	**p-value**	**OR (95% CI)**	**p-value**
Age	1.006 (0.958-1.056)	0.806		
Sex, Female	18.482 (5.806-58.833)	<0.001	12.882 (2.92256.796)	0.001
Smoking	0.094 (0.027-0.334)	<0.001	0.427 (0.078-2.339)	0.327
Histopathology, adenocarcinoma	2.192 (0.672-7.147)	0.193		
T stage	0.641 (0.401-1.025)	0.063		
N stage	0.933 (0.609-1.427)	0.748		
M stage	1.400 (0.541-3.623)	0.488		
Clinical stage	1.048 (0.565-1.943)	0.883		
Side, left	0.914 (0.344-2.428)	0.857		
Lobe, lower	1.179 (0.459-3.032)	0.732		
Operation	1.479 (0.350-6.248)	0.595		
SUV_{mean}, ≤6.3	2.316 (0.878-6.109)	0.090		
SUV_{max}, ≤10.9	2.578 (0.976-6.811)	0.056		
MTV, ≤24.9	3.370 (1.281-8.865)	0.014	1.503 (0.336-6.717)	0.594
TLG, ≤408.1	5.116 (1.401-18.689)	0.014	1.157 (0.182-7.381)	0.877
Normalized SUV, ≤5.59	4.592 (1.624-12.984)	0.004	1.320 (0.284-6.143)	0.724

OR: Odds ratio, CI: Confidence interval, EGFR: Epidermal growth factor receptor

Association between KRAS status, clinical features, and PET/CT results

Adenocarcinoma (p=0.001) percentage was significantly higher in the KRAS-mt group than in the KRAS-wt group. We found no significant differences between the KRAS mutation groups in terms of age, sex, smoking status, pack years, T stage, N stage, M stage, clinical stage, side, lobe, or surgical treatment. In addition, there were no significant differences between the KRAS mutation groups in terms of SUV_{mean}, SUV_{max}, MTV, TLV, liver-SUV_{mean}, and normalized SUV (Table 5).

Logistic regression analysis revealed that adenocarcinoma was the only factor associated with the presence of KRAS-mt (OR: 10.667, 95% CI: 2.351-48.396, p=0.002).

Discussion

In light of the advances and diversity in the treatment of NSCLC, such as TKIs, it is crucial to identify favorable mutations in the early stages of the disease. Although genetic analysis is undoubtedly the best approach, the use of molecular profiling may be limited due to various factors, including difficulty in obtaining sufficient tumor tissue, unavailability of genetic analyses, and the invasiveness of the procedure (4,6). The current study reported that it may be possible to obtain information regarding the presence/absence of relevant mutations by evaluating metabolic parameters obtained from ^{18}F-FDG PET/CT imaging performed at baseline. MTV and TLG were found to have statistical significance in distinguishing patients with and without EGFR-mt, but not KRAS-mt. Although the overall accuracy values were not excellent for distinguishing the presence of EGFR mutations, TLG had exceptional sensitivity and NPV, indicating notable utility in detecting and ruling out the presence of favorable EGFR mutations among patients with NSCLC.

In our study, similar to the literature, female sex, low smoking percentage, as well as PET parameters such as MTV and TLG obtained from the primary lesion, were significantly lower in patients with EGFR-mt compared with those without (7,11,12). Additionally, the MTV and TLG values obtained from the initial imaging of the primary lesions were significantly lower in the EGFR-mt group.

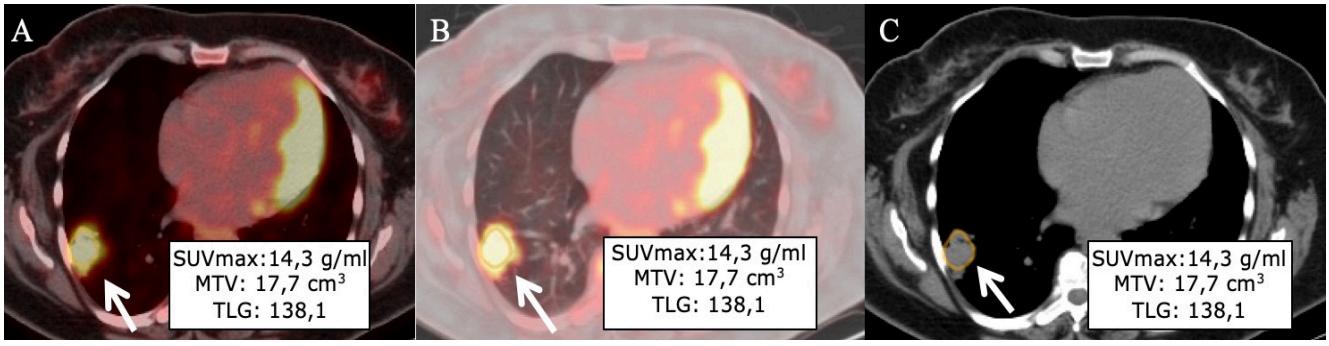

Figure 4. Pretreatment ^{18}F-FDG PET/CT images of a patient with NSCLC and EGFR mutation, demonstration of SUVmax, MTV, and TLG values derived from the primary lung tumor (arrow) on axial fused images (a,b) and axial CT images (c)

^{18}F-FDG PET/CT: Fluorine-18-fluorodeoxyglucose positron emission tomography-computed tomography, NSCLC: Non-small-cell lung cancer, EGFR: Epidermal growth factor receptor, MTV: Metabolic tumor volume, TLG: Total lesion glycolysis, CT: Computed tomography

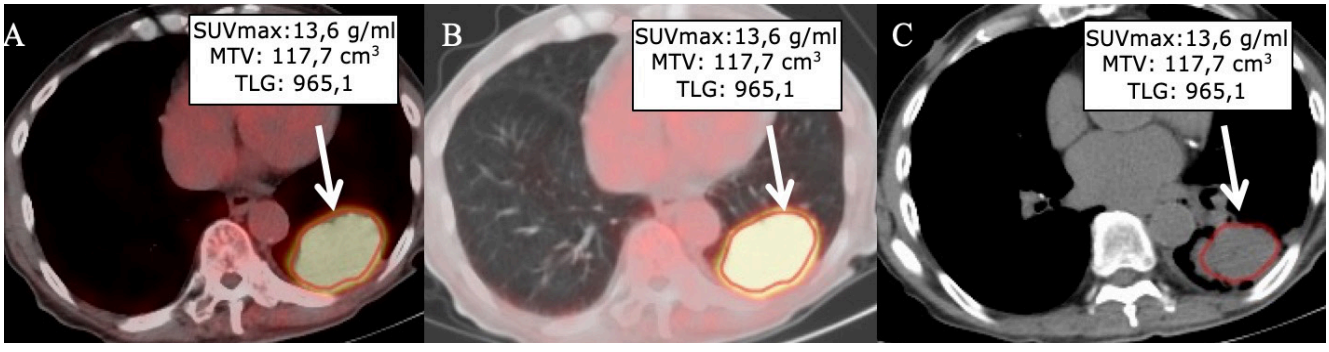

Figure 5. Pretreatment ^{18}F-FDG PET/CT images of a patient with NSCLC without EGFR mutation, demonstration of the SUVmax, MTV, and TLG values derived from the primary lung tumor (arrow) on the axial fused images (a,b) and axial CT images (c)

^{18}F-FDG PET/CT: Fluorine-18-fluorodeoxyglucose positron emission tomography-computed tomography, NSCLC: Non-small-cell lung cancer, EGFR: Epidermal growth factor receptor, MTV: Metabolic tumor volume, TLG: Total lesion glycolysis, CT: Computed tomography

Table 5. Summary of variables associated with KRAS mutation			
	KRAS mutation		
	Negative (n=65)	**Positive (n=34)**	**p-value**
Age	62.92±9.59	63.03±9.95	0.959†
Sex			
Male	47 (72.31%)	30 (88.24%)	0.120§
Female	18 (27.69%)	4 (11.76%)	
Smoking	47 (79.66%)	29 (93.55%)	0.126¶
Pack year	30 (15-45)	35 (20-50)	0.341‡
Histopathology			
Adenocarcinoma	39 (60.00%)	32 (94.12%)	0.001§
SCC	26 (40.00%)	2 (5.88%)	
T stage			
T1	3 (4.62%)	2 (5.88%)	1.000#
T2	17 (26.15%)	8 (23.53%)	
T3	12 (18.46%)	6 (17.65%)	
T4	33 (50.77%)	18 (52.94%)	
N stage			
N0	9 (13.85%)	5 (14.71%)	0.685§
N1	11 (16.92%)	4 (11.76%)	
N2	18 (27.69%)	7 (20.59%)	
N3	27 (41.54%)	18 (52.94%)	
M stage			
M0	27 (41.54%)	18 (52.94%)	0.385§
M1	38 (58.46%)	16 (47.06%)	
Clinical stage			
Stage I	2 (3.08%)	2 (5.88%)	0.664#
Stage II	3 (4.62%)	2 (5.88%)	
Stage III	22 (33.85%)	14 (41.18%)	
Stage IV	38 (58.46%)	16 (47.06%)	
Side			
Right	38 (58.46%)	25 (73.53%)	0.208§
Left	27 (41.54%)	9 (26.47%)	
Lobe			
Upper	34 (52.31%)	19 (55.88%)	0.221#
Middle	6 (9.23%)	0 (0.00%)	
Lower	25 (38.46%)	15 (44.12%)	
Operation	5 (7.69%)	5 (14.71%)	0.305¶
SUV_{mean}	6.3 (4.7-10.6)	6.5 (5.2-9.3)	0.897‡
SUV_{max}	11.0 (8.1-17.7)	11.45 (8.4-16.9)	0.909‡
MTV	33.0 (17.7-71.9)	41.4 (12.4-83.0)	0.912‡
TLG	281.88 (84.68-534.60)	261.60 (74.80-642.60)	0.848‡
Liver-SUV_{mean}	2.00±0.39	1.99±0.51	0.834†
Normalized SUV	5.59 (4.19-9.13)	6.32 (4.62-7.52)	0.757‡

Descriptive statistics were presented by using mean ± standard deviation for normally distributed continuous variables, median (25^{th}-75^{th} percentiles) for non-normally distributed continuous variables, and frequency (percentage) for categorical variables.
†Student's t-test, ‡Mann-Whitney U test, : chi-square test, #Fisher-Freeman-Halton test, : Fisher's exact test, KRAS: Kirsten rat sarcoma viral oncogene homolog gene, SCC: Squamous cell carcinoma, MTV: Metabolic tumor volume, TLG: Total lesion glycolysis

This relationship is likely due to the effect of EGFR on glucose transporters in the cell membrane via downstream pathways that may affect tumor glucose metabolism. In previous studies, different results were presented when examining the role of SUV_{max} in predicting EGFR mutation in patients with NSCLC.

Most studies have reported higher frequencies of EGFR mutations in patients with lower SUV values (12,13). However, a study consisting of Asian patients with advanced lung adenocarcinoma reported that patients with higher SUV_{max} values were more likely to carry EGFR-mt (14). Wang et al. (15) also reported significantly higher SUV_{max} values in patients with NSCLC with EGFR mutations compared with those without mutations, which they attributed to increased glucose uptake. These varying results suggest that SUV_{max} values in the present study may be affected by a multitude of factors, such as patient characteristics and imaging technique. As such, we believe that incorporating parameters like MTV and TLG could be beneficial for the evaluation and management of this patient group. Additionally, ethnic differences in patient groups and other uncontrolled genetic mutations in the EGFR-wt group may also cause these differences. Liu et al. (16), supporting this perspective and similar to our study, found that MTV values were lower in the EGFR-mt. However, the authors did not find a correlation between SUV_{max} and mutation status, thereby providing credibility to the perceived impact of similar underlying reasons. The authors suggested that this may be due to the semiquantitative nature of SUV data, which might vary depending on the PET scanner, fasting time, plasma glucose level, and selected ROIs (16).

In contrast to our findings, Minamimoto et al. (8) found that SUV_{max} values of primary lesions were predictive of EGFR-mt, whereas MTV and TLG were not, suggesting that gene mutations were unassociated with tumor size or volume. However, most of the patients included in their study (70.2%) had clinical stages of IA or IB, whereas 91% of the patients in our study had stage III and IV. The difference in the severity of patients is an important factor that could explain the contrasting results (8).

Our study demonstrated no correlation between ^{18}F-FDG uptake and KRAS-mt status, which is consistent with several other studies previously reported in the literature (8,17). Interestingly, Caicedo et al. (18), in their study enrolling patients with stage III and IV NSCLC, reported that SUV values were significantly higher in the KRAS-mt group than in the KRAS-wt group. Further studies with larger cohorts are required to clarify the associations between PET parameters and KRAS-mt states.

Since there are many variables affecting SUV_{max} values, such as body size and amount of tracer injected, standardization of SUV_{max} by assessing liver SUV data, as well as other approaches, is known to reduce variability. It has been reported to improve prognosis prediction and treatment response assessment in NSCLC (19). Mak et al. (20) reported that normalized SUV_{max} values of the primary tumor (normalized for SUV of blood in the pulmonary artery) were predictive of EGFR mutation. In our study, similar to the SUV values, there was no significant difference between the mutation groups when we used normalized SUV values. Since the utility of this approach has not been validated in this particular patient group, more studies are needed to determine whether normalized SUV values differ from other metabolic parameters in predicting genetic mutations in NSCLC.

In our study, based on the exceptionally high NPV of TLG in predicting EGFR-mt (91.6% for a cut-off point of 408.1), it may be feasible to suggest that early ^{18}F-FDG PET/CT imaging data can be used to primarily predict the absence of EGFR mutations, thereby facilitating the noninvasive identification of patients that will not respond to TKI in the early stages of the disease. With this approach, treatment options suitable for the appropriate patient group can be determined, and unnecessary treatments can be prevented when genetic analyses are unavailable or cannot be performed. Prospective studies with a larger number of patients are needed to confirm this finding.

Study Limitations

One of the limitations of our study was its retrospective design, which included a relatively small number of patients. Second, we could not report the prognostic value of ^{18}F-FDG PET/CT because the prognosis of the patients was not yet determined. Despite these limitations, our study has identified a non-invasive ^{18}F-FDG PET/CT parameter that can predict patients with NSCLC who are unlikely to respond to treatment.

Conclusion

Our study revealed that it is possible to noninvasively predict the presence/absence of EGFR mutations in the early period of the disease using the MTV and TLG values extracted from baseline ^{18}F-FDG PET/CT imaging. Since the sensitivity and NPV of TLG were exceptionally high, it may be possible to rule out the presence of EGFR mutations using this approach. It is evident that these results are relevant in cases in which genetic detection is not feasible and could prevent unnecessary treatments by identifying patients without EGFR-mt who do not demonstrate favorable response to TKIs.

Ethics

Ethics Committee Approval: This study was approved by the Ethics Committee of University of Health Sciences Turkey, Basaksehir Cam and Sakura City Hospital (date: July 2023, approval no.: 2023.07.296).

Informed Consent: Informed consent was obtained from all participants included in the study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: O.V.T., N.B., Concept: O.V.T., Design: O.V.T., Data Collection or Processing: O.V.T., N.B., Analysis or Interpretation: O.V.T., Literature Search: O.V.T., Writing: O.V.T., N.B.

Conflict of Interest: No conflicts of interest were declared by the authors.

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Original Article

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Modulation of Apoptosis and ER Stress Markers in Hepatocellular Carcinoma Cells by Irinotecan, Hesperidin, and Piperine

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Abstract

Aim: Hepatocellular carcinoma (HCC) remains a leading cause of cancer-related mortality, and there is a pressing need to explore novel therapeutic strategies that enhance the efficacy of existing treatments. This study aims to investigate the effects of irinotecan (IRT), hesperidin (HSP), and piperine (PIP) on HCC (HepG2), focusing on their modulation of apoptosis-related genes and endoplasmic reticulum (ER) stress markers.

Methods: This study is an in vitro experimental study. IC50 values for IRT, HSP, and PIP were determined using MTT cell viability assays. Researchers performed total RNA extraction and quantitative PCR to assess mRNA levels of *Bad, Bax, and p53* (apoptosis-related genes) and ATF4, CHOP, and GRP78 (ER stress markers).

Results: Irinotecan significantly upregulated the expression of *Bad, Bax, and p53* genes, as well as ER stress markers such as ATF4, CHOP, and GRP78. Hesperidin-enhanced apoptotic gene expression and exacerbated ER stress. Piperine attenuated IRT-induced apoptosis and suppressed ER stress markers.

Conclusion: Combining IRT with HSP enhanced apoptosis and ER stress in HepG2 cells, suggesting synergistic potential against HCC. Conversely, IRT combined with PIP reduced apoptotic response and ER stress markers, possibly compromising IRT's efficacy. These findings highlight complex interactions between chemotherapeutic agents and natural compounds, warranting further exploration in combination therapies.

Keywords: Irinotecan, hesperidin, piperine, carcinoma, hepatocellular, apoptosis, endoplasmic reticulum

Introduction

Hepatocellular carcinoma (HCC) is the sixth most common cancer type globally and the third leading cause of cancer-related deaths, with an average of more than 740,000 new diagnoses per year (1). HCC, originating from liver cells, poses a substantial global health burden primarily linked to chronic viral hepatitis infections, alcohol-related liver diseases, and non-alcoholic fatty liver disease (NAFLD/NASH). Molecular studies highlight chromosomal instability, genetic mutations, and epigenetic changes as pivotal in HCC pathogenesis, influencing oncogene activation and tumor suppressor gene inactivation. Cellular processes such as enhanced proliferation, impaired apoptosis, increased angiogenesis, and metastatic potential further underscore the complexity of HCC progression (2). Immune responses within the tumor microenvironment play a critical role in HCC development and treatment outcomes, paving the way for innovative

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therapies like immunotherapy (3). Understanding these multifaceted aspects is crucial for advancing effective management strategies against HCC.

Irinotecan (IRT) is one of the selective topoisomerase 1 (Topo1) inhibitors, including camptothecin, topotecan, idarubicin, daunorubicin, doxorubicin, and etoposide. Topo1 is an enzyme that alleviates torsional strain in DNA by inducing temporary single-strand breaks. Irinotecan, a Topo1 inhibitor, prevents the religation of these breaks, leading to DNA damage and ultimately inducing apoptosis in cancer cells. This mechanism underscores the therapeutic efficacy of IRT in cancer treatment, particularly in targeting rapidly proliferating cells. Although IRT was one of the most important drugs in the treatment of colon cancer for approximately 15 years between 1994 and 2008, its medical use continues today (4). Irinotecan causes human HCC cells to undergo apoptosis by activating p53 with its active metabolite. Irinotecan induces apoptosis in cancer cells by altering gene expression. Key genes involved in this process include p53, BAX/BCL-2, caspases, and NF-κB. The effects of IRT on gene expression promote cell death and inhibit tumor growth.

Flavonoids can be categorized into six primary classes based on their structure: flavan-3-ols, flavones, flavonols, flavanones, isoflavones, and anthocyanins (5). Among these subclasses, hesperidin (HSP) is identified as a flavanone compound. Hesperidin and its derivatives are characteristic compounds found in citrus fruits belonging to the Rutaceae family, including orange (Citrus sinensis), grapefruit (Citrus paradisi), tangerine (Citrus reticulata), lime (Citrus aurantifolia), and lemon (Citrus limon) (6). There have been many recent studies showing that HSP has anticancer activity (7). There are many studies showing that piperine (PIP), an alkaloid isolated from the piper nigrum plant, has an anticancer and enhancing effect of existing chemotherapeutics (8).

Hesperidin, a flavonoid, has been associated with various health advantages and has shown potential effects in regulating the expression of pro-apoptotic genes such as Bad, Bax, and p53. Piperine, an alkaloid found in spices, is recognized for its anti-cancer effects, with research focusing on its ability to modulate the expression of apoptosis-related genes like Bad, Bax, and p53 (9). These studies provide foundational insights into understanding the cellular effects of HSP and PIP and evaluating their potential therapeutic applications.

In recent years, the combination of chemotherapeutic agents with natural compounds has gained attention as a promising approach to enhance the efficacy of cancer treatments. However, the combination of IRT with natural compounds like HSP and PIP has not been extensively investigated. This study aims to address this research gap by evaluating the effects of IRT in combination with HSP and PIP in hepatocellular carcinoma cells, providing new insights into potential therapeutic strategies for improving cancer treatment outcomes.

Methods

Compliance with Ethical Standards

This study was conducted using commercially obtained HepG2 cell lines. No patient samples or primary cell cultures were used. Therefore, ethical approval was not required.

Chemicals and Reagents

Irinotecan and HSP were purchased from Cayman Chemical Company (Michigan, USA, Cat No: 14180); PIP was purchased from Sigma-Aldrich (Darmstadt, Germany, Cat No.: P49007) (Michigan, USA, Cat No: 18646). HCC cell line (HepG2) was purchased from American Type Culture Collection (HB-8065, Manassas, VA, USA). All primers were synthesized by Bioligo Biotechnology (Ankara, Turkey).

Cell Culture

The HepG2 cell line was cultured in Dulbecco's Modified Eagle's Medium containing 10% fetal bovine serum, 5% L-Glutamine, and 1% penicillin-streptomycin. Cells were maintained at 37 °C in a humidified incubator with 5% CO_2.

Determination of IC50 doses with the MTT Cell Viability Test

Cells were seeded at a density of 10^4 cells per well in 96-well plates. Cells were treated with different concentrations of IRT (1 µM, 10 µM, 25 µM, 50 µM, 100 µM, 250 µM, 500 µM, and 1000 µM) and different concentrations of HSP and PIP (1 µM, 10 µM, 50 µM, 100 µM, 200 µM, 400 µM, 800 µM, and 1000 µM). Then, 10 µL of MTT solution (5 mg/mL, SERVA, Heidelberg, Germany) was added to each well and incubated for 4 hours at 37 °C with 5% CO_2. After the incubation period, 100 µL of dimethyl sulfoxide (DMSO) was added to each well, and the absorbance of each well was measured at 570 nm using an automatic multiplate reader (Epoch, Biotek, USA). IC50 values were calculated using GraphPad Prism version 8.0.1 (GraphPad Software, Inc., CA, USA) (10). Each analysis was performed in triplicate.

Total RNA Extraction, Reverse Transcription, and Quantitative Polymerase Chain Reaction

After completion of the 48-hour incubation, cells were harvested and washed with cold PBS. For mRNA expression level, total RNA was isolated with the GeneJET RNA Purification Kit (Thermo Scientific Catalog No.: K0731). The quantity and purity of isolated RNAs were analyzed using the Epoch Take3 plate instrument (Agilent, USA). Then, complementary DNA (cDNA) synthesis was

performed according to the manufacturer's instructions (Biorad Cat No: BR1708891). Each 20 µL PCR reaction consisted of 1 µL of cDNA, 10 µL of 2X SYBR Green PCR Master Mix (prepared as 1X according to the manufacturer's guidelines), and specific primers, with a final concentration of 100 ng per reaction. 1 µg total RNA was used as a template in the PCR performed with reverse transcriptase. Then, 1 µL of cDNA was taken from each sample and the appropriate amount of SYBR Green PCR Master Mix was added according to the forward and reverse primer protocols. All stages were carried out under cold chain and sterile conditions. Expression levels of target genes were normalized using the housekeeping gene β-Actin. Gene expression values were then calculated with the REST2009 program by applying the ΔΔCt method using the equation RQ=2−ΔΔCt, according to (11). Primer sequences used in PCR reactions and PCR conditions are described in Table 1. Each assay was performed in quadruplicate.

Statistical Analysis

The data obtained within the scope of the study were analyzed using GraphPad Prism version 8.0.1 (GraphPad Software, Inc., CA, USA). The data was analyzed to determine whether it showed a normal distribution using the Kolmogorov-Smirnov test. Since the data showed normal distribution, one-way ANOVA test, one of the parametric tests, was used for the comparison of three or more groups. P<0.05 was considered the level of statistical significance.

Results

IC50 doses of bioactive compounds

It was found that IC50 values of IRT were 56.02 µM, HSP was 150.7 µM and PIP was 51.14 µM for 48 hours of treatment (Figure 1).

Findings on mRNA expression levels of genes that play a role in apoptotic processes

Compared to the control group, mRNA expression levels of the *Bad* gene increased 3.231-fold (p=0.002) in the IRT group, 8.351-fold (p=0.008) in the HSP group, and 3.044-fold (p=0.005) in the PIP group. The mRNA expression levels of *Bad* gene were also increased 5.227 times (p=0.004) in the IRT+HSP combination group and

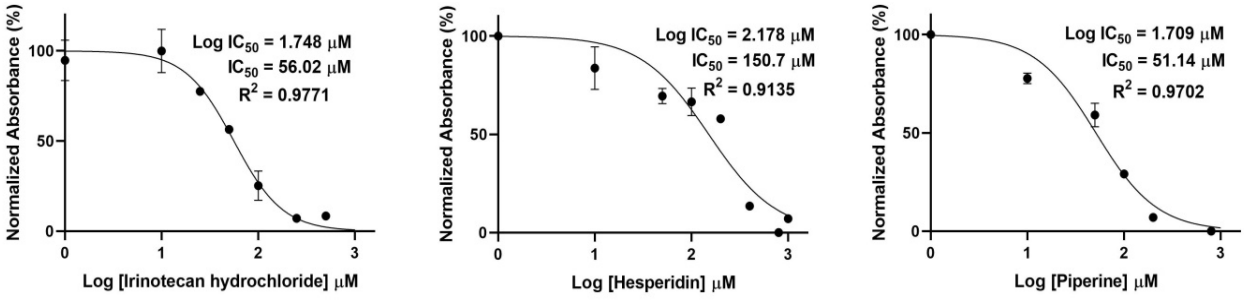

Figure 1. IC50 doses of IRT, HSP and PIP
IRT: Irinotecan, HSP: Hesperidin, PIP: Piperine

Table 1. Oligonucleotide primer sequences and PCR programs

Genes	Primer sequences (5' → 3')	RT-PCR Programs	Cycle
β-Actin	F-5' CTCCATCCTGGCCTCGCTGT 3' R-5' GCTGTCACCTTCACCGTTCC 3'	95˚C-30s/60˚C-1m/72˚C-30s	35
Bax	F-5' CATGAAGACAGGGGCCCTTT 3' R-5' AAACACAGTCCAAGGCAGCT 3'	95˚C-30s/59˚C-1m/72˚C-30s	35
Caspase3	F-5' GTGCTACAATGCCCCTGGAT 3' R-5' GCTGGATGCCGTCTAGAGTC 3'	95˚C-30s/59˚C-1m/72˚C-30s	35
p53	F-5' CCTCTCCCCAGCCAAAGAAG 3' R-5' GAAGTGGGCCCCTACCTAGA 3'	95˚C-30s/59˚C-1m/72˚C-30s	35
ATF4	F- 5' GGGACAGATTGGATGTTGGAGA 3' R- 5' ACCCAACAGGGCATCCAAGT 3'	95˚C-30s/57˚C-1m/72˚C-30s	35
CHOP	F- 5' TGCTTCTCTGGCTTGGCTGAC 3' R- 5' CCAAGGGAGAACCAGGAAACGG 3'	95˚C-30s/60˚C-1m/72˚C-30s	35
GRP78	F- 5' GGTGACCTGGTACTGCTTGATG 3' R-5' CCTTGGAATCAGTTTGGTCATG 3'	95˚C-30s/57˚C-1m/72˚C-30s	35

ATF4: Activating transcription factor 4, GRP78: Glucose-regulated protein 78, CHOP: C/EBP homologous protein

1.84 times (p=0.019) in the IRT+PIP group, in comparison with the control. *Bax* gene mRNA levels were upregulated by IRT (0.492-fold, p<0.001), PIP (0.443-fold, p<0.001), and IRT+HSP (0.472-fold, p<0.003), while in the HSP group, a 0.227-fold change (p<0.006) was found to be similar to the control group (p<0.86). Compared to the control group, *p53* gene mRNA expression levels increased 1.576-fold (p=0.007) in the IRT group, 3.195-fold (p=0.007) in the HSP group, 1.428-fold (p=0.039) in the PIP group, and 2.097-fold (p=0.005) in the IRT + HSP group. The +PIP group was found to be similar to the control group (p=0.113) (Table 2, Figure 2).

Findings on mRNA expression levels of genes playing a role in ER stress

The mRNA expression levels of the *ATF4* gene showed a 1.058-fold increase (p=0.413) in the IRT group, 1.202-fold (p=0.062) in the HSP group, 1.088-fold (p=0.213) in the PIP group, and 0.963-fold (p=0.707) in the IRT+HSP group compared to controls. Conversely, *ATF4* gene expression was downregulated by 0.563-fold (p=0.006) in the IRT+PIP group compared to controls. For the *CHOP* gene, mRNA levels were significantly increased to 4.42-fold (p=0.001) in the IRT group, 4.55-fold (p=0.004) in the HSP group, 2.263-fold (p=0.014) in the PIP group, and 2.535-fold (p=0.001) in the IRT+HSP group compared to controls. No significant difference was found in the IRT + PIP group compared to controls (p=0.123). In contrast, *GRP78* gene expression increased by 1.257-fold (p=0.01) in the IRT group and decreased by 0.6-fold (p=0.009) in the IRT+PIP group, with no significant changes observed in the HSP (p=0.774) and IRT+HSP (p=0.955) groups compared to controls (Table 2, Figure 3).

Discussion

The existence of IRT resistance is known, especially in the treatment of colon cancer (12,13) and HCC (14), negatively affecting treatment strategies. Therefore, there is a need for research that will enhance the treatment efficacy of IRT and make the cells more chemosensitive during treatment.

Irinotecan application alone increased CHOP and GRP78 mRNA expression levels, which are ER stress markers (p=0.001 and p=0.01, respectively), compared to the control group, but had no significant effect on ATF4 (p=0.062). In addition, while IRT application alone

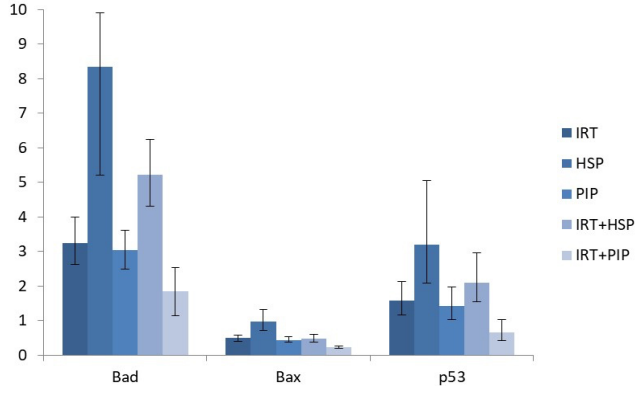

Figure 2. mRNA expression levels of apoptosis-related genes
IRT: Irinotecan, HSP: Hesperidin, PIP: Piperine

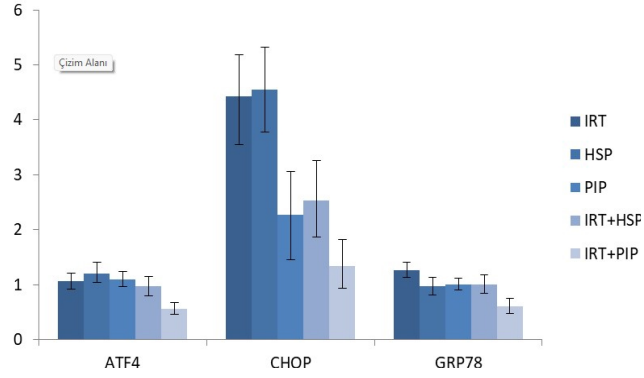

Figure 3. mRNA expression levels of ER stress-related genes
IRT: Irinotecan, HSP: Hesperidin, PIP: Piperine

Table 2. Comparision of mRNA expression levels of genes

Gene expression levels						
Groups	**Apoptosis related genes**			**ER stress related genes**		
	Bad	**Bax**	**P53**	**ATF4**	**CHOP**	**GRP78**
IRT	3,231**	0.492***	1,576**	1,058	4,42**	1,257*
HSP	8,351**	0.978	3,195**	1,202	4,55**	0.975
PIP	3,044**	0.443***	1,428*	1,088	2,263*	0.999
IRT+HSP	5,227**	0.472**	2,097**	0.963	2,535***	0.993
IRT+PIP	1.84*	0.227**	0.658	0.563**	1,334	0.6**

mRNA expression levels are given as fold increase/decrease. *p<0.05, **p≤0.01 and ***p≤0.001. Fold increases/decreases and statistical analyses were performed using REST 2009 (Qiagen) software. IRT: Irinotecan, HSP: Hesperidin, PIP: Piperine, Bad: Bcl-2-associated death promoter, Bax: Bcl-2-associated X protein, ATF4: Activating transcription factor 4, CHOP: C/EBP homologous protein, GRP78: Glucose-regulated protein 78

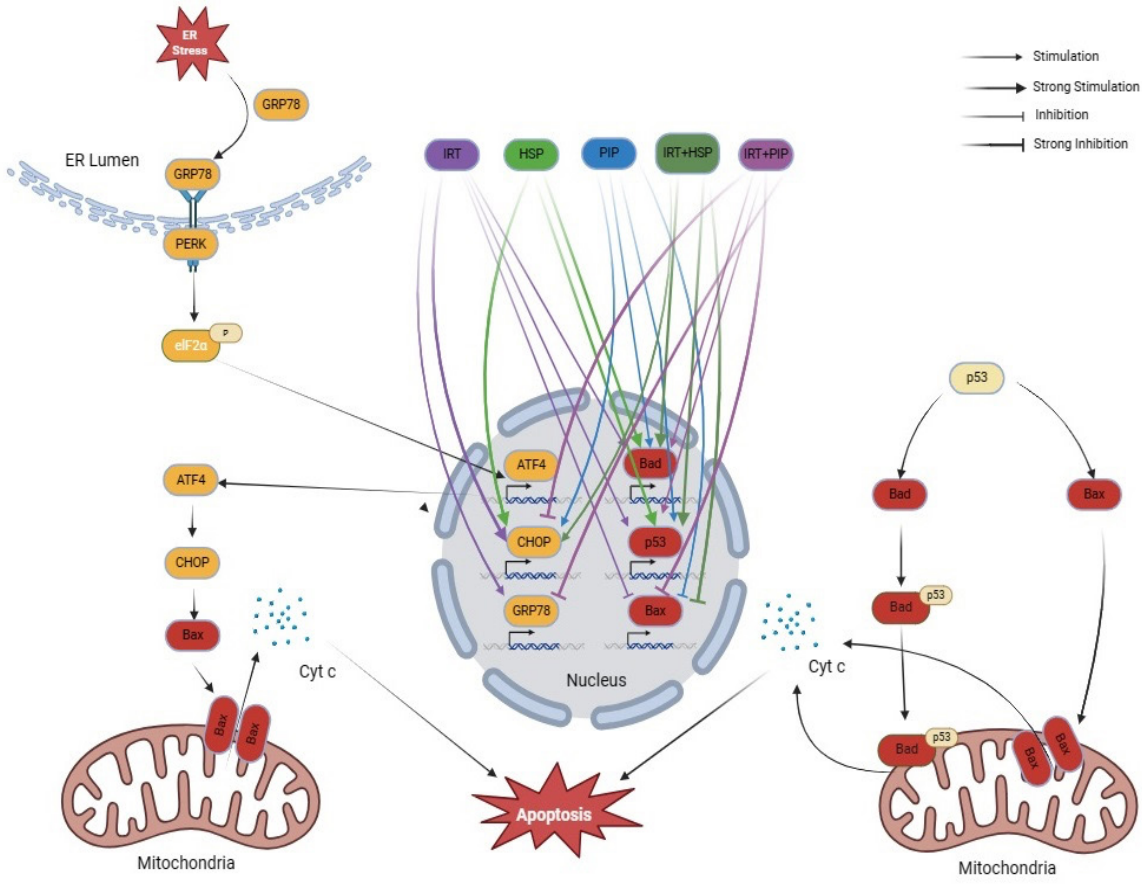

Figure 4. Effects of IRT, HSP and PIP on ER stress-mediated and p53-mediated apoptosis. It was created using Biorender
IRT: Irinotecan, HSP: Hesperidin, PIP: Piperine

increased CHOP mRNA expression compared to the control group (p=0.001), as a result of the combination of IRT with PIP, CHOP mRNA expression level decreased to the level of the control group (p=0.123) (Figure 4). The combination of IRT with PIP resulted in the suppression of ATF4 and GRP78 mRNA expression levels, which are ER stress markers, in HCC compared to the control group (p=0.006 and p=0.009, respectively), and the CHOP mRNA expression level decreased to the level observed in the control group (p=0.123). There are data in the literature that contradict our findings (15). Although we showed in our study that PIP does not promote IRT on genes responsible for ER stress, the literature also shows that the use of PIP increases chemosensitivity to 5-fluorouracil in resistant colon cancer (16). Piperine is a potent inhibitor of permeability glycoprotein (P-glycoprotein 1) and cytochrome P450. P-glycoprotein 1 is also known as multidrug resistance protein 1 (MDR1) or CD243 and is an important protein that pumps many foreign substances out of the cell that may be harmful and is inhibited by PIP. Microsomal cytochrome P450 overproduction causes endoplasmic reticulum stress (17). In this regard, the observation that the ER stress markers were reduced in the groups where PIP, a cytochrome P450 inhibitor, was applied, compared to the control group, was found to be compatible with the literature.

It has been shown that HSP induces CHOP protein expression in ovarian cancer cells (18), and it inhibits the proliferation of HeLa cells through ER stress-mediated apoptosis (19). In this regard, although the mRNA expression levels of ATF4 and GRP78, which are ER stress markers, did not differ significantly from the control group in the HSP group (p=0.062 and p=0.774, respectively), the CHOP mRNA expression level was found to be higher than the control group. This result was found to be compatible with literature data (p=0.004).

Shrivastava et al. (20) suggested that PIP has anti-apoptotic properties, and we confirmed this finding in

our study. While IRT alone increased the mRNA expression levels of the *Bad* and *p53* genes (p=0.002 and p=0.007, respectively), HSP enhanced this effect more than IRT alone (p=0.008 and p=0.007, respectively). In the IRT+HSP group, both Bad and p53 mRNA expression levels were higher than in the IRT group (p=0.004 and p=0.005, respectively). Piperine, on the other hand, either eliminated or reduced the effect of IRT alone on apoptotic markers. Bax mRNA expression levels were lower than those of the control group in all groups except the HSP group (Figure 4). Although no anti-cancer effects were observed with the combined use of PIP and IRT in HCC in our study, some studies in the literature have reported anti-cancer effects when PIP is used alone. For example, it has been shown to induce apoptosis in oral cancer (21,22), gastric cancer (23), and breast cancer (24). Similarly, our study found that PIP increased apoptotic markers when used alone but reduced the apoptotic effects of IRT when used in combination. A literature review revealed no studies addressing the combination of IRT with PIP.

Study Limitations

This study has some limitations. Firstly, it focuses solely on mRNA expression levels without examining protein levels and activities, which limits the understanding of the functional effects of gene products on cellular processes. The cell culture conditions may not fully replicate the in vivo environment, and the specific characteristics of the cell line used may limit the generalizability of the results. Due to financial constraints, the scope of the study was kept narrow. Despite these methodological difficulties, this study is a preliminary study that can be cited in subsequent studies.

Conclusion

The findings of our study indicate significant differences between the use of IRT alone and its combination with HSP or PIP. The combination of IRT with HSP enhanced the expression of apoptosis-related genes, such as Bad, Bax, and p53, thereby supporting IRT's pro-apoptotic effects in HCC. In contrast, the combination of IRT with PIP showed a reduced effect on ER stress markers, including ATF4, CHOP, and GRP78, compared to IRT alone. This suggests that PIP may inhibit the anti-tumor activity of IRT by suppressing the expression of genes responsible for ER stress. These findings highlight the potential of HSP to augment IRT's therapeutic efficacy, while PIP appears to mitigate its anti-tumor effects.

Ethics

Ethics Committee Approval: This study was conducted using commercially obtained HepG2 cell lines. No patient samples or primary cell cultures were used. Therefore, ethical approval was not required.

Informed Consent: No patient samples or primary cell cultures were used.

Authorship Contributions

Concept: S.S., K.K.K., C.Y., S.C., Design: S.S., K.K.K., C.Y., S.C., Data Collection or Processing: S.S., K.K.K., C.Y., S.C., Analysis or Interpretation: S.S., K.K.K., C.Y., S.C., Literature Search: S.S., K.K.K., C.Y., S.C., Writing: S.S., K.K.K., C.Y., S.C.

Conflict of Interest: No conflicts of interest were declared by the authors.

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Original Article

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The Relationship of Sirtuin 1 and Sirtuin 2 Expression with Clinicopathological Parameters in Non-Small-Cell Lung Carcinomas

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Abstract

Aim: Recent studies have shown that sirtuin signaling pathway activation could be a potential therapeutic target for future therapies and biomarkers for predicting prognosis in cancer. We aimed to investigate the association between Sirtuin 1 (SIRT1) and Sirtuin 2 (SIRT2) immunohistochemical (IHC) protein expression in tumors and clinicopathological parameters and survival profiles in non-small cell lung cancer (NSCLC) patients.

Methods: This retrospective observational study reviewed lung resections (lobectomy, segmentectomy, wedge resection, and pneumonectomy) of 186 patients diagnosed with NSCLC (adenocarcinoma, squamous cell carcinoma, and adenosquamous carcinoma). SIRT1 and SIRT2 expression was classified as high and low expression based on the extent and intensity of IHC staining.

Results: When there was advanced disease, a large tumor, and metastasis to lymph nodes, low levels of SIRT1 and SIRT2 expression were found. A positive correlation exists between high SIRT2 expression and longer overall survival. If SIRT1 and SIRT2 were both overexpressed (+/+), longer survival times were observed than in the group in which either of them was low (+/-, -/+, -/-) (p=0.01).

Conclusion: Our research suggests that SIRT1 and SIRT2 may suppress tumor growth in NSCLC and serve as positive prognostic indicators.

Keywords: Lung, non-small-cell carcinoma, Sirtuin 1, Sirtuin 2, prognosis, immunohistochemistry

Introduction

Non-small cell lung carcinoma (NSCLC), which mainly includes squamous cell carcinoma (SCC) and adenocarcinoma (ADC), accounts for approximately 80% of all lung cancer cases (1). Sirtuins (SIRTs) are a class of proteins with nicotinamide adenine dinucleotide (NAD+)-dependent protein deacetylase and/or ADP ribosyltransferase activities (2). SIRTs are involved in the metabolism, genomic stability, cell cycle, cell division, transcriptional editing, and pathogenesis of various diseases, such as metabolic diseases (2,3). SIRTs function as either tumor promoters or suppressors, participating in various processes, such as autophagy, apoptosis, and energy metabolism (2,3). Recent studies have shown that sirtuin signaling pathway activation could be a potential therapeutic target for future therapies and biomarkers for predicting prognosis in cancer.

We hypothesized that the expression of Sirtuin 1 (SIRT1) and Sirtuin 2 (SIRT2) in NSCLC would be associated with other prognostic parameters and survival. We aimed to examine the relationship between SIRT1 and SIRT2 expression levels, clinicopathological parameters, and survival in patients diagnosed with NSCLC. It is our contention that data on SIRT1 and SIRT2 expression

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in NSCLC may be beneficial for both prognostic and therapeutic purposes.

Materials and Methods

Compliance with Ethical Standards

University of Health Sciences Turkey, Kartal Dr. Lutfi Kirdar City Hospital Clinical Research Ethics Committee approval was obtained with the decision dated 22.07.2020 and numbered 2020/514/182/10, and it was supported by the Health Sciences University Scientific Research Projects Unit with the project code number 2020/097. All procedures conformed to the principles of the Declaration of Helsinki (as revised in 2013).

Data Collection

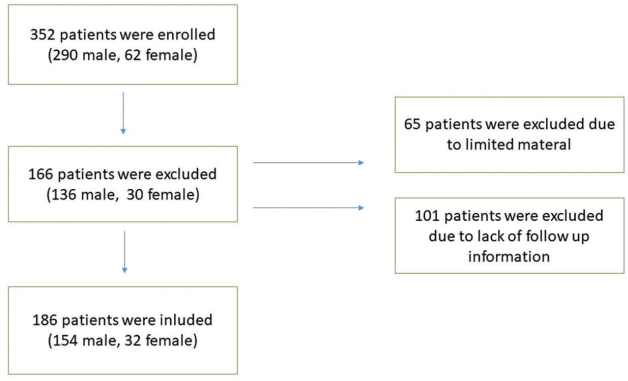
Lung resection (lobectomy, segmentectomy, wedge resection, and pneumonectomy) materials from 186 patients diagnosed with NSCLC (ADC, SCC, adenosquamous carcinoma) diagnosed in our pathology department between 2014 and 2018 were included in the study.

The clinicopathological parameters of the cases were extracted using the hospital automation system, and the clinical staging of the patients was calculated using the 8th Edition TNM classification of the "America Joint Committee on Cancer/Union for International Cancer Control (AJCC/UICC)" in NSCLC (4). The age, tumor size, location, histological subtype, number of tumor foci, bronchial involvement, pleural, lymphovascular, and perineural invasion, TNM stages, metastasis/relapse status, and survival data of the patients were recorded. SIRT1 and SIRT2 expressions were investigated immunohistochemically (IHC) and classified as low (low) and high (high) expression.

The inclusion criteria for the study were diagnosis of NSCLC and the presence of sufficient tumor tissue for IHC analysis of SIRT1 and SIRT2 expression. Cases with only needle biopsies and cases with limited material were excluded from the study. In addition, if paraffin blocks belonging to the patient in the pathology archive were absent, if there was a second primary tumor, and/or if the patient's follow-up information required for survival analysis could not be obtained, the case was excluded (Graph 1).

IHC Staining and Assessment

Hematoxylin & Eosin-stained sections of the cases, formalin-fixed and paraffin-embedded blocks, were removed from the archive and examined. A paraffin block that mostly consisted of tumor tissue and normal tissue was selected for each case.



Graph 1. Flow chart of patient selection

IHC staining was performed using "Ventana Medical System-Benchmark Ultra/ISH Staining". The following procedures were performed using the Ultraview Universal DAB Detection Kit: Sections from paraffin blocks were taken on 4-m-thick, positively charged slides. The mixture was kept in an oven at 70 °C for 1 h. The slides were transferred to a Benchmark Ventana Ultra IHC device. Antigen recovery was performed with ethylene diamine tetraacetic acid at pH 8 (CC1). Antibody incubation: SIRT1 (B-7, mouse monoclonal antibody, Santa Cruz Biotechnology, sc-74465, 1/300) and SIRT2 (A-5, mouse monoclonal antibody, Santa Cruz Biotechnology, sc-28298) with a primary antibody duration of 2 hours, 1/100) were applied. Harris Hematoxylin (Ventana Medical Systems) was applied for 16 min for background staining, and bluing reagent (bluing solution) (Ventana Medical Systems) was applied for 4 min. The slides were washed with detergent water, rinsed in absolute alcohol twice, dried, and covered with a xylol-based sealer. Internal and external controls were selected based on the SIRT1 and SIRT2 staining scores of tissues from the Human Protein Atlas (http://www.proteinatlas.org/) (5,6).

Staining scores and SIRT1 and SIRT2 expression levels based on the extent and intensity of staining were calculated using a semiquantitative scoring method, as reported in previous studies (2,7,8). The percentage of positive cells was considered as the extent of staining (0=0, 1=1-25%, 2=26-50%, 3=51-75%, 4=76-100%). The staining intensity was evaluated by comparing it with that of a known external positive control (0, negative; 1, weak; 2, moderate; 3, strong; Figures 1-4). The final IHC score was calculated by multiplying these two values. Based on this score, SIRT1 and SIRT2 expression were classified as high (score >3) and low expression (score ≤3). The cut-off value for the score was obtained by averaging the high and low expressions.

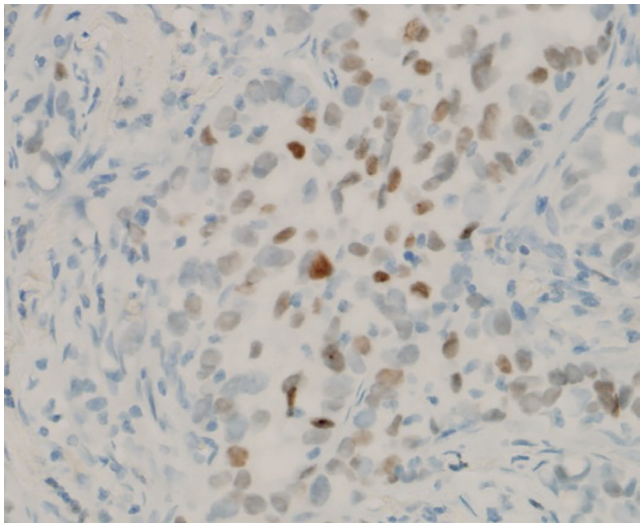


Figure 1. Adenocarcinoma; SIRT1 IHC X400, strong nuclear staining
IHC: Immunohistochemical, SIRT1: Sirtuin 1

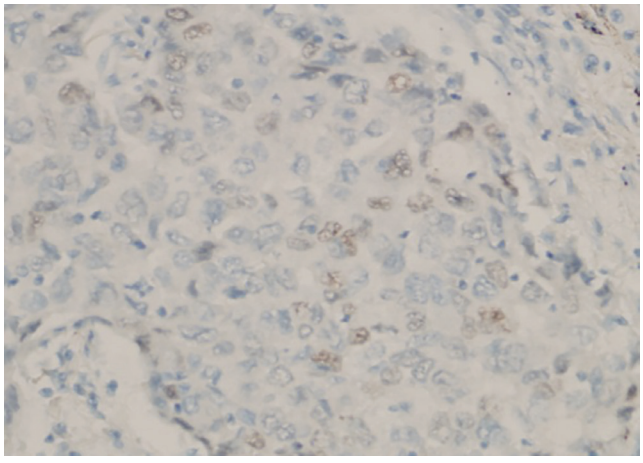


Figure 2. Adenocarcinoma, SIRT1 IHC X400
Adenocarcinoma, SIRT1 IHC X400, moderate staining

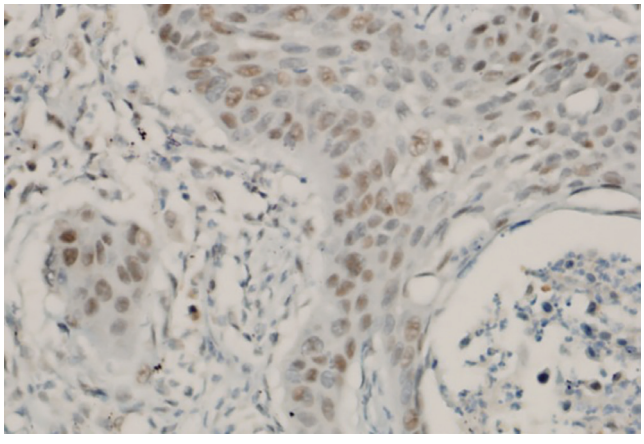


Figure 3. Squamous cell carcinoma, SIRT2 IHC X-400, showing strong staining

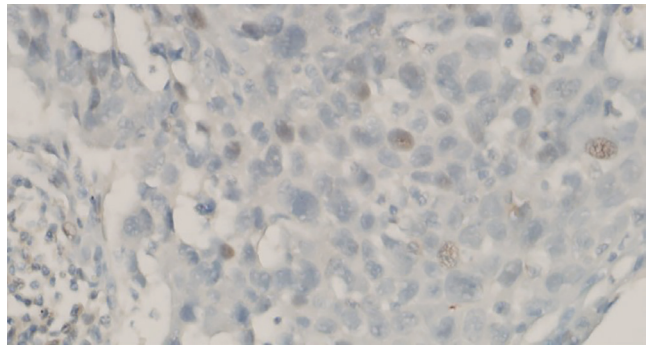


Figure 4. Squamous cell carcinoma, SIRT2 IHC X400
Squamous cell carcinoma, SIRT2 IHC X400, moderate staining

Statistical Analysis

In the data analysis, identifier statistics were presented as frequencies, percentages, averages, and standard deviations. In this study, chi-square (X^2) analysis was used for proportional comparisons of SIRT 1-2 scores according to the characteristics of patients and the Fisher test was performed in the corrections. The independent sample t-test was performed to compare SIRT 1-2 scores with patients' measurements. An independent sample variant analysis was used to examine the difference in patient measurements of SIRT 1-2 compliance ratios. The Sidak test was performed to determine if groups detected differently in the variance analysis. X^2 analysis was applied for proportional comparisons of SIRT 1-2 compliance rates according to the characteristics of the patients, and the Fisher's exact test was performed in the corrections. Progression-free survival and overall survival analyses were performed using the Kaplan-Meier method, and the effects of clinical and pathological features on survival were compared with the log-rank method. In this study, p-values less than 0.05 were considered statistically significant (α=0.05). The SPSS 22.0 package program was used for the statistical analysis.

Results

In the study, 154 (82.8%) of the 186 patients were male and 32 (17.2%) were female. The ages varied between 46 and 80 (average 67.89±8.30). Eighty-seven (46.8%) ADCs, 89 (47.8%) SCCs, and 10 (5.4%) adenosquamous carcinoma cases were included. Metastases were detected in 31.7% of the patients in subsequent clinical follow-ups, of which 24 (40.6%) were in the lung and 13 (22.03%) in the brain. Other rare sites of involvement included the mediastinal lymph nodes, liver, adrenal glands, pleura, and pancreas. The median overall survival was 70 months, with a median disease-free survival of 44 months. Table 1 presents the demographic and clinicopathological features of the patients.

Table 1. Clinicopathological parameters of patients with non-small-cell lung carcinoma			
Parameters		**Number**	**%**
Gender	Male	154	82.8%
	Female	32	17.2%
Pathological diagnosis	Adenocarcinoma	87	46.8%
	Adenosquamous carcinoma	10	5.4%
	Squamous cell carcinoma	89	47.8%
Histologic subtypes of adenocarcinoma	Acinar Solid Lepidic Papillary Micropapillary Could not be evaluated due to artifact detection	44 25 10 5 2 1	23.7% 13.4% 5.4% 2.7% 1.1% 0.05%
Histologic subtypes of squamous cell carcinoma	Keratinized Nonkeratinized Bazaloid	44 42 3	49.43% 47.19% 3.37%
Histologic subtypes of adenosquamous carcinoma	Moderately Mild	7 3	70% 30%
Location	Right upper lobe	58	31.20%
	Left upper lobe	50	26.90%
	Right lower lobe	31	16.70%
	Left lower lobe	20	10.80%
	Left lung	9	4.80%
	Right lung	7	3.80%
	Right middle lobe	6	3.20%
	Lower lobe	1	0.50%
	Rigt lobe	1	0.50%
	Left lower and upper lobe	2	1.1%
	Upper lobe	1	0.50%
Type of surgery	Bilobectomy		3.2%
	Lobectomy	148	79.6%
	Pneumonectomy	28	15.1%
	Segmentectomy	2	1.1%
	Wedge resection	2	1.1%
Vascular invasion	Absent	114	61.3%
	Present	72	38.7%
Perineural invasion	Absent	143	76.9%
	Present	43	23.1%
Bronchial involvement	Absent	133	71.5%
	Present	53	28.5%
Pleural invasion	Absent	141	75.8%
	Present	45	24.2%
Metastasis/Recurrence	Absent	127	68.3%
	Present	59	31.7%
T	T1A	43	23.1%
	T1B	32	17.2%
	T2A	62	33.3%
	T2B	22	11.8%
	T3	27	14.5%

Table 1. Continued			
Parameters		**Number**	**%**
N	N0	131	70.4%
	N1	34	18.3%
	N2	21	11.3%
Stage	1A	60	32.3%
	1B	35	18.8%
	2A	42	22.6%
	2B	26	14.0%
	3A	21	11.3%
	3B	2	1.1%
Survival	Dead Alive	79 107	42.5% 57.5%

Correlations of SIRT1 and SIRT2 with Clinicopathological Parameters

Low levels of SIRT1 and SIRT2 were present in 81.7% (n=152) and 76.9% (n=143) of patients, respectively, while high levels were observed in 18.3% (n=34) and 23.1% (n=43), respectively (Figures 1-4). No significant differences were observed between SIRT1 and SIRT2 expression and histological types of vascular, perinural, and pleural invasion. As presented in Table 2, patients with bronchial involvement had lower SIRT1 and SIRT2 expression than those without bronchial involvement (p=0.03, p=0.04). In the metastasizing/recurring patient group, the SIRT1 and SIRT2 scores also remained lower compared with the non-metastasized group (p = 0.01 and p=0.02, respectively).

The study demonstrated notable variations in the levels of SIRT1 scores across the various N groups; N2 patients exhibited a higher proportion of low SIRT1 expression scores (p=0.04). Conversely, no significant difference was observed in SIRT2 scores across the N levels (p=0.19).

Compared with the cases in the other stages, patients in stage 1A showed higher SIRT1 expression (p=0.01), whereas patients in stages 1A and 1B showed higher SIRT2 expression (p=0.01). Regarding the patients who received neoadjuvant treatment, statistically more patients had lower SIRT1 and SIRT2 scores than those who did not receive neoadjuvant treatment (p=0.04, p=0.04).

The tumor diameters of patients with low SIRT1 and SIRT2 expression were found to be larger, with a statistically significant difference for only SIRT1 (p=0.04).

Regarding overall survival, although a numerically longer survival was found in those with a high SIRT1 score (median 55 vs. 51 months), this difference did not reach statistical significance (p=0.706). When the relationship between SIRT2 score and survival was examined, lower survival times were found in cases with low expression (median 62 versus 47 months), and this difference was statistically significant (p=0.000). Regarding the overall survival times according to histological subtypes, high SIRT2 expression was significantly associated with high survival in the ADC and SCC groups (p=0.025, 0.019, respectively) (Table 3). However, the disease-free survival period showed no significant correlation between SIRT1 and SIRT2 scores (p=0.277).

Regarding age, patients showing high levels of expression for SIRT1/SIRT2 (+/+) were older than those with low expression (-/+, +/-, -/-) (p=0.01). Compared with the second group, the tumor sizes were smaller (p=0.03) and life expectancies were longer (p=0.01) in the older group. Additionally, bronchial invasion rates and metastasis/recurrence states were lower in the "+/+" group than in the other groups (p=0.04, p=0.03). In addition, the "+/+" group had higher T1B levels than the other T levels.

Discussion

The sirtuin family of lysine deacetylase comprises seven members in humans and has been subject to evolutionary conservation. Its role in regulating a multitude of physiological and pathological processes, including aging, cancer, inflammation, and metabolism, is of significant importance (9-13). Sirtuins are involved in a number of significant biological processes, including aging, stress response, vitality, differentiation, metabolism, apoptosis, and cell survival, due to their catalytic activities. However, the mechanisms of action of sirtuin remain incompletely understood because of their dual roles as both tumor promoters and suppressors in different tumors (2,9-14).

SIRT1 and its Effect on NSCLC

A review of the literature revealed conflicting findings regarding the existence of differences in the histologic

Table 2. Correlations of SIRT1 and SIRT2 with clinicopathological parameters

Parameters		SIRT1 EXPRESSION				SIRT2 EXPRESSION				P (SIRT1)	P (SIRT2)
		High		Low		High		Low			
		Number	%	Number	%	Number	%	Number	%		
Gender	Male	31	20.1%	123	79.9%	35	22.7%	119	77.3%	0.01*	0.32
	Female	3	9.4%	29	90.6%	8	25.0%	24	75.0%		
Bronchial involvement	Absent	29	21.8%	104	78.2%	35	26.3%	98	73.7%	0.03*	0.04*
	Present	5	9.4%	48	90.6%	8	15.1%	45	84.9%		
Metastasis/ Recurrence	Absent	27	21.3%	100	78.7%	33	26.0%	94	74.0%	0.01*	0.02*
	Present	7	11.9%	52	88.1%	10	16.9%	49	83.1%		
Neoadjuvant therapy	Absent	33	19.1%	140	80.9%	41	23.7%	132	76.3%	0.04*	0.04*
	Present	1	8.3%	11	91.7%	1	8.3%	11	91.7%		
N	N0	26	19.8%	105		32		99		0.04*	0.19
	N1	7	20.6%	27		7		27			
	N2	1	4.8%	20		4		17			
Stage	1A	18	30.0%	42	70.0%	18	30.0%	42	70.0%	0.01*	0.01*
	1B	3	8.6%	32	91.4%	9	25.7%	26	74.3%		
	2A	6	14.3%	36	85.7%	7	16.7%	35	83.3%		
	2B	4	15.4%	22	84.6%	5	19.2%	21	80.8%		
	3A	1	4.8%	20	95.2%	4	19.0%	17	81.0%		
	3B	2	100.0%	0	0.0%	0	0.0%	2	100.0%		
Tumor diameter (cm)		3.05±2.1		3.77±2.14		3.34±1.96		3.73±2.2		0.04*	0.09

*Significant correlation at the $p<0.05$. X^2: Chi-square analysis was used for proportional comparisons of SIRT 1-2 scores according to the characteristics of patients, and the Fisher's exact test was performed in the corrections.

Table 3. The relationship between SIRT 1 and SIRT2 and overall survival according to pathological diagnosis

Pathological diagnosis	SIRT1 EXPRESSION		SIRT2 EXPRESSION		P (SIRT1)	P (SIRT2)
	High	Low	High	Low		
	mOS (95% CI) (month)	mOS (95% CI) (month)	mOS (95% CI) (month)	mOS (95% CI) (month)		
Adenocarcinoma	55 (46-64)	46 (42-50)	61 (54-68)	45 (39-51)	0.706	0.025*
Adenosquamous carcinoma	65 (-/-)	39 (15-63)	65 (23-107)	35 (14-56)	0.919	0.322
Squamous cell carcinoma	51 (38-64)	57 (50-64)	65 (53-77)	53 (46-60)	0.339	0.019*

*mOS: median overall survival, CI: confidence interval
Overall survival was analyzed using the Kaplan-Meier method, and the effects of clinical and pathological features on survival were compared using the log-rank method

types of NSCLC. Some studies have reported higher SIRT1 expression in ADCs than in SHCs (15,16). Nevertheless, some studies have not identified a significant correlation between sirtuin expression levels and histologic types (16,17). In our series, although no significant difference in SIRT1 expression levels was observed between the ADC and SHC groups, it is noteworthy that SIRT1 expression was higher in the ADC group.

Noh et al. (17) and Gharabaghi (18) reported a correlation between elevated SIRT1 expression and increased tumor size, lymph node metastasis, and tumor stage. Gong et al. (15) reported that high SIRT1 expression is associated with low T stages. In our study, SIRT1 expression was lower in patients with a large tumor size, advanced tumor stage, and N2 lymph node metastasis. The presence of bronchial invasion, which is an important prognostic marker, and SIRT1 expression status were also evaluated. The results demonstrated that SIRT1 expression was lower in patients with bronchial invasion than in those without it. These findings support the hypothesis that SIRT1 acts as a potential tumor suppressor in NSCLC.

In a study by Gong et al. (15), the relationship between SIRT1 and distant metastasis was investigated, and it was found that patients with metastasis exhibited

high SIRT1 expression. In our study, low SIRT1 expression levels were observed in patients with metastasis, whereas high expression was identified as a favorable prognostic parameter.

In studies examining the correlation between sirtuin expression levels and survival, Lee et al. (19) observed a decline in survival rates as SIRT1 expression levels increased. A meta-analysis by Chen et al. (20) included seven eligible studies from diverse geographical locations, encompassing a larger patient cohort. In four studies, a negative correlation was observed between SIRT1 expression and survival (20). Nevertheless, three studies reported no correlation between SIRT1 expression and survival (20). In our study, although subjects with higher SIRT1 expression levels had a numerically longer survival, this difference did not reach statistical significance (p=0.706).

SIRT2 and its Effect on NSCLC

In the study by Gao et al. (21) investigating the protein expression and prognostic value of SIRT2 in patients with nonmetastatic NSCLC, no association was found between SIRT2 and clinicopathologic parameters, such as TNM stage and histologic type. However, survival time was found to be decreased in patients with high SIRT2 expression levels (21). In our study, high SIRT2 expression was observed in early-TNM stage tumors as well as in cases without bronchial invasion or metastasis. Our findings support the hypothesis that SIRT2 acts as a tumor suppressor.

In our study, the overall survival rate was significantly lower in patients with lower SIRT2 expression levels (median 62 months versus 47 months, p=0.000). Li et al. (22) observed significantly lower SIRT2 and higher Skp2 levels in NSCLC samples compared with normal tissue. In this study, a similar result was observed to that of our series, in which patients with NSCLC and low SIRT2 levels exhibited significantly shorter overall survival (22).

Upon examination of the correlation between SIRT2 expression and survival rates across histologic subtypes, patients with high SIRT2 expression exhibited prolonged survival times. The observed difference was statistically significant in the ADC and SHC groups (p<0.05), but not in the adenosquamous carcinoma patient groups (p=0.322).

A comprehensive meta-analysis was conducted by Gong et al. (15) to investigate the clinical significance and potential molecular mechanisms of all sirtuins in lung cancer. The study revealed that patients exhibiting elevated levels of SIRT1 and/or SIRT2 demonstrated markedly diminished disease-free and overall survival compared with patients with diminished levels of at least one sirtuin. In this study, we created SIRT1 and SIRT2 combinations and examined their concordance. We found that cases with high expression for both markers had smaller tumor sizes, lower T stages, and lower progression/recurrence rates compared with other groups. Furthermore, survival times were higher in the SIRT1+/SIRT2+ group than in the other groups. These findings were statistically significant (p=0.01). A review of the literature revealed a paucity of studies in which SIRT1 and SIRT2 protein levels were evaluated in combination and used as measures of disease-free survival. In this regard, our study makes a contribution to the existing literature and provides an illustration of the distinction in the mechanism of action of sirtuin. Our findings are consistent with those of previous studies, indicating that the combined use of both is a more significant prognostic marker. However, in contrast to the literature, we observed that cases with high expression exhibited a superior prognosis.

Study Limitations

It should be noted, however, that the analysis was not without limitations. First, the planned analyses in the ADC and SCC subgroups could not be performed. Second, patients lacking sufficient clinical knowledge and follow-up were excluded from the study, resulting in a reduction in the sample size. The absence of data on treatment modalities and the prevalence of comorbidities also diminished the statistical power of our study. In addition, the present study employed only IHC and used retrospectively available tissue samples obtained from paraffin blocks. Use of multiple methods, such as gene expression measured via qPCR or RT-PCR, protein content measured via Western blotting, blood enzyme levels measured via ELISA, and fluorometric or sirtuin activity assays (23) enhance the reliability of the results. Despite the aforementioned limitations, this study represents one of the few investigations in which SIRT1 and SIRT2 are evaluated collectively, with the objective of identifying a correlation between the clinical values of patients with NSCLC and this approach, which lends a distinctive value to our work.

Conclusion

Our study provides evidence supporting the hypothesis that high SIRT1 and SIRT2 levels may act as tumor suppressors in NSCLC. It can be used as a positive prognostic marker in the context of therapeutic planning for NSCLC. Further research is required to confirm these findings. Well-designed prospective studies with larger cohorts and more comprehensive clinical data are necessary to advance this field of study. The application of supplementary techniques to reinforce and corroborate the IHC approach may be advantageous for the assessment of expression levels.

Ethics

Ethics Committee Approval: Local ethics committee approval was obtained with the decision dated 22.07.2020 and numbered 2020/514/182/10, and it was supported by the University of Health Sciences Turkey, Scientific Research Projects Unit with the project code number 2020/097.

Informed Consent: This is a retrospective observational study.

Footnotes

Authorship Contributions

Concept: F.C.K., N.B.O., S.S., C.C.B., Design: F.C.K., N.B.O., S.S., C.C.B., Data Collection or Processing: F.C.K., N.B.O., Analysis or Interpretation: F.C.K., N.B.O., S.S., C.C.B., Literature Search: F.C.K., S.S., Writing: F.C.K., N.B.O., S.S., C.C.B.

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Original Article

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Frequency of Fibromyalgia in Patients with Chronic Hepatitis C Virus Infection and Its Relationship with Vitamin D Levels and Quality of Life

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Abstract

Aim: The literature on the effects of the fibromyalgia syndrome (FMS) association with hepatitis C virus (HCV) on quality of life and vitamin D levels is limited. We aimed to evaluate the frequency of fibromyalgia in patients with chronic hepatitis C virus (HCV) and also to analyze the relationship between the presence of fibromyalgia and vitamin D parameters.

Methods: This controlled, cross-sectional study of 123 participants (72 patients with HCV and 51 in the control group) was conducted in a tertiary hospital. The study enrolled age- gender and BMI-matched control groups. Patients were diagnosed with fibromyalgia and arthritis as per the 2010 American College of Rheumatology diagnostic criteria. Fibromyalgia Impact Questionnaire (FIQ), Nottingham Health Profile (NHP) and the level of vitamin D were performed in all patients.

Results: The presence of fibromyalgia, the level of vitamin D and the score of FIQ were statistically different between HCV (+) and control groups. The score of social isolation was higher in the HCV (+) group, whereas all other scores were similar in both groups according to the NHP scale. Based on the presence or absence of fibromyalgia, all scores of FIQ and NHP scales were higher in a subgroup with fibromyalgia. This study documented a prevalence of 56.9% of FMS in patients with HCV (+) in comparison to the control group

Conclusion: This study documented a high prevalence of 56.9% of FMS in patients with HCV (+) in comparison to the control group. While emphasizing raising awareness of fibromyalgia, its relationship with HCV, vitamin D, quality of life, age, gender, BMI, and tender points was revealed via regression analysis.

Keywords: Fibromyalgia, hepatitis C virus, vitamin D, quality of life

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Introduction

Fibromyalgia syndrome (FMS) is characterized by diffuse musculoskeletal pain, fatigue, and sleep disorders that negatively affect quality of life (1). Chronic hepatitis C virus infection (HCV) is one of society's most common viral infections. HCV plays a role in the pathogenesis of autoimmune, rheumatological, and musculoskeletal diseases (2). Chronic viral infections trigger FMS symptoms through inflammatory mediators, cytokines, and low IGF-1 levels (3). Studies have found that the prevalence of FMS in patients with HCV infection is 1.9-57% (4). However, these studies focused on tender points in the FMS diagnostic criteria. After the FMS diagnostic criteria were changed in 2010, tender point examination was removed from the FMS diagnostic criteria. Symptoms are mainly diagnosed according to the widespread pain index (WPI) and symptom severity (SS). A prevalence study should be conducted according to these new criteria.

The prevalence of vitamin D deficiency ranges from 46% to 92% in chronic HCV infection population screening (3,5). A study examining vitamin D's effect on liver fibrosis reported that low vitamin D levels were observed in 80%. Vitamin D deficiency is also linked to liver fibrosis and cirrhosis progression (5). Vitamin D levels have also been shown to decrease in chronic diseases, and this relationship has been documented in increasingly different studies on a daily basis (6). Vitamin D deficiency, which affects quality of life, is frequently observed in chronic diseases and adds synergy to people's suffering and considerably reduces their quality of life (3,6). While vitamin D maintains normal bone mineralization by affecting the absorption of minerals such as calcium from the intestine, it is a powerful biomodulator that affects cell metabolism and differentiation as a powerful biomodulator with vitamin D receptor activation (7).

Serum vitamin D levels were significantly lower in patients with FMS than in the control group. Vitamin D deficiency contributes to the progression of chronic hepatitis in HCV. Low vitamin D levels are associated with fibrosis in HCV. Low vitamin D levels can cause muscle weakness and sarcopenia (8). Studies have reported high vitamin D deficiency rates in patients with chronic liver disease. However, the literature on the effects of the FMS association with HCV on quality of life and vitamin D levels is limited (9).

We aimed to evaluate the frequency of FMS in patients with HCV according to the 2010 diagnostic criteria and to analyze the relationship between FMS and vitamin D deficiency and quality of life.

Materials and Methods

Compliance with Ethical Standards

A local university ethics committee approved our study protocol, and approval was received from the Erciyes University Ethical Committee before starting the study (date: 16.06.2017, decision number: 2017/330). All participants provided written informed consent, and the study was conducted in accordance with the Human Rights Declaration.

Study Design

A controlled, cross-sectional study. People 18-65 were admitted to the Physical Therapy and Rehabilitation outpatient clinic of Kayseri City Education and Research Hospital in Turkey between July 2019 and January 2020. A total of 123 patients (98 female, 25 male) were included in the study, of whom 72 (58 female, 14 male) patients were classified into the hepatitis C virus (+) group, and 51 (40 female, 11 male) patients were classified into the HCV (-) control group.

Patients with positive HCV-RNA and liver biopsy results in the infectious diseases outpatient clinic were included in the study. Those with newly diagnosed acute hepatitis C infection, any hepatitis other than HCV, end-stage liver cirrhosis, uncontrolled systemic disease (another bacterial or viral infection, congestive heart failure, renal failure, epilepsy, organ transplantation), congenital or acquired physical deformities (kyphoscoliosis, scoliosis, agenesis, amputation), the affected musculoskeletal system due to neurological diseases (stroke, muscular dystrophy, Guillain-Barre), pregnant and lactating patients, or a history of trauma in the last week were not included. Patients who met the inclusion criteria were referred to the Physical Medicine and Rehabilitation (PMR) outpatient clinic and included in the study.

HCV (-) patients who applied to the PMR outpatient clinic for any reason (due to waist, neck, shoulder, hip, knee, ankle, or joint pain) who met the exclusion criteria of this study were included in the control group. Age, gender, and body mass index (BMI)-matched control group participants were enrolled in the study.

HCV was diagnosed in patients with antibodies against HCV (anti-HCV) and serum HCV ribonucleic acid (RNA). Detailed rheumatological and neurological examinations were performed to differentially diagnose the patients. A specialist physician in physical medicine and rehabilitation diagnosed patients with FMS according to the 2010 American College of Rheumatology (ACR) diagnostic criteria (10).

Interventions

Patients' age, gender, height, weight, HCV disease duration, 2010 diagnostic criteria, FIQ, and NHP scores were questioned. A fasting venous blood sample was collected after an overnight fast of at least 8 h for biochemical investigations, and samples were processed at the hospital laboratory on the same day. The Chroma Systems Kit on the Agilent 1200 device was used with

HPLC in 0.5 mL of plasma taken into a tube with EDTA and 25 (OH) vitamin D levels were studied. Regarding 25 (OH) D vitamin status, those below 20 ng/mL were considered vitamin D deficient, those between 21-29 ng/mL, vitamin D insufficient, and those above 30 ng/mL, with sufficient vitamin D levels. The HCV (+) and HCV (-) control groups were compared for all these parameters. In addition, as a subgroup analysis in the HCV (+) group, patients with and without FMS were compared for the same parameters.

Diagnosis of FMS

According to ACR 2010 diagnostic criteria, the WPI and SS scale were used (10). The WPI shows the number of areas where the patient has experienced pain in the past week. Each region in which pain is felt gets 1 point. Score ranges from 0 to 19. In the SS scale, the severity of 1) fatigue, 2) waking up tired in the morning, 3) cognitive symptoms, and 4) somatic symptoms in the last week were assessed. These four items were scored between 0 and 3 (0=normal, 1=mild, 2=moderate, and 3=severe). The total score ranges from 0 to 12. For diagnosis of FMS, the WPI must be seven or more and SS 5 or more, or the WPI must be 3-6 and SS 9 or more. The symptoms should have been ongoing for at least three months, and there should be no other disease to explain the pain.

The Fibromyalgia Impact Questionnaire

The Fibromyalgia Impact Questionnaire (FIQ), whose Turkish validity was performed by Sarmer et al. (11), calculated patients' functional status and disease progression. The first item of the scale comprises 10 Likert-type questions. Participants were asked to select the days to determine "absence from job" and "exposure to disease" in the second and third items. The scores provided are adapted to ten. The seven questions are also based on selecting the corresponding points on an equivalent visual scale. The score interval is 0-100; a higher score indicates a higher level of physical disability.

The Nottingham Health Profile

The Nottingham Health Profile (NHP) is a self-reported questionnaire that measures perceived health status. According to a Turkish validation study of the NHP, it is a reliable and validated psychometric scale, which includes 38 questions (answered yes/no) that form six statements of distress: pain (eight items), physical mobility (eight items), energy (three items), sleep (five items), social isolation (five items), and emotional reactions (nine items). The item scores were used to calculate the final index of distress. "0 points" indicates no restrictions, and "100 points" indicates the presence of all listed restrictions (12).

Statistical Analysis

The SPSS 22.0 (IBM Corp., Armonk, United States of America) software was used for statistical analysis. Frequencies are expressed as percentages. The chi-square test was used to compare categorical data. The Shapiro-Wilk test and histogram graphs determine whether numeric (digital) data are distributed normally or not. Numeric (digital) data relating to independent groups with a normal distribution were compared using Levene's test and independent samples t-test. Variables with normal distribution are expressed in mean ± standard deviation. If the distribution is abnormal, the Mann-Whitney U test is used. Variables with abnormal distribution are expressed as median (1^{st}-3^{th} quartile). $P<0.05$ is accepted and considered statistically significant.

Results

There were no statistically significant differences between the HCV+ and control groups in terms of gender, age, BMI, vitamin D status (normal, deficiency, insufficiency), and vitamin D ($p>0.05$). Both groups were also compared regarding FMS, vitamin D levels, FIQ, and NHP according to the 2010 criteria (Table 1). When those with and without FMS were compared as a sub-analysis in the HCV (+) group, there was again no statistically significant difference in terms of gender, age, BMI, symptom duration, vitamin D levels, and status ($p>0.05$). FIQ and NHP scores were significantly different (Table 2).

The group's mean age with hepatitis C virus (+) was 58.38±8.2 years, whereas that of the control group was 58.82±9.79 years. The mean body mass index of the group with HCV (+) was 31.98±5.3 kg/m², whereas that of the control group was 30.36±4.31 kg/m². This difference in age, sex, and BMI was insignificant (respectively, $p=0.783$, $p=0.951$, $p=0.075$). The presence of FMS, vitamin D level, and Fibromyalgia Impact Questionnaire score were significantly different between the two groups ($p=0.031$, $p<0.001$, $p=0.004$). The social isolation score was higher in the HCV (+) group. In contrast, scores of pain, physical mobility, energy, sleep, emotional reactions, and total data were similar in both groups, according to the results calculated with the Nottingham Health Profile scale analysis (respectively, $p=0.027$, $p=0.057$, $p=0.959$, $p=0.064$, $p=0.794$, $p=0.129$, $p=0.407$). The results are detailed in Table 1.

Patients with HCV (+) were examined in two subgroups based on the presence or absence of FMS. Age, BMI, disease duration, and vitamin D levels were similar in both subgroups (respectively, $p=0.783$, $p=0.530$, $p=0.915$, $p=0.413$). All subgroup patients with FMS (41 subjects) were female, whereas those without FMS included 17 females and 14 males. All FIQ and NHP scale scores were higher in the FMS subgroup. The results are detailed in Table 2.

Table 1. Clinical data of patients with HCV infection and controls			
	HCV (+) (n=72)	**Control (n=51)**	**p-value**
Sex (F/M), n (%)	58/14 (80.6/19.4)	40/11 (78.4/21.6)	0.951
Age (year), mean ± SD	58.38±8.20	58.82±9.79	0.783
BMI (kg/m²), mean ± SD	31.98±5.30	30.36±4.31	0.075
Fibromyalgia (+/-), n (%)	41/31 (56.9/43.1)	19/32 (37.3/62.7)	**0.031**
Vitamin D (µg/L), median (per 25-75)	11.7 (7.6-20.1)	16.4 (13.0-25.4)	**<0.001**
Vitamin D status, n(%)			
Deficiency	53 (73.6)	28 (54.9)	0.082
Insufficiency	14 (19.4)	15 (29.4)	
Normal	5 (6.9)	8 (15.7)	
FIQ, median (per 25-75)	47.93 (22.9-64.5)	35.66 (14-55)	**0.004**
NHP, median (per 25-75)			
Pain	40.2 (10-78.7)	56.86 (26-100)	0.057
Physical mobility	32.56 (14.5-54.2)	31.29 (21.4-54.5)	0.959
Energy	76 (0-100)	39.2 (0-100)	0.064
Sleep	49.65 (12.6-77.6)	50.37 (12.6-77.6)	0.794
Social isolation	22.01 (0-61.5)	0 (0-42.1)	**0.027**
Emotional reactions	37.97 (9.8-81.5)	23.29 (0-71.8)	0.129
Total	273.4 (133.9-405.1)	240.19 (88.7-353.2)	0.407

P<0.05 considered statistically significant. Depending on normality, the independent samples t-test or Mann-Whitney U test was used
F: Female, M: Male, SD: Standard deviation, BMI: Body mass index, FIQ: Fibromyalgia impact questionnaire, NHP: Nottingham Health Profile

Table 2. Subgroup analysis of patients with HCV infection according to the presence of FMS			
	Fibromyalgia (+) (n=41)	**Fibromyalgia (-) (n=31)**	**p-value**
Sex (F/M), n (%)	41/0 (100/0)	17/14 (54.8/45.2)	-
Age (year), mean ± SD	59.15±8.72	57.35±7.47	0.783
BMI (kg/m²), mean ± SD	32.33±5.52	31.53±5.04	0.530
Duration of symptoms Median (per 25-75)	72 (24-120)	66(24-120)	0.915
Vitamin D (µg/L), median (per 25-75)	11.4 (7.2-19.7)	13.3 (8-20.1)	0.413
Vitamin D status, n (%)			
Deficiency	31 (75.6)	22 (71.0)	0.322
Insufficiency	6 (14.6)	8 (25.8)	
Normal	4 (9.8)	1 (3.2)	
NHP, median (per 25-75)			
Pain	67.35 (36.3-89.5)	9.99 (0-37.5)	**<0.001**
Physical mobility	41.86 (31.3-54.5)	11.54 (0-41.9)	**<0.001**
Energy	100 (69.6-100)	0 (0-100)	**<0.001**
Sleep	77.63 (37.4-77.6)	16.1 (12.6-77.6)	**0.014**
Social isolation	22.53 (22-62.7)	0 (0-42.7)	**0.025**
Emotional reactions	62.58 (22-87)	9.76 (0-56.3)	**0.003**
Total	349.65 (243.6-422.9)	116.38 (38-293.6)	**<0.001**

P<0.05 considered statistically significant. Depending on normality, the independent samples t-test or Mann-Whitney U test was used
F: Female, M: Male, SD: Standard deviation, BMI: Body mass index, NHP: Nottingham Health Profile

Discussion

In this study, according to the 2010 ACR diagnostic criteria, the frequency of FMS was higher in HCV (+) patients than in HCV (-) patients at 56.9%. Vitamin D levels were statistically significantly lower in HCV (+) patients than in HCV (-) individuals. However, when a subgroup analysis of HCV (+) patients was performed, there was no significant difference in vitamin D levels between patients with and without FMS. In HCV (+) patients, the FIQ median and NHP social isolation values. When patients with and without FMS were compared, there was a statistically significant decrease in all NHP scores. Pain, physical mobility, energy, sleep, and emotional reactions, which are fibromyalgia-related factors, were found to be significant for HCV infection.

Our results showed that the frequency of FMS was higher in HCV (+) patients than in HCV (-) patients. In previous studies, the prevalence of FMS in patients with HCV infection ranged from 4% to 57% (13). Our study revealed a significantly higher prevalence rate of 56.9% compared with the control group. Based on our study, which has relatively more recent data than previous studies, it can be concluded that the frequency of FMSs is increasing daily. These results may have been influenced by the high level of scientific awareness, media interest, and the frequently changing definition of FMS, along with psychological disorders. In addition, several conditions, such as socioeconomic factors, demographic data, and genetic differences, may affect FMS. People with HCV infection may have extrahepatic symptoms, such as arthralgia, marked arthritis, painful paresthesias, and peripheral neuropathy, or they may meet the FMS criteria in 25% of cases (13,14). In addition, a recent study mentioned common non-rheumatic medical conditions that mimic FMS, in addition to chronic infections such as HCV (2). These conditions include vitamin B12, C, and D deficiencies; iron and magnesium deficiencies; thyroid dysfunction; obesity; obstructive sleep apnea; neuropathies; chronic infections such as HCV; cancer; and celiac disease; lipid-lowering drugs; bisphosphonates; aromatase inhibitors; and isotretinoin use. However, it may be extremely difficult to distinguish between FMS-like conditions that can be attributed to these conditions and pure FMS (2,13). One of the important limitations of our study is that we did not question all of these factors. Despite this limitation, the fact that vitamin D levels were measured was a strength of our study. The significantly lower vitamin D levels in HCV (+) patients in our study suggest that the prevalence of FMS is increasing. The 2010 FMS diagnostic criteria should also be reconsidered for these conditions.

FMS is a syndrome of unknown cause characterized by chronic widespread musculoskeletal pain lasting more than 3 months, often accompanied by symptoms such as fatigue, non-restorative sleep, cognitive impairment, short-term memory deficit, headache, irritable bowel syndrome, anxiety, and depression (15). FIQ is a scale commonly used in patients with FMS, and its higher detection is considered significant in support of FMS.

Along with increased FMS, the median FIQ score was higher in HCV (+) patients than in HCV (-) ones. The disease duration was longer in patients with FMS in our study, although this difference was not statistically significant (Table 2). We believe that FMS should be added to comorbidities that may occur as the disease duration increases.

Vitamin D deficiency is common among patients with HCV infection and is directly related to disease severity (16). It is also recommended as a supplement to treat HCV infection (3,17). It is also emphasized that vitamin D deficiency plays a role in inflammatory responses via tumor necrosis factor inhibition in chronic liver diseases caused by HCV, and it has been shown that patients with HCV may have a poor prognosis (13,18). According to the findings compatible with the literature we obtained in our study, vitamin D levels should be examined in patients with HCV and replaced if there is a deficiency.

Data on the poor quality of life in patients with chronic hepatitis and cirrhosis are available in previous studies (19,20). Even when HCV (+) people are asymptomatic, significant deterioration in quality of life occurs. In a study conducted by Honrubia-López et al. (21), 86 patients were evaluated using the SF-36 scale, and lower scores were obtained than healthy controls. In another study of patients with chronic viral hepatitis evaluated using the NHP scale, the main areas of worse quality of life were decreased energy, sleep, and emotional reactions (22). Unlike in our study, only the median score of NHP-social isolation was higher in HCV (+) patients than in the control group.

FMS is more common in women (23). Not only compatible with the literature but also surprisingly in our study, there were no male patients among the 41 HCV (+) patients with FMS. As expected, in line with these findings, the median FIQ score was higher in participants with FMS.

Although vitamin D deficiency increases FMS symptoms, FMS patients generally have vitamin D levels similar to those of healthy individuals (20). In this study, vitamin D levels were significantly different in the presence of HCV and not in the presence of FMS. From this, we can conclude that HCV, rather than FMS, affects vitamin D levels.

Generally, all NHP subscale values for patients with FMS are higher than those of the healthy population in studies evaluating the NHP scale (24,25). In our study, patients with HCV (+) were evaluated and divided into

two subgroups according to the presence or absence of FMS. The median scores in all sections of the NHP scale were higher in patients with FMS. Multiple analyses on whether there is FMS or not, only its relationship with NHP-emotional reactions can be revealed.

Study Limitations

We did not intend to determine the effect of HCV transmission route and HCV genotype on FMS symptoms. We included HCV (-) individuals who visited the PMR outpatient clinic for any reason (neck, shoulder, waist, hip, knee pain, joint pain) as the control group. We did not have a control group of completely healthy individuals. Our study's strength lies in its use of the 2010 criteria. It is known that HCV infection alone can affect quality of life. However, we would like to emphasize that the presence of FMS and/or vitamin D deficiency affects the quality of life of HCV. Patients should also be evaluated in this regard.

Conclusion

This study documented a prevalence of 56.9% of FMS in patients with HCV (+) in comparison to the control group. Other painful conditions that resemble fibromyalgia syndrome should also be taken into account. Vitamin D levels were observed to be lower in HCV patients. The quality of life in patients with HCV is primarily limited by social isolation; however, the presence of FMS may further diminish quality of life across various domains, including pain, physical mobility, energy levels, sleep, and emotional responses.

Ethics

Ethics Committee Approval: A local university ethics committee approved our study protocol, and approval was received from the Erciyes University Ethical Committee before starting the study (date: 16.06.2017, decision number: 2017/330).

Informed Consent: All participants provided written informed consent, and the study was conducted in accordance with the Human Rights Declaration.

Footnotes

Authorship Contributions

Surgical and Medical Practices: H.T.C., F.G.U.D., U.S.T., H.S., D.K.G., E.G., F.S., C.K., S.T.S., H.K.T., Concept: H.T.C., F.G.U.D., U.S.T., H.S., D.K.G., E.G., C.K., H.K.T., Design: H.T.C., F.G.U.D., H.S., D.K.G., E.G., F.S., C.K., S.T.S., Data Collection or Processing: H.T.C., F.G.U.D., U.S.T., H.S., D.K.G., E.G., F.S., C.K., S.T.S., H.K.T., Analysis or Interpretation: H.T.C., F.G.U.D., U.S.T., D.K.G., E.G., F.S., C.K., S.T.S., H.K.T., Literature Search: H.T.C., U.S.T., H.S., D.K.G., E.G., F.S., C.K., S.T.S., H.K.T., Writing: H.T.C., F.G.U.D., U.S.T., H.S., F.S., C.K., H.K.T.

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Original Article

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Bibliometric and Altmetric Analysis of the 100 Most Cited Articles on Piriformis Syndrome

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Abstract

Aim: A bibliometric analysis is used to quantitatively analyze scientific production and thematic development in a given research area. We aimed to analyze the 100 most cited articles on Piriformis syndrome (PS).

Methods: This observational, descriptive, and retrospective study used a bibliometric technique. Using the word PS the top 100 (T100) most cited articles in the Web of Science between 1975 and 2023 were identified. In the bibliometric analysis, the title, number of authors, author names (first authors and corresponding authors), year and country of publication, number of citations, citation index, genre, subject, Q classification, h-index, impact factor, and publication area were recorded.

Results: The total number of citations in the T100 article list ranged from 7 to 202. Most T100 articles were published between 2001 and 2005 (n=22). The country that produced the most articles was the United States, and the most cited author was Fishman. Clinical studies accounted for the majority of the top 100 articles (n=44). The majority of the T100 articles (n=93) were published in journals with expanded science citation indexes, and the most common field of publication was orthopedics (n=29). The altimetric attention score for the articles ranged from 1 to 435. The altimetric attention score was not available for 54 articles.

Conclusion: The results of this study provide insight into the level of attention that the scientific community and social media platforms pay to the most cited articles in PS. Further research using larger databases to examine interactions across countries is needed.

Keywords: Piriformis syndrome, bibliometric analysis, altmetric analysis, citation

Introduction

The diagnosis of Piriformis syndrome (PS), which causes pain in the lower back, hips, and upper back of the thigh, is not very clear. Compression of the piriformis muscle on the sciatic nerve plays a role in the etiology. Many factors, including trauma and anatomical anomalies, may play a role in the occurrence of PS. PS is diagnosed based on clinical signs and symptoms; as agreed, clinical criteria have yet to be established (1,2). The prevalence of PS is not well known, and it is believed that the syndrome is underdiagnosed. In contrast, there are also claims of overdiagnosis (3). This may be due to inadequate diagnostic criteria.

Bibliometric analysis is a method used to quantitatively analyze scientific production and thematic development in a particular research field. In any scientific field, citation analysis is a quantitative, bibliometric method that examines the pattern and frequency of citations. Citation analysis is a method for evaluating the influence of particular published works on science and trends in current research. In the scientific field, frequently cited articles present significant new findings. At the same time, these articles demonstrate a growing interest in a

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particular field. In academia, citation analysis is effective for guiding future studies by identifying unstudied areas and introducing novice researchers to the most salient topics in a given field (4-6). When searching for high-quality articles on a particular topic, the criteria usually considered are the journal's impact factor, number of citations, and h-index. Based on the scientific data found in relevant articles, these criteria provide useful information. In addition, social media platforms also play a role in the promotion of medical literature. Thus, altimetric are used to determine the metrics of an article on social media platforms (7,8).

We hypothesized that the most cited articles on PS present diagnostic challenges and new treatment approaches and that they ranked first in terms of the number of citations and altimetric scores. We also hypothesized that most of the most cited articles on PS were published in the United States. In this study, we analyzed the 100 most cited articles (T100) related to PS in the last 30 years and evaluated the relationship between the total number of citations/citation index and the altimetric attention score. Thus, this study aimed to contribute to future studies by identifying popular topics or deficiencies in this field.

Materials and Methods

Study Design and Search Strategy

The Web of Science, accessible at http://apps.webofknowledge.com, is a reliable scientific database. In addition to conducting general literature searches, the tool also offers citation index searches, which are useful for assessing the academic significance of an article in a specific field. We performed a PS search of the Web of Science database from 1975 to 2023 on November 28, 2023. The search term used was "Piriformis Syndrome". Articles were organized by sorting based on citation count, with those with higher citation counts placed at the top. The data used in our study were sourced from published articles; therefore, ethics committee approval was not necessary.

Article Selection

Two reviewers (BTD and MO) independently analyzed the abstracts and full texts to identify the 100 (T100) most cited articles on PS. In case of disagreement between the two reviewers, consensus was sought through two other researchers (FB and EA). The examiners were physicians. Articles were included in this study regardless of publication type. Because of the limited number of publications on PS. Articles focusing on PS were included.

Data Extraction

In the bibliometric analysis, the title, number of authors, author names (first authors and corresponding authors), year and country of publication, number of citations, citation index, genre, subject, Q classification, h-index, impact factor, and publication area were recorded. The article was deemed to be from the country of the responsible author (9). The citation index is calculated as the total number of citations in the article divided by the number of years since the article was published (10). The "Altmetric attention score" was obtained through the "Altmetric it" function on the Altmetric.com website (https://www.altmetric.com). The impact of an article is assessed by considering the number of citations as well as the number of views and downloads on social platforms (11,12). The stages of this study are presented in Figure 1.

Statistical Analysis

Statistical analysis was performed using IBM SPSS version 22.0 software (IBM Corp., Armonk, IL, USA). The Kolmogorov-Smirnov/Shapiro-Wilk test was used to determine whether the distribution was normal. For quantitative variables, mean ± standard deviation values or median [minimum (min.)-maximum (max.)] values are presented when performing descriptive analyses. For categorical variables, frequencies and percentages are provided. The relationships between quantitative variables were assessed using the Spearman or Pearson's correlation tests. An r-value <0.3 indicates a weak relationship, 0.3-0.7 a moderate relationship, and >0.7 indicates a strong relationship (13). P-values of 0.05 were accepted as statistically significant results.

Results

In this study, articles published between 1975 and 2023 were searched using the Web of Science database by searching the keyword "Piriformis syndrome". T100 articles on PS were published between 1981 and 2021. When the T100 articles were analyzed by year, most articles were published between 2001 and 2005 (n=22) and then between 2006 and 2010 (n=19) (Figure 2).

T100 articles were published by 21 countries. Almost half of the T100 articles were published in the USA (n=47), followed by South Korea (n=8). Table 1 shows the countries with ≥3 published articles. In total, T100 articles were published in 68 journals. Two or more articles were published in 15 of these journals (Table 2). Most articles were published in Muscle & Nerve (n=7) and Archives of Physical Medicine and Rehabilitation (n=6). Of the journals that published ≥2 articles, 11 were from the United States. 2 were from Germany, one was from France, and 1 was from England (Table 2). The IF (impact factor), Q classification, and H index of the journals publishing ≥2 articles are presented in Table 2.

The number of authors for the T100 articles ranged from 1 to 11. Approximately one-third of the T100 articles (n=32) were written by ≥5 authors. The most

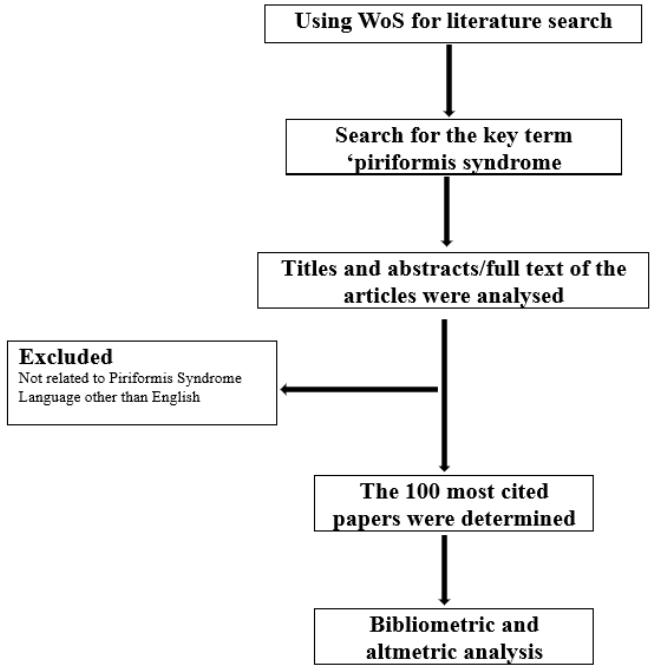

Figure 1. Flowchart of the study
WoS: Web of Science

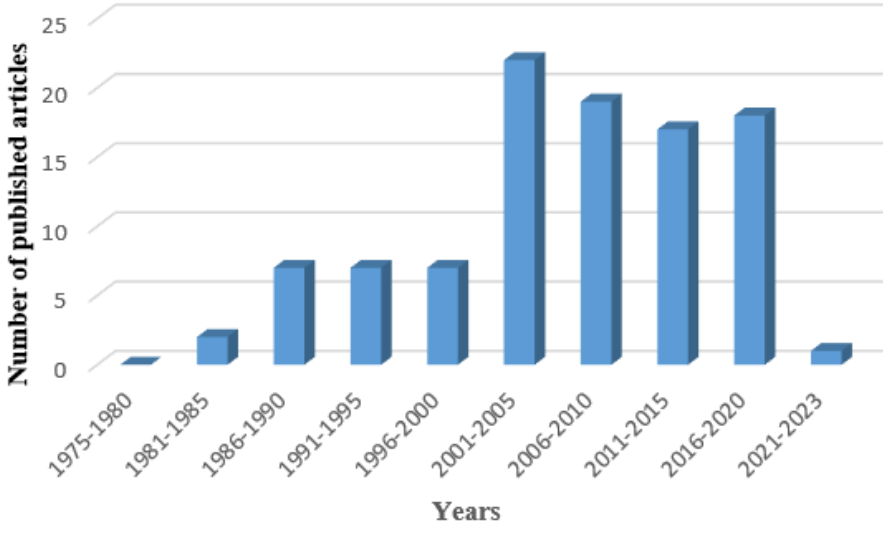

Figure 2. Number of articles published by year

cited author was Fishman LM, who served as the first and corresponding author in 6 articles.

The mean number of citations of the T100 articles was 35.1±32.2 and the median number of citations was 26.5 (min.=7, max.=202). The mean altimetric attention score of the T100 articles was 18.9±64.7 with a median value of 4 (min.=1, max.=435). The altimetric attention score could not be obtained for the 54 articles.

The most cited article in T100 was "Sciatica of Nordic origin and piriformis syndrome: diagnosis by magnetic resonance neurography and interventional magnetic resonance imaging with outcome study of resulting treatment" by Filler et al. (14), published in 1990; this article also had the highest citation index. The article with the highest altimetric attention score was "Diagnosis and Management of Piriformis Syndrome: An Osteopathic

Approach" by Boyajian-O'Neill et al. (15), which was published in 2008. However, this article was ranked 13th in terms of the number of citations.

When T100 articles were analyzed according to their content, publications sharing information about diagnosis and treatment were in the first rank in terms of the number of citations (14,16,17). In terms of the altimetric attention score, a review mentioning the osteopathic approach in the diagnosis and treatment of PS ranked first (15). In PS, when T100 articles were analyzed, the most frequently emphasized topics were treatment (n=57), diagnosis (n=45), etiology (n=22), and symptoms (n=9). There was a moderately significant correlation between the altimetric attention score and the citation index ($p<0.05$, $r=0.312$) (Figure 3). No significant correlation was found between the altimetric attention score and the total number of citations ($p>0.05$, $r=0.076$) (Table 3). Of the T100 articles, 44 were clinical studies, 5 were clinical guidelines, 29 were case reports, 3 were letters, and 19 were reviews.

The majority of the T100 articles (n=93) were published in journals with expanded science citation indexes. When we looked at the fields in which T100 articles were published, orthopedics (n=29) ranked first, clinical neurology (n=28) ranked second, and surgery (n=22) ranked third (Figure 4).

Table 1. Countries where T100 papers were published (n≥3)

Country	Number of articles
US	47
South Korea	8
Canada	6
Italy	5
Japan	5
Germany	3
Spain	3
Turkey	3

Discussion

This is the first study to conduct a bibliometric and altimetric analysis of T100 articles on PS. The results of this study reveal advances and areas of interest in PS. Bibliometric analysis quantitatively performs articles published during a given period. Citation analysis is a widely used bibliometric analysis method that identifies highly cited publications. This will help identify advances and areas of interest in the relevant subject. This can shed light on future research. Although citation analysis is a frequently used method to assess article quality, it has some disadvantages (18). It has been reported that the number of citations is affected by many factors, such as the journal in which the article was published (19,20) and the geographical origin of the author (21,22).

As the time elapsed since the article was published increases, the number of citations may increase accordingly (22). However, in this article, we found that most T100

Table 2. Journals in which T100 articles have been published (n≥2)

Journal	Number of articles	IF*	Q** Category	H** Index	Country of the journals
Muscle and Nerve	7	3.4	Q2	159	USA
Archives of Physical Medicine and Rehabilıtation	6	4.3	Q1	206	USA
American Journal of Physical Medicine & Rehabilitation	4	3.0	Q1	112	USA
European Radiology	4	5.9	Q1	164	Germany
Orthopedics	4	1.1	Q2	73	USA
Journal of Bone and Joint Surgery-American Volume	3	5.3	Q1	290	USA
Surgical and Radiologic Anatomy	3	1.4	Q2	67	France
Journal of Neurosurgical Spine	2	2.8	Q1	113	USA
European Spine Journal	2	2.8	Q1	155	Germany
Journal of the American Osteopathıc Association	2	-	-	-	USA
Neurosurgery	2	4.8	Q1	215	USA
Clinical Orthopaedıcs and Related Research	2	4.2	Q1	225	USA
Regional Anesthesia and Pain Medicine	2	5.1	Q1	119	England
PM&R	2	2.1	Q2	79	USA
Journal of Chiropractic Medicine	2	0.9	Q3	26	USA

*IF: Impact factor, 2022 Journal Citation Reports, Web of Science Group, **2022 SCImago Journal and Country Rank

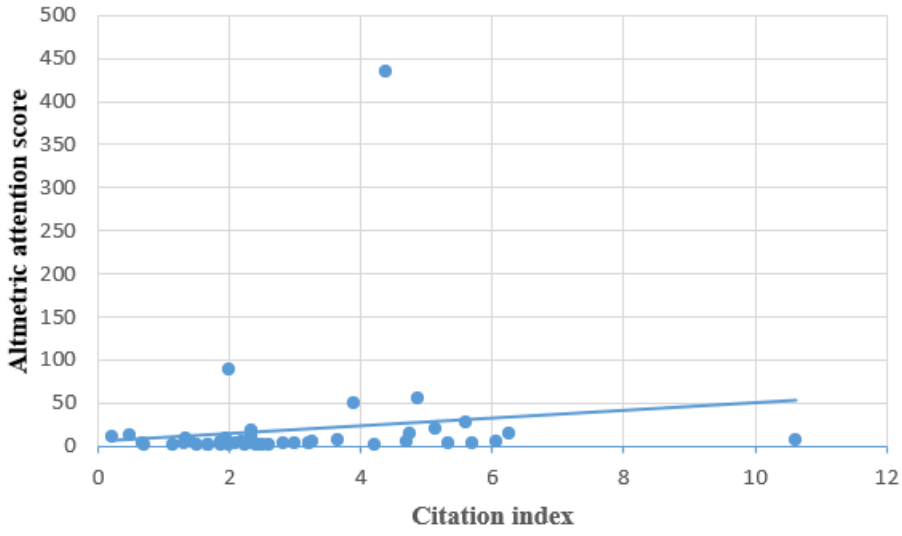

Figure 3. Correlation analysis between the altimetric attention score and citation index

Table 3. Correlation analysis between citation parameters and the altmetric attention score			
		Citation index	**Number of citations**
Altmetric attention score	r	0.312*	0.076
(Pearson correlation tests) *p<0.05			

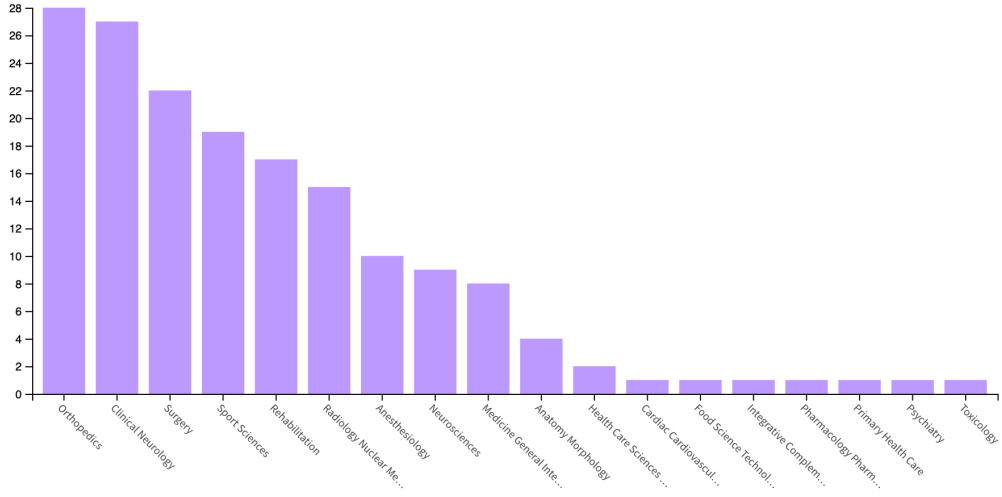

Figure 4. Distribution of articles according to fields in which they are published

articles on PS were published between 2001 and 2005, which may be related to developments in scientific fields. Previously, T100 articles on rheumatoid arthritis and acute kidney injury were published in a similar year range (10,22).

Due to the widespread use of social media in recent years, altimetric analysis can be used as an alternative to the impact factor of citations from web-based journals (23). Altmetric analysis evaluates not only the number of citations of the article but also the number of downloads, views, and social media value of the articles as an alternative to impact factor and other evaluation methods (12). In this study, altimetric scores could not be obtained for 54 of the articles. This shows that the impact of T100 articles on digital media is insufficient. It is clear that there is a need for more digital discussion on this topic.

The majority (n=47) of T100 articles on PS were published in the United States, as in many other fields.

This result is consistent with our hypothesis. This was followed by South Korea with 7 articles. In the study by Bağcıer et al. (11) on fibromyalgia syndrome, Quan et al. (24) on systemic lupus erythematosus, and Goebel et al. (25) on dental fluorosis, T100 articles were mostly published in the United States. This may indicate that scientific quality is high in the United States (11), but a previous study has shown that journals tend to publish articles submitted from their own regions (9). In fact, in this study, the majority of journals that published ≥2 articles were from US journals.

The 100 articles with the highest number of citations for PS were identified. Articles were included in this study regardless of publication type. The most cited article in publications about PS had 202 citations. In this study, a total of 29 case reports were found in T100 articles. This shows that authors benefit from case-based articles rather than comprehensive studies during the publication production phase. Therefore, there is undoubtedly a need for high-quality publications in this field. It is evident from previous bibliometric studies that the number of citations of T100 articles is much higher than that of PS (10,11,26,27). This may be due to the fact that PS is not sufficiently recognized, and authors conduct less research on this subject because of insufficient awareness. However, PS has a considerable rate in patients with low back pain, and it has been reported that it constitutes 17.2% of low back pain (28).

Primary PS arising from anatomical variants of nerve and muscle in the etiology of PS corresponds to approximately 15% of all PS cases (29). However, a study included in T100 articles reported that the anatomical variant of the nerve was not associated with PS (30). Although this study was in the last rank according to the number of citations, it was in the second rank according to the altimetric attention score. Secondary PS is more common due to secondary factors, such as trauma (31). PS may develop after trauma to the hip region was mentioned in one article among the T100 articles and ranked fourth according to the number of citations (32).

Since there are no agreed criteria for the diagnosis of PS, diagnosis is based on clinical signs and symptoms (2). The diagnosis of PS has been a subject of curiosity for researchers for years. In fact, the article with the highest number of citations in T100 articles mentioned the diagnosis of PS (14,16). These articles were ranked first in terms of number of citations and citation index. However, they were not ranked first in terms of the altmetric attention score. They secured the 8th and 15th positions, respectively. The article with the highest altimetric attention score also mentioned the diagnosis and treatment of PS (15). The fact that these articles mention the diagnosis of PS ranked first in terms of the number of citations and altimetric scores supports our hypothesis. Conservative treatment should be attempted first for PS, and local anesthetic and steroid injections may be used if conservative treatment and medical treatment fail. If these treatments fail, surgical methods may be considered (33). The 3 most-cited articles also mentioned local anesthesia and steroid administration for PS. Botulinum toxin, another invasive treatment method, has been shown to reduce pain during PS treatment (34). In fact, botulinum toxin was a popular treatment in T100 articles. In 12 articles, botulinum toxin was mentioned. However, the cost of botulinum toxin is higher than local anesthetics. Botulinum toxin applications should be used in patients who are resistant to first-line treatments (35).

Study Limitations

There are some limitations to this study. First, only the Web of Science database was searched for the articles. Google Scholar, etc., databases were not used. However, one of the most widely used databases for bibliometric analysis is Web of Science (36-39). Second, only articles published in English were analyzed. Third, self-citation was not considered. Fourth, the interaction between countries was not examined. Fifth, such studies usually include articles with long publications (40). Thus, high-quality articles may be overlooked. Sixth, case reports and letters were also included, but the number of articles with ≤20 citations was 44. Despite these limitations, this study is the first bibliometric and altimetric analysis of PS, which is an important cause of low back pain and sciatic pain. Thus, it may shed light on future research.

Conclusion

In this study, we analyzed T100 articles on PS according to their level of interest in science and social media. The topics of most interest in PS were treatment and diagnosis. When the number of citations for the T100 articles was analyzed, similar studies in the literature were excluded. This may be due to the low awareness of PS. It is believed that this study will contribute to the design and production of new diagnostic studies. In addition, new studies using languages other than English and from different databases are needed.

Ethics

Ethics Committee Approval: As the data for our study were sourced from published articles, ethics committee approval was not necessary.

Informed Consent: The data used in our study were sourced from published articles.

Footnotes

Authorship Contributions

Concept: B.T.D., F.B., E.A., Design: B.T.D., M.O., Data Collection or Processing: B.T.D., M.O., F.B., Analysis or Interpretation: B.T.D., F.B., E.A., Literature Search: B.T.D., M.O., E.A., Writing: B.T.D., M.O., F.B., E.A.

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Original Article

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Effects of Prognostic Nutrition Index, Neutrophil/Lymphocyte Ratio, and C-reactive Protein/Albumin Ratio on Prognosis Undergoing Open Heart Surgery

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Abstract

Aim: Deficiencies in preoperative nutritional status increase the incidence of negative postoperative events. In this study, we aimed to investigate the effect of the prognostic nutrition index (PNI), neutrophil-to-lymphocyte (N/L) ratio, and C-reactive protein (CRP)-to-albumin (CRP/Alb) ratio on postoperative prognosis in patients undergoing open-heart surgery.

Methods: Patients undergoing primary open-heart surgery in our hospital from December 2021 to August 2023 were screened for PNI, N/L ratio, and CRP/Alb ratios, along with durations in intensive care, total hospitalization, and 30-day mortality rates. Binary logistic regression and robust regression analyses were used for the statistical analysis of this cross-sectional study.

Results: The study included a total of 437 cases. All patients had a mean PNI of 49.35±7.70. A one-unit increase in PNI value reduced intensive care duration by 0.495 units (p=0.049), while it reduced discharge duration by 0.101 units (p<0.001). A one-unit reduction in PNI value increased mortality by 1.07 times (p=0.002). The other variables showed no significant effects on intensive care duration, total hospitalization, and 30-day mortality rates.

Conclusion: PNI, a marker of inflammatory and immune processes, may be a beneficial variable for estimating postoperative prognosis among patients undergoing open heart surgery.

Keywords: PNI, N/L ratio, CRP/Alb ratio, open-heart surgery, prognosis

Introduction

Coronary artery bypass graft surgery (CABG), valve surgery, and aortic graft surgery are the most common open-heart surgeries performed in adults. Despite new surgical techniques and technological advances, the risk of complications after cardiac surgery remains high (1). The postoperative period is of critical importance for all open-heart surgeries, and patient-related correctable factors, especially during the postoperative period, may favorably affect all outcomes, including postoperative mortality.

In general, low albumin levels are a strong marker of mortality in patients with poor prognosis, chronic inflammation, and associated cardiovascular disease. Lymphocytes are important peripheral blood elements that indicate immune function in patients and may be indicative of prognosis in critical diseases. They are used to predict poor prognosis in clinical situations such as coronary artery disease (CAD) and heart failure (2). The prognostic nutrition index (PNI), calculated using serum albumin and peripheral total lymphocyte counts, is a relatively new score that has been included in clinical practice. While the PNI was initially used to assess preoperative nutritional conditions and surgical complications in patients with malignancies, it is now used to assess the prognosis of patients with autoimmune diseases, other surgical diseases, and a variety of different clinical conditions (3,4).

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The aim of this study, which was performed with open-heart surgery patients, a patient group known to have high surgical and anesthesia risks, was to determine whether predicting factors with the potential to impact postoperative prognosis in the preoperative period would be beneficial or not. We hypothesized that the effects of the PNI, neutrophil/lymphocyte (N/L) ratio, and C-reactive protein (CRP)-to-albumin (CRP/Alb) ratio, which were simply calculated preoperatively, on the duration of intensive care unit duration (ICU duration), total hospitalization (discharge duration), and 30-day mortality rates in the postoperative period.

Materials and Methods

Ethical Approval and Study Design

Permission for this retrospective study was obtained from the Ordu University Clinical Research Ethics Committee (approval no.: 2023/224, date: 01.09.2023). The study included all adults undergoing primary open-heart surgery, including CABG, valve surgeries, and aorta surgeries with median sternotomy and cardiopulmonary bypass from December 1, 2021, to August 31, 2023. Patients younger than 18 years, repeat cases, emergency cases, surgeries without cardiopulmonary bypass, and exitus patients within 24 hours postoperatively were excluded from the study.

Anesthesia Management

All patients received general anesthesia using the same medications and methods routinely used in cardiovascular anesthesia administration: IV induction with 0.1 mg/kg midazolam, 3-5 mcg/kg fentanyl, and 1-2 mg/kg propofol (until loss of eyelash reflex) and 0.6-0.8 mg/kg rocuronium, followed by endotracheal intubation. After this, anesthesia was maintained in volume control mode with 2% sevoflurane + 50% oxygen and a 50% dry air mixture to ensure ETCO2 was 35-40 mm Hg. When cardiopulmonary bypass began, inhalation anesthesia was discontinued. During cardiopulmonary bypass, 0.05 mg/kg midazolam, 1 mcg/kg fentanyl, and 0.2 mg/kg rocuronium at 30-minute intervals were administered for anesthesia maintenance. After the end of cardiopulmonary bypass, maintenance continued with 2% sevoflurane + 50% oxygen and 50% dry air mixture titrated according to the hemodynamic situation and arterial gas oxygenation to ensure ETCO2 was 35-40 mmHg until transfer to the ICU.

Cardiopulmonary Bypass Process

Anticoagulation was provided by 300 U/kg heparin, as routine for CPB. During surgery, the pump blood flow rate was BSAx2.4 L/min for isothermic patients and BSAx2.2 L/min for patients with mild hypothermia, and the mean blood pressure was kept at 50-80 mmHg. On exiting the pump, heparin was antagonized with a 1:1 ratio of protamine sulfate. At the end of surgery, patients were transferred to the cardiovascular surgery ICU unit with or without vasopressor/inotrope support according to the hemodynamic requirements.

Data Collection

Patient age, sex, ASA, comorbid diseases, type of operation, surgical duration, postoperative ICU duration, discharge duration, and 30-day mortality outcomes were recorded. PNI, N/L ratio, CRP/Alb ratio, and other routine laboratory values were recorded from peripheral venous blood samples taken routinely during the preoperative period.

Statistical Analysis

Data were analyzed using IBM SPSS V23 and the R program. Continuous variables were presented as mean (± standard deviation) and median (mininum-maximum), whereas categorical variables were presented as counts (n) and percentages. The fit to the normal distribution was investigated using the Kolmogorov-Smirnov test. Investigation of risk factors affecting mortality using binary logistic regression analysis. The factors affecting discharge duration were investigated without normal distribution using robust regression analysis. The significance level was taken as $p<0.050$.

Results

For this study, data from a total of 461 patients were screened. Twenty-four patients with missing data were excluded from the study. The study included 437 patients. The mean age of patients included in the study was 63.63±10.37 years. The most common diagnosis was CABG surgery (76%). The mean surgical duration was calculated as 225.46±60.94 minutes. The mean ICU duration was 61.37±75.13 hours and the mean discharge duration was 6.15±3.72 days. Additionally, the 30-day mortality rate was 7.8%. According to laboratory values, the mean white blood count was 8.83±4.97, the mean hematocrit (Htc) was 45.55±127.20, the mean neutrophil count was 6.00±4.39, and the mean lymphocyte count was 1.93±0.78. The mean CRP was 17.27±34.21 and the mean albumin was 39.97±5.68. The mean PNI value was found to be 49.35±7.70, with a mean N/L ratio of 4.16±5.23 and a mean CRP/Alb ratio of 0.50±1.07 (Table 1).

As a result of an investigation of factors affecting ICU duration with robust regression analysis, the regression model was found to be statistically significant ($F=3.992$, $p<0.00$). A one-unit increase in the PNI value reduced

ICU duration by 0.495 (p=0.049). At the same time, for patients with mortality, the ICU duration was 12.8 days longer than that for patients without mortality (p=0.048). Other variables were not found to have statistically significant effects (Table 2).

Table 1. Descriptive statistics of the variables

	Mean±SD/n	Median (Min.-Max.)/%
Age†	63.63±10.37	65.00 (29.00-87.00)
Gender‡		
Female	134	30.4
Male	307	69.6
Diagnosis‡		
CABG	335	76
Valve surgery	97	22
Aort surgery	41	9,3
Number of comorbidities†	1.63±0.93	2.00 (0.00-6.00)
Comorbidities‡		
CAD	184	41.7
DM	78	17.7
HT	289	65.5
COPD/Asthma	51	11.6
CHF	55	12.5
Renal disease	19	4.3
Tyroid disease	9	2
Neurological disease	34	7.7
Surgery duration†	225.46±60.94	215.00 (50.00-660.00)
ICU duration	61.37±75.13	48.00 (5.00-1200.00)
Discharge duration*	6.15±3.72	5.00 (1.00-50.00)
Thirty day mortality‡		
None	403	92.2
Present	34	7.8
WBC *	8.83±4.97	8.11 (3.22-90.40)
Htc†	45.55±127.20	40.40 (23.70-2708.00)
Neutrophil*	6.00±4.39	5.09 (1.64-72.80)
Lymphocyte*	1.93±0.78	1.85 (0.37-5.49)
CRP*	17.27±34.21	4.17 (0.02-248.45)
Alb*	39.97±5.68	40.70 (18.00-52.80)
ALT*	24.26±41.24	17.00 (2.00-728.00)
AST*	27.81±39.25	19.00 (0.29-575.00)
PNI†	49.35±7.70	50.00 (13.60-71.10)
N/L ratio†	4.16±5.23	2.62 (0.56-64.43)
CRP/Alb ratio†	0.50±1.07	0.10 (0.00-9.20)

*Indicates normal distribution, †indicates non-normal distribution and ‡indicates categorical variables.
CABG: Coronary artery bypass graft surgery, CAD: Coronary artery disease, CHF: Congestive heart failure, WBC: White blood cell, Htc: Hematocrit, CRP: C-reactive protein, Alb: Albumin, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, PNI: Prognostic nutritional index, N/L ratio: Neutrophil/lymphocyte ratio, CRP/Alb ratio: C-reactive protein/albumin ratio, Min.-Max.: Minimum-maximum

The regression model created as a result of robust regression analysis for factors affecting discharge duration was found to be statistically significant (F=11.287, p<0.001). A one-unit increase in PNI reduced discharge duration by 0.101 (p<0.001). An increase in the number of comorbid diseases increased the discharge duration by 0.181 units (p=0.021). A one-unit increase in the surgical duration increased the discharge duration by 0.003 units (p=0.004). The other variables did not have statistically significant effects (Table 3).

Risk factors affecting mortality were investigated using binary logistic regression analysis in univariate and multivariate models. According to the univariate model, as age increased, mortality increased by 1.057 times (p=0.004). A reduction in the Htc value was observed to increase the mortality risk (Odds ratio=0.92; p=0.006). According to the analysis, a one-unit reduction in PNI values increased the mortality risk by 1.07 times (1/0.935) (p=0.002). An increase in ICU duration increased the mortality risk by 1.005 times (p=0.013). Investigation of the multivariate model results revealed that an increase in age increased the mortality risk by 1.058 times (p=0.009). Other variables were not found to have statistically significant effects (Table 4).

Discussion

This study revealed significant correlations between low PNI values and adverse postoperative events after cardiac surgery, defined as prolonged ICU time, prolonged discharge time, and increased 30-day mortality rate. Open-heart surgery is a process that may stimulate oxidative stress and inflammation, which are significant for postoperative complications. PNI was first reported to have potential prognostic value in patients undergoing gastrointestinal surgery (6,7). Severity of cardiac pathology, comorbid diseases, age, and other similar factors significantly affect postoperative morbidity and mortality (1).

Variations in leukocyte subtypes examined in the preoperative period, particularly the N/L ratio, may provide significant prognostic information for the postoperative period (8). Recent studies have proposed that an increase in the perioperative N/L ratio is associated with poor outcomes in adult heart surgery cases (9,10). In this study, there was no significant correlation between N/L ratios and postoperative ICU duration, discharge time, and 30-day mortality rates. This result may be explained by the fact that the patients included in the study had no additional pathologies other than cardiac pathologies that could cause abnormalities in peripheral blood values, and emergency patients with poor general condition were excluded from the study. Surgical stress suppresses cellular immunity as a result of inflammatory responses in all patients who undergo surgery. In addition to damaging

the host's defense mechanism, this suppression can cause the overproduction of inflammatory mediators. Serum CRP, an acute-phase protein, is released by the liver in response to inflammation and is associated with poor prognosis after CABG in patients with CAD. Reduced levels of the negative acute phase protein of albumin increase blood viscosity and platelet activation, worsen endothelial function, and are associated with negative cardiovascular events. The inflammatory parameter of the CRP/Alb ratio is superior to CRP or albumin alone for determining the inflammatory status in a variety of cardiovascular diseases (7). In our study, the mean CRP/Alb ratio was 0.50±1.07 in all patients, and this ratio was not significantly correlated with postoperative ICU duration, discharge time, and 30-day mortality rates. Although our patients were in the critically ill group, we believe that the prognosis may not have been affected by changes in the CRP/Alb ratio because they were operated on under relatively elective conditions.

Table 2. Investigation of factors affecting ICU duration

	β1 (95% CI)	S. data	β2	t	p	VIF
Constant	44,745 (4,835-84,656)	20,303		2,204	0.**028**	
Age	0.248 (-0.042-0.538)	0.148	0.080	1,679	0.094	1,108
Htc	0.016 (-0,007-0,040)	0.012	0.067	1,362	0.174	1,163
PNI	-0,495 (-0.987- -0.002)	0.251	-0.112	-1,974	**0.049**	1,563
N/L ratio	0.000 (-0.665-0.665)	0.338	0.000	0.001	1,000	1,398
CRP/Alb ratio	3,741 (-0.164-7,645)	1,986	0.113	1,883	0.060	1,744
Number of comorbidities	0.480 (-2,757-3,717)	1,647	0.014	0.291	0.771	1,096
Surgery duration	-0.001 (-0.052-0.050)	0.026	-0.002	-0.038	0.969	1,117
Mortality (Reference: none)	12,834 (0.127-25,542)	6,465	0.094	1,985	**0.048**	1,084

Robust regression analysis. F=3,992, p<0.001, R^2=%14,82, β1: Unstandardized beta coefficient, β2: Standardized beta coefficient
ICU: Intensive care unit, CI: Confidence interval, Htc: Hematocrit, PNI: Prognostic nutrition index, N/L: Neutrophil-to-lymphocyte, CRP/Alb: C-reactive protein-to-albumin

Table 3. Robust regression analysis of factors affecting discharge time (Survivors)

	β1 (95% CI)	S. data	β2	t	p	VIF
Constant	9,382 (7.57-11,194)	0.922		10,180	0.000	
Age	0 (-0.013-0.014)	0.007	0.002	0.040	0.968	1,083
PNI	-0.101 (-0.125- -0.077)	0.012	-0.442	-8,226	**<0.001**	1,475
N/L ratio	0.006 (-0.025-0.037)	0.016	0.019	0.379	0.705	1,244
CRP/Alb ratio	0.126 (-0.034-0.285)	0.081	0.075	1,552	0.122	1,197
Number of comorbidities	0.181 (0.028-0.334)	0.078	0.105	2,321	**0.021**	1,046
Surgery duration	0.003 (0.001-0.006)	0.001	0.135	2,923	**0.004**	1,097

Robust regression analysis. F=11,287, p<0.001, R^2=%24,3, β1: Unstandardized beta coefficient, β2: Standardized beta coefficient
CI: Confidence interval, PNI: Prognostic nutrition index, N/L: Neutrophil-to-lymphocyte, CRP/Alb: C-reactive protein-to-albumin

Table 4. Logistic regression analysis of mortality risk factors

	Mortality		Univariate		Multiple	
	No	Yes	OR (95% CI)	p	OR (95% CI)	p
Age	63.26±10.34	68.65±9.49	1,057 (1,018-1,097)	**0.004**	1,058 (1,014-1,104)	**0.009**
Htc	46.30±133.04	36.80±6.47	0.92 (0.867-0.976)	**0.006**	0.979 (0.897-1,068)	0.631
PNI	49.66±7.38	45.37±10.18	0.935 (0.896-0.976)	**0.002**	0.955 (0.888-1,027)	0.215
N/L ratio	4.02±5.16	5.64±5.79	1,038 (0.992-1,087)	0.107	0.987 (0.91-1.07)	0.749
CRP/Alb ratio	0.48±1.07	0.68±1.15	1,141 (0.882-1,476)	0.315	0.888 (0.597-1,323)	0.561
Number of comorbidities	1.62±0.92	1.71±1.12	1,098 (0.76-1,587)	0.617	1,273 (0.817-1,984)	0.286
Surgery duration	225.32±60.71	228.18±67.36	1,001 (0.995-1,006)	0.796	1 (0.994-1,007)	0.899
ICU duration	57.34±68.47	112.68±122.86	1,005 (1,001-1,009)	**0.013**	1,003 (1-1,006)	0.077

Binary logistic regression analysis
ICU: Intensive care unit, CI: Confidence interval, Htc: Hematocrit, PNI: Prognostic nutrition index, N/L: Neutrophil-to-lymphocyte, CRP/Alb: C-reactive protein-to-albumin

Deficiencies in preoperative nutritional status increase the incidence of adverse postoperative events, such as increased risk of infection, prolonged mechanical ventilator support, and prolonged hospital stay. In patients with cardiac pathology, malnutrition is associated with fluid retention, inflammation, and neurohormonal activation, leading to poor prognosis in cardiac patients (11). In such cases, the preoperative nutritional index is a valuable marker of postoperative prognosis. Many nutritional indices are used in clinical practice; however, most of these indices use multiple parameters and are relatively difficult to calculate. Onodera's PNI score, however, is a simple index that can be derived using only absolute albumin and absolute lymphocyte counts, requires less time to calculate, and can be used routinely (12,13). One potential mechanism underlying the prognostic impact of PNI is that low PNI reflects hypoalbuminemia. Serum albumin was used to assess disease severity, progression, and prognosis. Another important factor is the important role of lymphocytes in the immunity of patients (14). PNI was initially proposed to assess the perioperative immunologic status and surgical risk in patients undergoing gastrointestinal surgery; however, it is currently used to assess prognosis in many clinical situations (14,15).

Currently, PNI is used for critical illnesses such as heart failure and malignancies (16,17). Recent studies have shown a significant association between decreased PNI levels and increased mortality rates in patients with cardiovascular conditions who undergo CABG surgery and that PNI is an important predictor of mortality in this group of patients (18).

Among other factors affecting surgical outcomes, preoperative nutritional status has the potential to influence outcomes. Despite its proven prognostic value for abdominal and gastrointestinal surgery, studies investigating the impact of PNI in cardiac surgery are still not very extensive. Impaired preoperative nutritional status is associated with increased morbidity and mortality rates after cardiac surgery, prolonged hospital stay, and decreased postoperative quality of life (1). Malnutrition in the preoperative period is a risk factor for poor outcomes after orthopedic, percutaneous coronary, and cardiovascular surgeries; therefore, measuring and screening for malnutrition in surgical patients are necessary. In fact, many patients are malnourished during the preoperative preparation period, but this condition is often not recognized. Although there are different nutritional assessment tools in clinical practice, the PNI is very easy to calculate and apply in the preoperative period. A variety of studies in the literature have reported different threshold values for PNI in different diseases (2,19,20). The mean PNI value for patients included in our research was 49.35±7.70, and it was identified that increases or reductions in PNI values were effective for prognostic factors. Research defined PNI<45 as moderate or severe nutritional deficiency, and the cutoff value may be 45. It has been reported that low PNI increases the risk of adverse events by 2-fold. Koyuncu and Koyun (21) investigated the effect of preoperative PNI levels on postoperative 1-month mortality in patients undergoing CABG surgery and reported that the mortality risk increased in patients with PNI values below 39.1. Similarly, in their study on patients undergoing hip arthroplasty, Tuncez et al. (22) found that the postoperative period and total hospital stay were longer in patients with low PNI levels (≤38.4) than in patients with high PNI levels and reported that the PNI value is a modifiable risk factor affecting the survival of patients. However, despite these recommendations, the optimal threshold value of PNI for predicting long-term outcomes remains unclear (14). The increase in postoperative morbidity and mortality after low PNI may be explained by functional changes in the immunologic system due to surgical stress, which is associated with physiologic homeostatic changes related to proinflammatory cytokines. Several recent studies have reported that lower PNI levels are significantly associated with higher mortality and morbidity in patients with cardiovascular disease, including coronary artery disease (23,24). According to the results of our research, a one-unit increase in the PNI value increased mortality risk by 1.07 times. Arai et al. (25) analyzed 146 patients undergoing elective cardiovascular surgery and found that patients with good preoperative nutritional status had better postoperative cardiac rehabilitation, and this group of patients had shorter lengths of hospitalization. In a study conducted by Lin et al. (26) on aortic dissection cases, it was found that patients with low PNI had higher in-hospital mortality, longer duration of stay on mechanical ventilators, and longer duration of stay in the ICU. For these patients, PNI≤41.6 was significantly and strongly associated with postoperative in-hospital mortality in cases of aortic dissection, even after adjustment for other risk factors (26). Similarly, studies on pediatric open-heart surgery patients have reported that hypoalbuminemia (<3 g/dL) and lymphocytopenia (<3000 mL) are important markers for poor prognosis criteria, such as prolonged mechanical ventilator support, infections, and high morbidity and mortality. In a study, Wakita et al. (13) identified a PNI cutoff value of 55 as a reliable marker and that infants with PNI<55 were included in the postoperative risk group.

PNI has become a promising prognostic biomarker for several diseases because it reflects the inflammatory status on one hand and the nutritional status on the other (3). For PNI, predicted to show poor postoperative outcomes, including few parameters, is an important advantage (4,12). Available evidence suggests that a low PNI value

may be a predictive marker for overall prognosis and postoperative complications after a surgical procedure, and the investigation of nutritional and immunologic status through PNI may be a useful clinical approach (4,27). A retrospective study by Cadwell et al. (28) found that the preoperative PNI value in patients with geriatric cancer aged >75 years could independently predict six-month postoperative mortality regardless of age, frailty, American Society of Anesthesiologists Performance Scale (ASA-PS), and metastasis. The role of PNI in predicting prognosis has received considerable attention in the last decade. A study of 453 patients undergoing cardiovascular surgery showed that low PNI significantly increased the risk of postoperative complications and shorter survival. In this study, the investigators set the cutoff value for PNI as 48 and showed that PNI values above this limit were statistically associated with shorter stays in the ICU and shorter intubation times (29). In our study, a one-unit increase in the PNI value reduced the ICU stay by 0.495. Similarly, Lee et al. (30) reported that a low PNI value alone can predict early mortality and morbidity in adult patients undergoing primary cardiac surgery. The authors stated that a low PNI value was also associated with a longer duration of mechanical ventilation and longer ICU duration. According to the results of this study, PNI values were found to be significantly lower in non-survivors (30). A study by Kim et al. (31) involving 132 lung transplant recipients reported that the preoperative PNI score was a useful prognostic marker for identifying high-risk lung transplant recipients. In this study, the survival of the group with a higher PNI value was found to be higher, and they stated that useful information could be obtained to reduce postoperative morbidity and mortality with preoperative nutritional evaluation using PNI (31). The relationship between malnutrition and poor prognosis in critically ill and ICU patients has long been known. In a study conducted by Kosovali et al. (19) on patients with COVID-19, albumin and lymphocyte levels were found to be significantly lower in mortal patients, and the PNI value was ≤42 in these patients. In the period after cardiovascular interventions, patients at nutritional risk are more likely to have chronic obstructive pulmonary disease and impaired renal function than those who are not at risk of malnutrition (32,33). In our research, a one-unit reduction in PNI increased mortality by 1.07-fold. Considering the increase in the number of high-risk patients undergoing elective heart surgery, nutritional management practices are recommended, like serum albumin and nutrient supplementation, for cases with preoperative low albumin levels in postoperative enhanced recovery and postoperative rehabilitation protocols (ERAS protocols). The study by Gucu et al. (34) mentioned the importance of the PNI score for optimal care to help predict surgical outcomes and select the right strategies in the preoperative period. They recommended preoperative intervention by a cardiometabolic team consisting of a cardiologist, internist, dietician, cardiovascular surgeon, and clinicians from other provinces for patients with low PNI scores (34).

In a cohort of elderly patients admitted for acute decompensated heart failure, a significant increase in both short- and long-term mortality was reported with low PNI (11,26). A study conducted by Keskin et al. (35) on 644 patients with CABG found that patients with low PNI had a 12-fold higher long-term mortality rate than those with high PNI. They reported that PNI was an independent prognostic factor for mortality among patients undergoing CABG (35). When the literature is reviewed, low PNI values were independently associated with 30-day mortality, 1-year mortality, and overall mortality after cardiac surgery, as reported in a study by Tóth et al. (36). In their study on pediatric open-heart surgery cases, Wakita et al. (13) found that intensive care bed prices were higher than normal ward bed prices and that even a one-day reduction in intensive care time reduced overall hospital costs. In this study, the authors concluded that improving the PNI score after determining a low PNI score for patients would be beneficial both medically and in terms of cost. In our study, as the PNI value decreased, ICU duration and discharge times were prolonged. Although our study design did not include the issue of cost, evaluation of ICU durations and discharge times in a detailed cost analysis based on PNI in our study may be instructive for such studies. One of the most basic studies in the literature on PNI is Onodera's study, in which a PNI value of 40 was associated with an increased incidence of postoperative complications (5). In our study, when postoperative adverse events were characterized, prolonged ICU time, prolonged discharge time, and 30-day mortality rates were all significantly correlated with low PNI. Our study is consistent with the literature in that it reveals this correlation with low PNI.

Study Limitations

Our study has some limitations. First, the inherent limitations of this single-center retrospective observational study should not be ignored. Another related limitation is the small sample size; however, this may be explained by the fact that our hospital has recently opened a cardiovascular surgery unit, and the date range for data screening was narrow. Finally, due to the retrospective study design, we could not predict whether prognosis-related outcomes would have changed after correction of low PNI values in patients who received appropriate medical treatment.

Conclusion

In this study, it was determined that changes in PNI values examined preoperatively in cardiac surgery patients were associated with prolonged postoperative intensive care period, prolonged discharge time, and increased 30-day mortality rates. Identifying patients with poor immunity and nutrition with preoperative PNI value seems to be useful in terms of reducing morbidity and mortality on a patient basis and optimizing intensive care and general hospital services in terms of hospital services. PNI is a simple systemic prognostic marker that can be calculated with objective quantitative data, and we believe that it will be more accepted in the future for many clinical situations, such as our cardiac surgery patients.

Ethics

Ethics Committee Approval: Permission for this retrospective study was obtained from the Ordu University Clinical Research Ethics Committee (approval no.: 2023/224, date: 01.09.2023).

Informed Consent: Retrospective study.

Footnotes

Authorship Contributions

Surgical and Medical Practices: A.K., M.U., E.C., Concept: A.K., N.T., M.U., E.C., I.C., Design: A.K., N.T., E.C., Data Collection or Processing: N.T., M.U., M.E.D., Analysis or Interpretation: A.K., E.C., I.C., Literature Search: A.K., N.T., I.C., M.E.D., Writing: A.K., N.T., M.U., E.C., I.C., M.E.D.

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Original Article

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Bianchi Scrotal Orchiopexy Method: An Alternative Surgical Technique for Undescended Testicles

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Abstract

Aim: Palpable undescended testis (PUT) is a common urological condition. Traditional inguinal orchidopexy is widely performed in many clinics for the treatment of this pathology. However, crotal orchidopexy is an effective and safe surgical procedure for PUT. We aimed to evaluate the surgical outcomes of patients who underwent a single transverse scrotal incision for PUT.

Methods: The data of 212 patients who underwent scrotal orchidopexy for PUT between 2018 and 2022 at our clinic were retrospectively analyzed. Unilateral cases of PUT located between the outer and inner inguinal rings or distal to the outer inguinal ring were included in the study. The analysis also considered postoperative complications and final cosmetic outcomes.

Results: The average age of the patients was 3.26 years. Of these, 112 (52.8%) cases were right-sided. A hernia sac was present in 168 (79.2%) patients. The testis was located distal to the outer ring in 150 (70.8%) cases and between the outer and inner rings in 62 (29.2%) cases. Postoperative complications included scrotal edema in 8 (3.8%), wound infection in 5 (2.4%) cases, and recurrence of testicular ascent in 3 (1.4%). Cosmetic satisfaction was reported by the families of 208 patients (98.1%).

Conclusion: Scrotal orchiopexy is an effective technique for managing PUT located between the outer and inner inguinal rings or distal to the outer inguinal ring.

Keywords: Scrotal incision, orchidopexy, undescended testis, children

Introduction

Undescended testis (UT) is a prevalent congenital anomaly in male neonates (1). UT is observed in 1%–4.6% of full-term male babies by the age of 1 year (2). This rate is significantly higher in premature boys, with approximately one-third of them having UT on at least one side (3). Approximately 80% of the UT cases are considered palpable and are typically located at the external inguinal ring, upper scrotum, or inguinal canal location (1). Traditional inguinal orchiopexy, which requires two incisions, is an established technique that is considered the standard for the treatment of UT. This process allows for adequate mobilization of the testes and spermatic vessels and ligation of the associated hernia (1, 4). However, the most significant complications following orchidopexy include psychological problems, testicular atrophy, increased cancer risk, and infertility rate (5).

To reduce the potential morbidity associated with the combined method, Bianchi and Squire (6) described a technique in 1989 that could be performed through a single scrotal incision. This technique is claimed to have the advantages of a single incision, shorter surgical time, ease of dissection, faster recovery, less pain, good preservation of testicular position, and excellent cosmetic results (7).

Palpable undescended testis (PUT) is a common urological condition. Traditional orchidopexy is widely performed in many clinics for the treatment of this pathology. In this study, we aimed to evaluate the results of scrotal orchidopexy (Bianchi method) performed with a single high transverse scrotal incision in cases where the PUT is located between the outer and inner inguinal rings or distal to the outer inguinal ring.

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Methods

Compliance with Ethical Standards

The study was approved by the Tokat Gaziosmanpasa University Faculty of Medicine Clinical Research Ethics Committee (approval no.: 24-KAEK-147, date: 18.04.2024).

Study Design

Bilateral PUT cases, those previously operated on the same side, and those located at the inner inguinal ring were excluded from the study. Unilateral PUTs located between the outer and inner inguinal rings or distal to the outer inguinal ring were included. Data from 212 patients who satisfied the criteria were evaluated.

We conducted a retrospective study from 2018 to 2022 of patients with PUT treated with scrotal orchidopexy. Medical reports of patients who underwent surgical intervention were retrospectively analyzed. The study was conducted in accordance with the Declaration of Helsinki. While recording patient data, patients were informed that these data would be used for scientific purposes, and written informed consent was obtained from each patient.

All patients who underwent surgery for PUT had the following data recorded: age, operation time, presence of hernia sac, side of UT, number of cases of testicular ascent, testicular atrophy, other complications, and cosmetic satisfaction recorded.

Surgical Technique

General anesthesia with endotracheal intubation or laryngeal mask was administered. All patients received prophylactic third-generation cephalosporin (50-100 mg/kg). The examination of the UTs of the patients is performed again under anesthesia for preliminary evaluation. In this technique, the testes are brought out through the incision line at the upper border of the scrotum, parallel to the scrotal skin fold. After dissecting cremasteric fibers, vascular structures and the vas deferens are exposed. Gubernacular attachments are divided if present. The processus vaginalis is then ligated and separated at the level of the outer inguinal ring. Finally, the testis was fixed into the dartos pouch using an absorbable suture from the scrotal skin to the tunica albuginea at the base of the appendix. The dartos fascia was reapproximated, and a subcuticular closure was performed.

It was noted that in most cases, dissection within the canal could be performed solely through externalization without the need to open the anterior wall of the inguinal canal, allowing for ligation and separation of the processus vaginalis, similar to that in inguinal orchiopexy. Moreover, if sufficient cord length could not be achieved, a second incision in the inguinal region could be easily performed. Nevertheless, a second incision was not necessary in any of the patients (Figure 1).

A total of 212 cases underwent scrotal orchidopexy surgery for PUT. Patients who were followed up in the hospital for 1 day after the operation were examined again before discharge and were discharged with appropriate treatment. A check-up was recommended 1 week later. All patients were followed up at 1, 3, and 12 months after surgery. During follow-ups, testis sizes, potential early and late complications, and cosmetic satisfaction of the families were recorded.

Statistical Analysis

Statistical analyses were performed using the MedCalc (version 20.009; Ostend, Belgium) statistical package

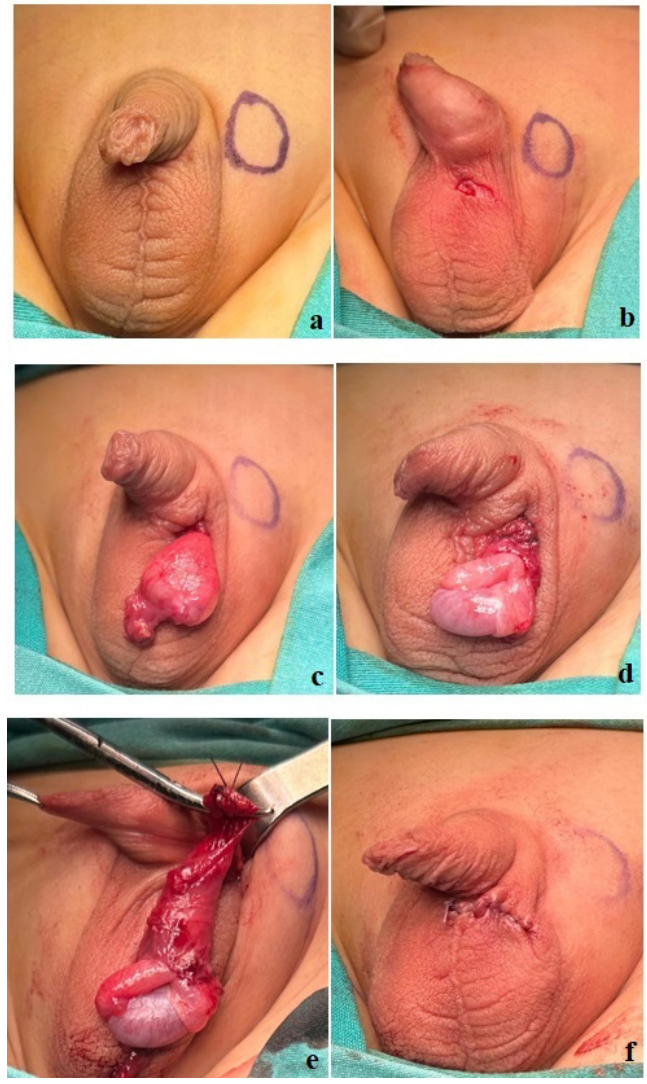

Figure 1. Stages of scrotal orchiopexy operation
a) Preoperative image showing the presumed locations of the external inguinal ring and testes. **b)** A preoperative image showing the presumed location of the scrotal incision. **c-e)** Intraoperative appearance of scrotal orchidopexy. **f)** Final cosmetic image

program. The number, mean, standard deviation, frequency, percentage, median, 25th, and 75th percentile values were used to describe the data statistically. In the evaluation of the numerical data, the Kolmogorov-Smirnov test was used to determine whether the groups conformed to the normal distribution. Fisher's exact test was used to evaluate categorical data. Significance level $p<0.05$ was taken for the interpretation of the results. Groups were presented graphically in the form of stacked percentage column charts.

Results

The mean age of the 212 patients who underwent scrotal orchidopexy was 3.26 years (1-8), and the mean operation time was 23.3 minutes. Of these, 112 (52.8%) cases were right-sided. A hernia sac was present in 168 (79.2%) and absent in 44 (20.8%) patients. The testis was located distal to the outer ring in 150 (70.8%) cases and between the outer and inner rings in 62 (29.2%) cases. Postoperative complications included scrotal edema in 8 (3.8%), wound infection in 5 (2.4%) cases, and recurrence of testicular ascent in 3 (1.4%). Testicular atrophy was not observed in any patient during follow-up at 1, 3, and 12 months postoperatively. However, no long-term complications or recurrences were observed during the 1-year follow-up period. In 208 patients (98.1%), cosmetic satisfaction was reported within the families (Table 1). No groin incision was performed in any patient. Statistically significant relationships were found between the location of the UT and its inguinal position as well as the presence of a hernia sac among patients who underwent scrotal orchiopexy. Specifically, the frequency of hernias was notably high in cases where the testis was located between the outer and inner rings ($p<0.001$) (Table 2).

All cases of scrotal edema and wound infection were resolved with medical treatment. Protrusions of the testicle were re-operated with the same scrotal incision and were then fixed to the scrotal sac. No groin incision was made in the reoperated patients, and there were no problems during follow-up.

Discussion

Orchiopexy is a common treatment procedure in the pediatric population (8). Early treatment of UT is crucial to prevent testicular degeneration, improve fertility, and facilitate the detection of malignancy (9). Traditionally, this procedure is performed through an inguinal procedure with a second incision in the scrotum for the insertion of the testicle. An inguinal incision is essential for adequate mobilization of the undescended testicle and ligation of the hernia sac. Through this incision, the inguinal canal can be easily accessed, cord structures can be identified, and the hernia sac can be dissected and treated with high-ligation. This procedure ensures that the spermatic cord is sufficiently long. A scrotal incision is made for placement of the testes into the dartos pouch and its fixation to the scrotum (10,11).

PUTs are palpable in the outer inguinal ring or more distal to it. The distance between the outer ring and the upper scrotum is short. Therefore, the idea of performing orchidopexy with a single scrotal incision was proposed. This technique was defined by Bianchi and

Table 1. Postoperative complications and cosmetic outcomes

		n	%
Hernia sac	**Seen**	168	79.2
	Not seen	44	20.8
Undescended testes	**Right**	112	52.8
	Left	100	47.2
Testicular location in the groin	**Location distal to the outer ring**	150	70.8
	Placement between the outer and inner rings	62	29.2
Scrotal edema	**Seen**	8	3.8
	Not seen	204	96.2
Testis atrophy	**Seen**	0	0
	Not seen	212	100.0
Wound infection	**Seen**	5	2.4
	Not seen	207	97.6
Testicular protrusion	**Seen**	3	1.4
	Not seen	209	98.6
Cosmetic satisfaction	**Satisfied**	208	98.1
	Not satisfied	4	1.9

Table 2. Relationship between undescended testis and testis position in the groin with hernia sac in patients undergoing scrotal orchiopexy

		Hernia Sac				p-value
		Seen		Not seen		
		n		n		
Undescended testes	Right	111	66.10%	1	2.30%	p<0.001*
	Left	57	33.90%	43	97.70%	
Testicular location in the groin	Location distal to the outer ring	111	66.10%	39	88.60%	p<0.001*
	Placement between the outer and inner rings	57	33.90%	5	11.40%	

*Significant difference at <0.05 level according to Fisher's Exact test

Squire (6) in 1989. The advantages of this method include a shorter surgical time, less postoperative pain, and fewer scars. The most suitable level for this method is around the outer inguinal ring or when the testes are located in the high scrotal region (6,7). This procedure is less invasive than the standard groin technique. It should be used in undescended testicles located distal to the outer inguinal ring (4,7) In our study, not only cases distal to the outer inguinal ring but also cases between the outer and inner inguinal rings were included, and this technique was successfully applied.

Hazebroek et al. (12) reported that in most cases of PUT, dissection of the cremaster and processus vaginalis allows sufficient length for the spermatic cord elements. Therefore, the testicles can be placed in the pouch of the dartos without tension. Callewaert et al. (13) suggested that the Bianchi method offers several advantages over the traditional inguinal procedure, owing to its faster operation and better cosmetic outcomes. The Bianchi method has been shown to be effective in the treatment of primary and secondary cryptorchidism, but the success of this process for inguinal hernias has not been confirmed (6,13). Redman (14) reported that the lack of widespread use of this technique is due to the complexity of ligation of the hernia sac with a high scrotal incision. However, the importance of ligation of the processus vaginalis in the treatment of UT remains controversial. Parsons et al. (15) reported in their scrotal orchiopexy series that an inguinal incision was necessary for ligation of the patent processus vaginalis, which was found in 20% of cases. On the other hand, several recent clinical studies on scrotal orchiopexy have found that, with traction on the sac, the patent processus vaginalis can be dissected from the cord structures through the inguinal canal (16,17). In another study, it was noted that dissection within the canal could be performed solely through externalization without the need to open the anterior wall of the inguinal canal, allowing for ligation and separation of the processus vaginalis, similar to that in inguinal orchiopexy. In our study, 79.2% of patients had a hernia sac. The hernia sacs were ligated through a single high scrotal incision. Excluding the patients who had previously undergone surgery on the same side and the cases in the inner inguinal ring and including the cases with PUT between the outer and inner inguinal ring and distal to the outer inguinal ring may have enabled us to reach this conclusion. However, the fact that all hernia sacs were ligated through a single high scrotal incision and that no inguinal incision was made in any case demonstrates the success of this technique.

In their series of 292 cases, Na et al. (18) reported that the success rate of single scrotal incision orchiopexy was 92.5% and that the hospital stay and operation times were shorter than those for traditional inguinal incision orchiopexy. Bassel et al. (16) reported that the average operation time was less than 20 minutes, and the success rate was 100%. In a similar study, Dayanç et al. (19) reported a success rate of scrotal incision orchidopexy of 94.4% with a short operating time. In our study, the operation time was compatible with the literature, and surgical (98.6%) and cosmetic success (98.1%) were higher. Despite the presence of a hernia sac in 79.2% of cases, it has been observed that the location of the palpable testes does not affect the success rate. In the 20-year transcrotal orchidopexy outcomes reported by Gordon et al. (7), 2.5% of patients required groin incision. In the study of Arena et al. (20), it was observed that 3.9% of cases initially approached transcrotally were converted to the inguinal approach. In our study, no patient underwent a transition to the traditional two-incision procedure.

Re-ascent postoperatively is a very important problem. According to the current literature, it is observed at rates ranging from 1% to 10%. In their study analyzing the effectiveness of inguinal and scrotal approaches in a series of 554 patients who underwent primary orchiopexy, Selin et al. (21) reported that the re-ascent rate was 7.2% and 3.1%, respectively, and that the surgical approach was not a predictive factor for re-ascent. In our study, the re-ascent rate was 1.4%, which was quite successful, according to the literature. In a series of 100 cases by Nazem et al. (22), scrotal and inguinal orchidopexy for PUT were compared. In their study, no difference was observed between the two methods in terms of testicular hypotrophy, testicular

atrophy, wound infection, and relapse rates. The study of Zouari et al. (23) reported that single scrotal-incision orchidopexy in PUT is an effective and reliable method. In the same study, 1% wound dehiscence, 5% scrotal hematoma, and 1% hernia recurrence were observed. In a similar study, Russinko et al. (24) reported an overall success rate of 98.8% and an overall complication rate of 4.7% for prescrotal orchidopexy. Our overall complication rate was also low (7.6%). In a systematic review by Novaes et al. (4), they reported that a single scrotal incision orchiopexy had a high efficacy rate, ranging from 88% to 100%. In our study, very successful results were obtained, including 98.1% cosmetic satisfaction.

Study Limitations

The limitations of this study include its small size, single-center, retrospective design, and short follow-up period. The results of the scrotal orchidopexy technique could not be evaluated using objective parameters. Additionally, the absence of a traditional inguinal orchidopexy group and the lack of a comparative analysis represent another significant limitation. Despite these limitations, our study demonstrates that scrotal orchiopexy is an effective and safe method for treating PUT across a broad age range in childhood.

Conclusion

Scrotal orchidopexy has some advantages; it is easy to learn and master. Minimal dissection and tissue destruction of the groin area. We conclude that this technique is safe and effective and can be an alternative to inguinal orchiopexy in cases in which the PUT is located between the outer and inner inguinal rings and distal to the outer inguinal ring. We would like to state that more prospective, randomized, controlled studies should be conducted to evaluate the efficacy of the scrotal orchiopexy technique.

Ethics

Ethics Committee Approval: The study was approved by the Tokat Gaziosmanpasa University Faculty of Medicine, Clinical Research Ethics Committee (approval no.: 24-KAEK-147, date: 18.04.2024).

Informed Consent: While recording patient data, patients were informed that these data would be used for scientific purposes, and written informed consent was obtained from each patient.

Footnotes

Authorship Contributions

Surgical and Medical Practices: K.Y., E.K., F.F., Concept: K.Y., E.K., F.F., Design: K.Y., E.K., F.F., Data Collection or Processing: K.Y., E.K., F.F., Analysis or Interpretation: K.Y., E.K., F.F., Literature Search: K.Y., E.K., F.F., Writing: K.Y., E.K., F.F.

Conflict of Interest: No conflicts of interest were declared by the authors.

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Original Article

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The Effects of Alexithymia on Self-Reflection and Insight in Major Depressive Disorder

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Abstract

Aim: We hypothesized that alexithymic depressive patients have low insight, which correlates with more severe depression and anxiety. In this context, we aimed to explore the correlation between insight and self-reflective abilities, alexithymia, as well as the presence and severity of major depression, which is the most diagnosed psychiatric disease in the world.

Methods: We accepted 80 patients diagnosed with major depression who were in outpatient care at our psychiatry clinic between September and December 2020, along with 80 healthy controls. We applied the Toronto Alexithymia Scale (TAS-20), Hamilton Anxiety Rating Scale, Hamilton Depression Rating Scale, and Self-Reflection and Insight Scale (SRIS) to all participants. This study was designed as a cross-sectional observational study.

Results: SRIS-insight score was found to be lower ($p<0.001$) in the patient group; higher scores were observed for difficulty in identifying feelings, difficulty in describing feelings, and the TAS-20 total score ($p<0.001$). TAS-20-total and subscales were found to be predicted by SRIS-insight in both groups ($p<0.001$; $p<0.01$).

Conclusion: When clinicians evaluate alexithymic patients with major depression, they need to consider this alongside symptom evaluation, as these patients may have low insight.

Keywords: Depressive disorder, alexithymia, anxiety, consciousness

Introduction

Major depression is a psychiatric disorder that impacts various aspects of life, including sleep, appetite, psychomotor activity, and sexuality (1). In Turkey, it ranks as the third most prevalent cause of disability (2). According to Taylor et al. (3), alexithymic traits involve difficulties in identifying and articulating emotions, trouble distinguishing between physical sensations and feelings of emotional arousal, limited imagination characterized by externally oriented cognition, and a lack of fantasy. Self-reflection, self-regulation, and insight involve establishing objectives, formulating strategies, implementing actions, evaluating progress, and deriving insights through comparison with the situations of others, as suggested by Grant et al. (4). Insight is not merely an understanding of the illness but a continuous process of awareness and comprehension of how the disease affects a person's interactions with the world (5). Early diagnosis and treatment of depression are crucial, especially to prevent disability and cognitive impairment. Studies have shown that recurrence rates are higher in depressive disorders where insight is low (6). Additionally, research has indicated that the presence and severity of depression negatively impact cognitive functions even during the first untreated depressive episode (7). Given these considerations, it is vital for clinicians to diagnose major depressive disorders early. Exploring how personality traits, such as alexithymia, self-reflection, and insight-key topics of our research-affect depression may provide valuable insights. We hypothesized that alexithymic depressive patients have low insight, which correlates with more severe depression and anxiety.

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Our study aimed to explore the correlation between the severity of alexithymia and the levels of self-reflection and insight among patients diagnosed with major depression. We sought to uncover associations between patients' depressive symptoms, anxiety levels, alexithymia severity, characteristics, insight, and self-reflection abilities.

Methods

Compliance with Ethical Standards

This study was approved by the Clinical Research Ethics Committee of University of Health Sciences Turkey, Haydarpasa Numune Training and Reseacrh Hospital (approval no.: 2020/KK/184, dated 31.08.2020). All participants provided informed consent.

Study Design

This study followed a cross-sectional observational design and commenced after receiving ethics committee approval. Figure 1 displays the study flowchart. Between September and December 2020, we conducted a study with 80 patients diagnosed with major depressive disorder, aged 18 to 65, randomly selected from the psychiatry outpatient clinic. The control group consisted of 80 individuals within the same age, education, and socio-economic range, confirmed to have no psychiatric diagnoses through the Structured Clinical Interview for the Diagnostic and Statistical Manual of Mental Disorders-V (DSM-V) (SCID-V).

Both groups completed the Sociodemographic and Clinical Data Form, Toronto Alexithymia Scale (TAS-20), Hamilton Anxiety Rating Scale (HAMA), Hamilton Depression Rating Scale (HAMD), and Self-Reflection and Insight Scale (SRIS).

Structured Clinical Interview for DSM-V

The Structured Clinical Interview for DSM-V (SCID-V), consisting of 10 modules and 32 diagnostic categories, is a clinician-administered scale used to assess a wide range of psychopathologies. These include psychotic disorders, mood disorders, substance use disorders, anxiety disorders, obsessive-compulsive spectrum disorders, post-traumatic stress disorder, and more (8).

Hamilton Depression Rating Scale

Developed by Hamilton, the Hamilton Depression Rating Scale (HAMD) has been widely used for over five decades to evaluate the severity of depressive symptoms. Scores range from 0 to 51, with higher scores indicating greater severity. Akdemir et al. (9) validated and adapted the scale for the Turkish population.

Hamilton Anxiety Rating Scale

The Hamilton Anxiety Rating Scale (HAMA) measures the severity of anxiety symptoms. Scores of 0-5 indicate no anxiety, 6-14 indicate mild anxiety, and 15 or more reflect significant anxiety. Yazıcı et al. (10) conducted a validity and reliability study for the Turkish version.

Toronto Alexithymia Scale

The Toronto Alexithymia Scale (TAS-20) is a 20-item scale that assesses alexithymia across three subscales: "externally oriented thinking", "difficulty describing

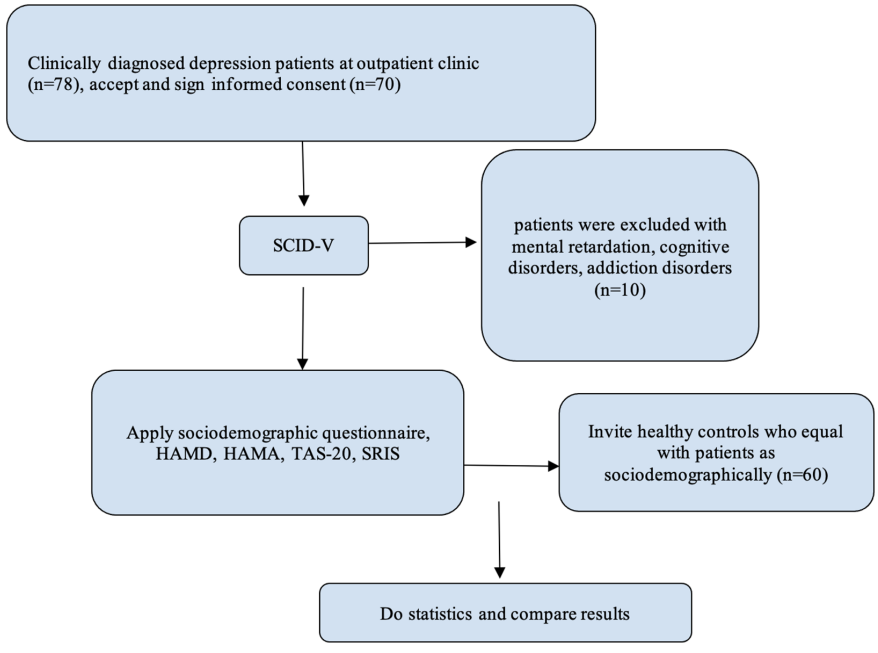


Figure 1. Our study's flow chart
SCID-V: Structured clinical interview for DSM-5

feelings", and "difficulty identifying feelings". Responses range from 1 to 5, with higher scores indicating greater alexithymia. The Turkish validity and reliability study was conducted for TAS-20 (11).

Self-Reflection and Insight Scale

The Self-Reflection and Insight Scale (SRIS) consists of 20 items assessing two subdimensions: insight and self-reflection. Six items measure self-reflection, while eight items evaluate insight. The validity and reliability of the scale have been established (12).

Statistical Analysis

Data were analyzed using IBM SPSS Statistics 22 (IBM SPSS, Turkey). The normal distribution of variables was assessed using the Kolmogorov-Smirnov test, Q-Q plots, and histograms. Descriptive statistical methods, including minimum, maximum, mean, standard deviation, median, frequency, and percentage, were used. Student's t-test was applied for comparing normally distributed quantitative data. The Mann-Whitney U test was used for non-normally distributed data. Qualitative data were analyzed with Fisher's exact test, the continuity (Yates) corrected chi-square test, and the Pearson's chi-squared test. The Pearson and Spearman's Rho correlation analyses assessed relationships between scale scores. Linear regression analysis examined the predictive value of scale scores. Statistical significance was set at $p<0.05$.

Results

Table 1 presents the sociodemographic data. An analysis of the scale data revealed that the sub-dimensions "difficulty describing feelings" and "difficulty identifying feelings," as well as the total TAS-20 score, were significantly higher in the patient group compared to the control group ($p<0.001$). However, no significant difference was found in the "externally oriented thinking" subscale between the two groups ($p=0.073$) (Graphic 1).

The SRIS-insight subscale mean score was significantly lower in the patient group ($p<0.001$), while no significant difference was observed in SRIS-self-reflection scores ($p=0.703$) (Graphic 2).

In both groups, a significant negative correlation was found between SRIS-insight and the TAS-20 total score, as well as its sub-dimensions ("difficulty describing feelings," "difficulty identifying feelings," and "externally oriented thinking") ($p<0.001$) (Table 2).

Regression analysis indicated that, in both groups, the TAS-20 total score and all its sub-dimensions were negatively predicted by SRIS-insight. Additionally, in the patient group diagnosed with major depression ($R^2=0.401$; $p<0.001$), the insight score more strongly predicted the TAS-20 total score and its sub-dimensions ($R^2=0.422$; $p<0.001$) (Tables 3 and 4).

Table 1. Socio-demographic datas

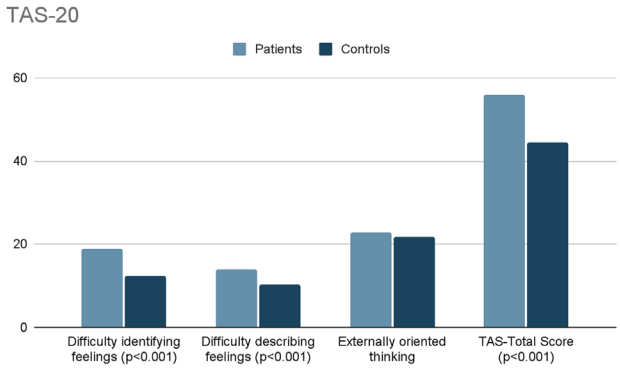
Socio-demographic features		Patients	Controls	Total	Test value	p-value
Age of marriage (years) (n=88)	**Min.-Max.**	13-33	18-33	13-33	2-2,642	0.011*
	Mean±SD	23.37±4.49	25.69±3.72	24.56±4.25		
Number of kids (n=88)	No kids	8 (18.6%)	18 (40%)	26 (29.5%)	15,989	0.112
	One kid	10 (23.3%)	11 (24.4%)	21 (23.9%)		
	Two kids	14 (32.6%)	10 (22.2%)	24 (27.3%)		
	Three and more	11 (25.6%)	6 (13.3%)	17 (19.3%)		
Have own room in childhood	Yes	38 (47.5%)	41 (51.3%)	79 (49.4%)	10.225	0.635
	No	42 (52.5%)	39 (48.8%)	81 (50.6%)		
Diagnosed a psychiatric disorder before	Yes	34 (42.5%)	15 (18.8%)	49 (30.6%)	**10,620**	**0.001****
	No	46 (57.5%)	65 (81.3%)	111 (69.4%)		
Have a family member who has somatic complainings	Yes	34 (42.5%)	35 (43.8%)	69 (43.1%)	0.025	0.873
	No	46 (57.5%)	45 (56.3%)	91 (56.9%)		
Apply to a doctor because of somatic complainings	Always/often	9 (11.3%)	3 (3.8%)	12 (7.5%)	**14,695**	**0.002****
	Sometimes	34 (42.5%)	36 (45%)	70 (43.8%)		
	Rarely	13 (16.3%)	2 (2.5%)	15 (9.4%)		
	None	24 (30%)	39 (48.8%)	63 (39.4%)		
Self injury behaviour	Yes	22 (27.5%)	4 (5%)	26 (16.3%)	**13,272**	**<0.001****
	No	58 (72.5%)	76 (95%)	134 (83.8%)		

*Pearson Correlation Coefficient, **p<0.01

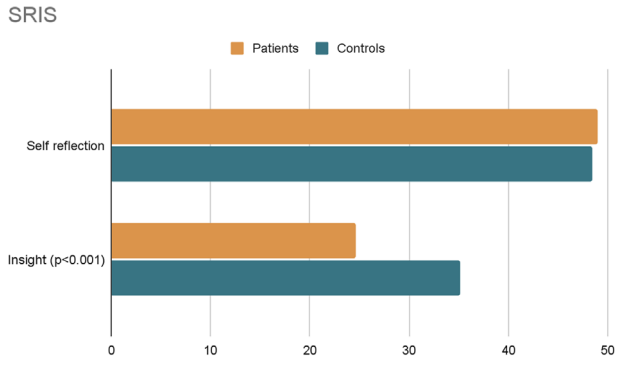
Discussion

Our findings indicate that alexithymia and its subdimensions increase as insight decreases. Regression analysis further demonstrated that low insight in depression is a strong predictor of higher alexithymia scores.

Although limited, studies examining the correlation between insight and alexithymia suggest a similar trend.



Graphic 1. Comparison of TAS-20 scores of study and control groups
Student's t-test, TAS-20: Toronto Alexithymia Scale-20



Graphic 2. Comparison of SRIS scores of study and control groups
Student's t-test, SRIS: Self Reflection and Insight Scale

Research in non-clinical populations has shown that insight decreases as alexithymia increases, as measured by the SRIS (4). While studies on insight have predominantly focused on psychotic disorders, other psychiatric disorders have also been explored (13-15). A study in Turkey similarly identified a negative correlation between increased insight and alexithymic traits (16). Consistent with these findings, our study revealed that higher levels of alexithymia were associated with reduced insight in both groups.

Interestingly, our study also showed that insight was a more potent predictor of alexithymia in patients with major depression. Unlike prior studies, our results indicated that all aspects of alexithymia, including "difficulty identifying feelings", "difficulty describing feelings", and "externally oriented thinking", increased as insight decreased in patients with depression.

The link between alexithymia and depression has been well-established in the literature, with various studies highlighting distinct but interrelated structures (17-19). A prospective study demonstrated that individuals with higher alexithymia levels are more likely to develop depression over time (20). A 2021 review also confirmed a strong correlation between alexithymia and depression severity (21). Furthermore, alexithymia has been found to play a critical role in the somatization of emotions in depression (22).

A study examining the relationship between alexithymia, anxiety, stress, and depression emphasized the importance of these findings for targeted treatments (23). However, similar to our study, other research found no significant association between depression and the "externally oriented thinking" sub-dimension of alexithymia (24,25).

Insight plays a critical role in the management of depression, as it is associated with treatment adherence (26,27). Studies suggest that patients with major depressive disorders who lack insight are more likely to experience recurrent episodes (28). Strong self-reflection skills, which involve frequent evaluation of thoughts, behaviors, and

Table 2. Correlation of Toronto Alexithymia Scale-20 with Self Reflection and Insight Scale scores in both groups

Scale/Questionnaire	Sub-scale	Group	Toronto Alexithymia Scale			
			Difficulty identifying feelings	Difficulty describing feelings	Externally oriented thinking	TAS-Total
			r; p	r; p	r; p	r; p
Self Reflection and Insight Scale (SRIS)	Self reflection	Patients	0.106; 0.351	0.029; 0.798	-0.225; 0.056	-0.028; 0.805
		Controls	0.038; 0.735	-0.062; 0.585	**-0.431; <0.001****	-0.194; 0.085
	Insight	Patients	**-0.554; <0.001****	**-0.407; <0.001****	**-0.301; <0.001****	**-0.627; <0.001****
		Controls	**-0.561; <0.001****	**-0.604; <0.001****	**-0.338; <0.001****	**-0.642; <0.001****

Pearson and Yates Chi-square test, **p<0.01

emotions, are linked to better emotional regulation and lower susceptibility to dysphoria and depression (29).

Interestingly, our study found that increased self-reflection in the non-depressed group predicted a reduction in externally oriented thinking. This finding aligns with the hypothesis that self-reflection can mitigate maladaptive thought patterns, although excessive self-reflection may also lead to anxiety and depressive symptoms (4,30).

In our study, as self-reflection increased in the non-depressed group, externally oriented thinking reduced; in fact, the increase in self-reflection skills predicted that the severity of externally oriented thinking would reduce.

Anxiety often accompanies depression and significantly affects the disease's prognosis (31). In patients with depression, insight levels were observed to decrease as psychotic-like experiences and anxiety levels increased, with anxiety mediating this relationship (32). In our study, we similarly observed that as anxiety levels increased in patients with depression, their insight levels decreased. This suggests that anxiety levels should be carefully considered when assessing patients with depression.

Table 3. Regression analysis of TAS-20 and SRIS in the patient group

TAS-20 (Dependent variables)	SRIS (Independent variables)	Coefficient	SE	t	p	CI 95%	
						Lower bound	Upper bound
Difficulty identifying feelings	Constant	25.251	3.519	7.176	**<0.001****	18.244	32.259
	Self reflection	0.147	0.064	2.286	**0.025***	0.019	0.275
	Insight	-0.548	0.086	-6.357	**<0.001****	-0.719	-0.376
Difficulty describing feelings	Constant	17.477	2.286	7.646	**<0.001****	12.925	22.029
	Self reflection	0.042	0.042	1.013	0.314	-0.041	0.126
	Insight	-0.227	0.056	-4.054	**<0.001****	-0.338	-0.115
Externally oriented thinking	Constant	30.967	2.444	12.671	**<0.001****	26.101	35.833
	Self reflection	-0.095	0.045	-2.132	0.056	-0.184	-0.006
	Insight	-0.139	0.060	-2.318	**0.023***	-0.258	-0.020
TAS-20 total	Constant	73.696	5.199	14.174	**<0.001****	63.342	84.049
	Self reflection	0.094	0.095	0.990	0.325	-0.095	0.284
	Insight	-0.913	0.127	-7.174	**<0.001****	-1.167	-0.660

SE: Standard error, CI: Confidence interval, *p<0.05, **p<0.01
TAS-20: Toronto Alexithymia Scale; SRIS: Self-reflection and insight scale

Table 4. Regression analysis of TAS-20 and SRIS in the control group

TAS-20 (Dependent variables)	SRIS (Independent variables)	Coefficient	SE	t	p	CI 95%	
						Lower bound	Upper bound
Difficulty identifying feelings	Constant	24.027	2.775	8.658	**<0.001****	18.501	29.553
	Self reflection	0.051	0.039	1.309	0.194	-0.026	0.128
	Insight	-0.403	0.066	-6.144	**<0.001****	-0.533	-0.272
Difficulty describing feelings	Constant	17.477	2.286	7.646	**<0.001****	12.925	22.029
	Self reflection	0.042	0.042	1.013	0.314	-0.041	0.126
	Insight	-0.227	0.056	-4.054	**<0.001****	-0.338	-0.115
Externally oriented thinking	Constant	34.914	2.617	13.342	**<0.001****	29.703	40.125
	Self reflection	-0.144	0.037	-3.945	**<0.001****	-0.217	-0.072
	Insight	-0.175	0.062	-2.835	**0.006****	-0.298	-0.052
TAS-20 Total	Constant	79.600	5.244	15.181	**<0.001****	69.159	90.042
	Self reflection	-0.085	0.073	-1.164	0.248	-0.231	0.061
	Insight	-0.887	0.124	-7.158	**<0.001****	-1.133	-0.640

*p<0.05, **p<0.01
SE: Standard error, CI: Confidence interval, TAS-20: Toronto Alexithymia Scale, SRIS: Self-Reflection and Insight Scale

Our study also found that self-reflection levels increased in patients with depression. However, Grant et al. (4) noted that self-reflection is not always an adaptive function; excessive self-reflection may lead to heightened anxiety and depressive symptoms. Another study observed that individuals who engaged in intense ruminative thinking over three days experienced greater self-doubt and developed a negative self-perception (33).

These findings may serve as both a theoretical framework and empirical support for current transdiagnostic treatments targeting depressive and anxiety symptoms. Such interventions could include methods designed to enhance patients' self-insight. In our study, increased self-reflection in the non-depressed group was associated with reduced externally oriented thinking. Furthermore, improvements in self-reflection skills were found to predict a reduction in externally oriented thinking severity.

Study Limitations

Our study has several limitations. First, it was conducted during the coronavirus disease-2019 pandemic. As a result, participant interviews were carried out under precautionary measures (e.g., maintaining social distancing and limiting interaction time), which may have constrained the depth and application of interviews and scales. Second, the use of self-report scales may have introduced bias, as these scales rely on patients' subjective evaluations, which can sometimes lead to exaggerated symptoms. Third, participants in the patient group were included regardless of their history of previous depressive episodes. The relationship between alexithymia and depression remains controversial; thus, it is unclear whether alexithymia preceded the depressive episode or emerged as a consequence. A prospective study might provide more clarity on this relationship, as well as on treatment adherence and attitudes toward the treatment team in psychiatric conditions.

Despite these limitations, our study has significant strengths. Insight has been explored in only a few studies outside of psychotic disorders, and its role in depression has received limited attention in the literature. Furthermore, while most studies employing the SRIS scale for insight research have focused on non-clinical samples, our study included a clinical sample, adding value to the findings. Lastly, to our knowledge, this is the first study in Turkey to examine insight in patients with depression using the SRIS scale.

Conclusion

Investigating the predictors of insight, an essential factor in the treatment of major depressive disorders, is of great importance. Our study demonstrated that assessing alexithymia can help predict not only the severity of depression and anxiety but also critical personality traits such as insight and self-reflection. These findings underscore the need for targeted interventions that address alexithymia, enhance insight, and promote self-reflection. Such interventions may improve treatment adherence and outcomes in patients with major depressive disorders. Further research, particularly longitudinal and interventional studies, is recommended to expand upon these findings and to develop more comprehensive treatment approaches.

Ethics

Ethics Committee Approval: This study was approved by the Clinical Research Ethics Committee of University of Health Sciences Turkey, Haydarpasa Numune Training and Reseacrh Hospital (approval no.: 2020/KK/184, dated 31.08.2020).

Informed Consent: All participants provided informed consent.

Footnotes

Authorship Contributions

Surgical and Medical Practices: A.Y.O., Concept: A.Y.O., M.Z.E., Design: A.Y.O., M.Z.E., Data Collection or Processing: A.Y.O., Analysis or Interpretation: A.Y.O., Literature Search: A.Y.O., Writing: A.Y.O., M.Z.E.

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Case Report

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Complete Heart Block Following Anaphylaxis: A Case Report and Literature Review

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Abstract

Allergic reactions can range from mild symptoms to life-threatening anaphylaxis, and they may involve significant cardiovascular consequences. Anaphylaxis can cause disturbances in the conduction system, including complete heart block. This case report describes a patient who developed complete heart block following the administration of cefixime, a third-generation cephalosporin. The condition required the insertion of a pacemaker during the patient's follow-up. This report underscores the importance of monitoring and managing cardiovascular effects in patients experiencing severe allergic reactions, particularly when using certain antibiotics like cefixime.

Keywords: Anaphylaxis, third-generation cephalosporin, complete heart block, Kounis syndrome

Introduction

Conduction disorders in the atrioventricular (AV) node may be transient, intermittent, or permanent. They may be due to physiological changes, such as increased vagal tone, or they may occur due to pathological causes, such as congenital ischemic heart disease, valve diseases, and iatrogenic drugs. Cases of AV block that develop due to drugs and require permanent pacemaker insertion have been reported in the literature (1,2). Currently, there are no specific case reports in the available literature directly associating cefixime with AV block. However, other cephalosporins, such as ceftriaxone, are associated with cardiovascular events, usually anaphylaxis or arrhythmias resulting from histamine release (3). Although cefixime is generally regarded as safe, rare cases of cardiovascular side effects, including conduction disorders, have been reported, similar to other antibiotics. This article presents a case of complete heart block induced by cefixime, a third-generation cephalosporin, which required pacemaker implantation during follow-up.

Case Presentation

A 60-year-old male patient presented to the emergency department in May 2022 with widespread rash, redness, itching, and fainting following cefixime administration. He had undergone prostate surgery for benign hyperplasia two weeks prior, and the doctor prescribed cefixime for postsurgical fever and chills. Upon his second presentation, he was referred to the cardiogenic shock clinic. Physical examination revealed bradycardia, hypotension, and altered consciousness. Electrocardiography revealed complete AV block, and the patient was subsequently taken to the coronary angiography laboratory (Figure 1). The patient was promptly managed with transvenous temporary pacemaker implantation via the femoral venous system. Coronary angiography was performed to rule out ischemic causes of complete heart block. The examination revealed no coronary lesions. Laboratory tests showed no abnormalities that could explain the AV block, and echocardiography did not reveal any valve pathology. During the patient's follow-up, it was noted that he had no previous drug allergies, and a complete

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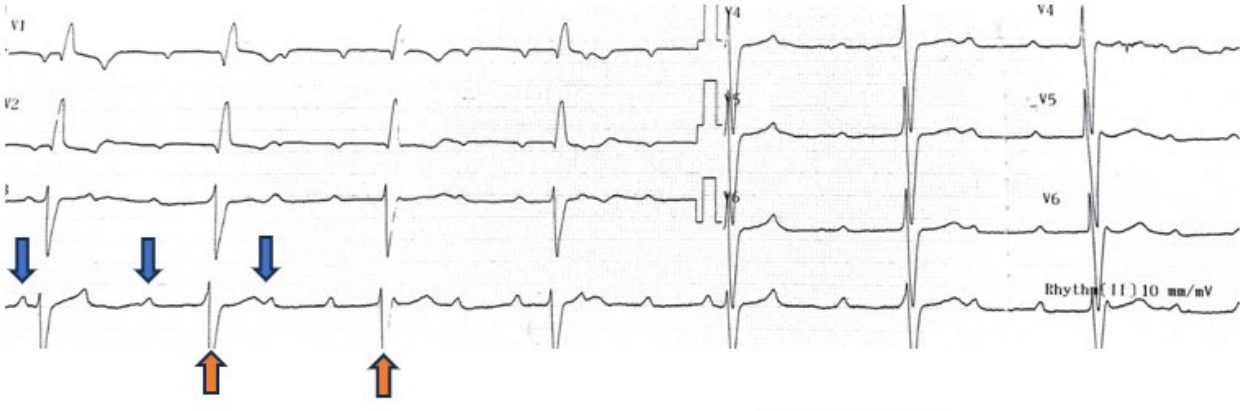

Figure 1. Admission electrocardiogram showing complete atrioventriculer block

heart block developed after the first dose of cefixime. The patient did not respond to atropine, and he was not receiving rate-limiting medications, such as beta-blockers, calcium channel blockers, or digoxin. Despite management with a temporary pacemaker for 5 days, complete AV block persisted. Consequently, they discharged him and implanted a permanent pacemaker. An informed consent form was obtained from the patient.

Discusion

AV block can be classified as physiological, pathological, idiopathic, or iatrogenic. In approximately 50% of cases, the etiology remains unknown and is classified as idiopathic. Iatrogenic AV block can occur after invasive procedures, such as cardiac surgery, catheter ablation, or transcatheter aortic valve replacement, as well as from medications that affect AV conduction. These include beta-blockers, calcium channel blockers, digoxin, adenosine, and antiarrhythmic drugs, although other non-cardiac medications may also affect conduction. Additionally, cases of acute coronary syndrome (Kounis syndrome) associated with anaphylaxis and mast cell activation due to antibiotics and other allergens have been documented (4,5). Complete AV block associated with Kounis syndrome has been previously reported in the literature (6). Studies on guinea pigs have demonstrated that anaphylactic reactions can adversely affect the conduction system. During anaphylaxis, the heart is one of the main organs that is affected. The chemicals that are released during this process can cause coronary vasospasm, arrhythmias, poor ventricular contractility, and negative inotropic effects. Histamine, through H1 receptor stimulation, causes a delay in AV conduction and constriction of epicardial coronary vessels (7). In addition to histamine, studies in guinea pigs have demonstrated that elevated endogenous adenosine levels resulting from anaphylaxis may also impair AV conduction and contribute to the development of heart block (8). The literature also reports cases of transient 2:1 AV blocks associated with contrast media-induced anaphylaxis (9). Anaphylaxis can have a wide range of clinical manifestations, ranging from mild allergic symptoms to severe cardiogenic shock following exposure to an allergenic agent. However, clinical conditions typically improve with the discontinuation of the offending substance (10). In our case, the AV block persisted. It is possible that the anaphylactic reaction intensified the underlying disease. Electrophysiological studies can be valuable in identifying organic conduction disorders.

Conclusion

This case highlights the potential for serious cardiovascular complications, such as complete heart block, following anaphylaxis and highlights the need to increase awareness of the cardiovascular effects associated with anaphylaxis.

Ethics

Informed Consent: An informed consent form was obtained from the patient.

Footnotes

Authorship Contributions

Surgical and Medical Practices: Y.A., A.S., Concept: N.A., Y.A., A.S., Design: N.A., Y.A., A.S., Data Collection or Processing N.A., Y.A., A.S., Analysis or Interpretation: Y.A., A.S., Literature Search: N.A., Y.A., A.S., Writing: N.A.

Conflict of Interest: No conflicts of interest were declared by the authors.

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Letter to the Editor

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Evaluation of ChatGPT's Performance in the Turkish Board of Orthopaedic Surgery Examination

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Dear Editor,

I read Ahmet Yigitbay intriguing work on the artificial intelligence tool ChatGPT. The widespread use and accessibility of artificial intelligence tools will certainly affect medical education. Currently, many medical students use artificial intelligence tools that convert voice recordings into text and pictures instead of taking notes in lectures (1). I believe that in the near future, artificial intelligence will have a place in all areas of the education process, such as learning, teaching, and evaluation. The fact that ChatGPT was not as successful as humans in answering the questions in this study conducted by Yigitbay suggests that these tools have not yet matured for widespread use.

In the method section of the study, the author stated that the TOTEK exam questions between 2019 and 2023 were asked to be answered via ChatGPT. It is understood that questions were asked of ChatGPT in 2024, the year this study was conducted. However, when analyzing by years in the results section, it is understood as if these questions were asked to ChatGPT every year between 2019 and 2023. This is particularly evident in the third paragraph of the Results. In the last sentence of this paragraph, it is stated that this variability may stem from changes in the datasets used to train the model, updates to the model itself, or differences in the complexity of exam questions across the years (2). Since all questions were asked of ChatGPT at the same time (2024), it cannot be considered that the artificial intelligence training data has changed.

Footnotes

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