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SDÜ Tıp Fakültesi Dekanlığı / 32260 ISPARTA
Tel: 0 246 2113714 - 2113230 - Fax: 0 246 2371165
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Contents

Research Articles

ORIGINAL RESEARCH

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The Effect of Central Lymph Node Dissection Before Thyroidectomy on Incidental Parathyroidectomy in Patients with Thyroid Cancer

Furkan SAVAS[1], Servet KOCAOZ[1], Mustafa Ömer YAZICIOGLU[1], Ahmet Fırat BOZDOGAN[1], Birol KORUKLUOGLU[2]

[1] Ankara City Hospital, Department of General Surgery, Breast and Endocrine Surgery Clinic, Ankara, Türkiye.
[2] Health Sciences University Faculty of Medicine, Department of General Surgery, Istanbul, Türkiye.

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Abstract

Objective
It is the fact that the incidence of thyroid cancer has been increasing recently. Thyroid cancer often tends to metastasize to the cervical lymph nodes and as a result, central region lymph node dissection increases the risk of incidental parathyroidectomy. In this study, the effect of two different surgical techniques on incidental parathyroidectomy (IP) has been investigated.

Material and Method
A total of 115 patients who underwent bilateral total thyroidectomy (BTT) and cervical neck dissection (CND) for thyroid cancer at the Department of General Surgery Clinics have been included in the study. Patients were divided into two groups according to the surgical technique used. The first group consisted of patients who underwent CND after thyroidectomy. The second group consisted of patients who underwent lateral neck dissection (LND) before CND or then underwent CND after ligation and transection of the middle thyroid vein and, if necessary, the superior thyroid artery.

Results
Incidental parathyroidectomy (IP) has been identified in the pathology specimen in 47.4% (54) of the patients. While IP was performed on 1 gland in 29.8% (34) of the patients in group 1, on 2 glands in 14% (16), and on 3 glands in 3.5% (4) of the patients, no IP was detected in group 2 ($p < 0.001$). It was observed that hypoparathyroidism did not develop in patients in the second group. However, 12.3% (14) of the patients who underwent BTT followed by CND developed transient hypoparathyroidism and 3.5% (4) of them developed permanent hypoparathyroidism. When the collected data is analyzed, it has been concluded that the tumor size and the diameter of the largest metastatic lymph node are significantly larger in men than in women ($p < 0.001$ and $p < 0.001$, respectively).

Conclusion
IP is commonly encountered in thyroid surgery. It is concluded that performing CND and LND before thyroidectomy might reduce IP.

Keywords: Thyroid carcinoma, lymph node metastasis, central compartment lymph node dissection, postoperative hypoparathyroidism, incidental parathyroidectomy

Correspondence: F. S. / opdrfurkansavas@gmail.com
Received: 13.02.2024 • Accepted: 25.11.2024
ORCID IDs of the Authors: F. S: 0000-0002-3963-7784, S.K: 0000-0002-0085-2380
Ö.Y: 0000-0001-6150-0226, A.F.B: 0000-0002-5609-6975, B.K: 0000-0003-4164-6898

Introduction

It is a known fact that well-differentiated thyroid cancers include papillary, follicular, and Hurthle cell carcinomas. Also, other thyroid cancers include medullary thyroid cancer (MTC) and anaplastic thyroid cancer (1, 2). Over the past decade, the incidence of papillary thyroid cancer (PTC), which accounts for 80-85% of thyroid malignancies worldwide, has steadily increased (3, 4). The prognosis of PTC is generally more favorable when it is compared to other thyroid malignancies (4, 5). In PTC, the incidence of cervical lymph node metastasis (CLNM) can reach 20-50% (6, 7). In PTC patients, CLNM is the primary risk factor for high recurrence rates (8, 9). The presence of CLNM is a positive predictive factor independent of tumor size (10, 11). Other factors influencing CLNM include gender (male), age (<45 years), bilaterality, extrathyroidal extension, multifocality, tumor size >1 cm, and the presence of capsular or lymph vascular invasion in the tumor (10-13).

MTC is a neuroendocrine neoplasm that originates from the parafollicular C-cells of the thyroid. It frequently metastasizes to cervical lymph nodes (14). Serum calcitonin levels are directly proportional to tumor size and the number of metastatic lymph nodes (15). Therefore, the decision to perform lymph node dissection is based on the assessment of serum calcitonin levels (16).

Some studies have reported that the female gender is an independent risk factor for hypoparathyroidism (17, 18), while other studies have failed to obtain similar results (19, 20). During central neck dissection (CND), temporary or permanent hypoparathyroidism is thought to occur due to traumatic or ischaemic damage (21, 22). This condition may also result from an incidental parathyroidectomy performed accidentally during lymph node dissection (23). In addition, whether CND is performed unilaterally or bilaterally may have different effects on transient and permanent hypoparathyroidism (24). However, some studies do not report a significant relationship between postoperative hypoparathyroidism and CND (25, 26). The short vertical length of the thyroid gland and the presence of small nodules increase the risk of incidental parathyroidectomy (27). Surgeon experience and higher volumes of thyroid surgery performed in surgical centers lead to a reduction in incidental parathyroidectomy (28). For these reasons, in this study, the effects of thyroid capsule invasion (CI), lymph vascular invasion (LVI), and multicentricity on LNM and the largest metastatic lymph node in patients undergoing bilateral total thyroidectomy (BTT) along with CND and/or lateral neck lymph node dissection (LND) for thyroid cancer have been investigated. Besides, the impact of two different surgical techniques on incidental parathyroidectomy (IP) has been evaluated.

Material and Method

The study has been conducted following the approval by the Clinical Research Ethics Committee on 21 June 2023 under decision number 3746. Firstly, 128 patients who underwent bilateral total thyroidectomy (BTT) and cervical neck dissection (CND) for thyroid cancer at the General Surgery Clinic between 1st March 2019 and 1st March 2023 have been included in the study. All patients enrolled in the trial were aged 18 years or older. The research has been conducted under the World Medical Association (WMA) Declaration of Helsinki Ethical Principles for Medical Research on Human Volunteers and related guidelines/regulations. Patients were informed about the study in advance. An informed consent was obtained from all patients who enrolled in the study. Ten patients were found to have undergone thyroid surgery for thyroid cancer at another medical center, and for this reason, they were excluded from the study. Of the remaining patients, a total of 115 patients had the necessary surgical and

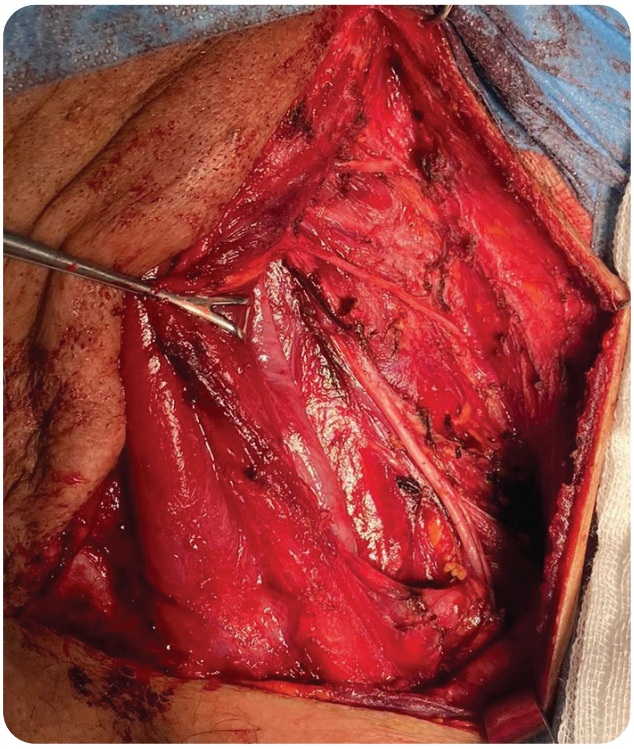

Figure 1
Operative view of the patient who first underwent left LND, before proceeding to left CND.

pathological reports and other data required for this study. Patients were divided into 2 groups according to the surgical technique used. The first group consisted of patients who underwent CND after thyroidectomy. The second group consisted of patients who underwent lateral neck dissection (LND) before CND and or then underwent CND after ligation and transection of the middle thyroid vein and, if necessary, the superior thyroid artery (Figure 1-2).

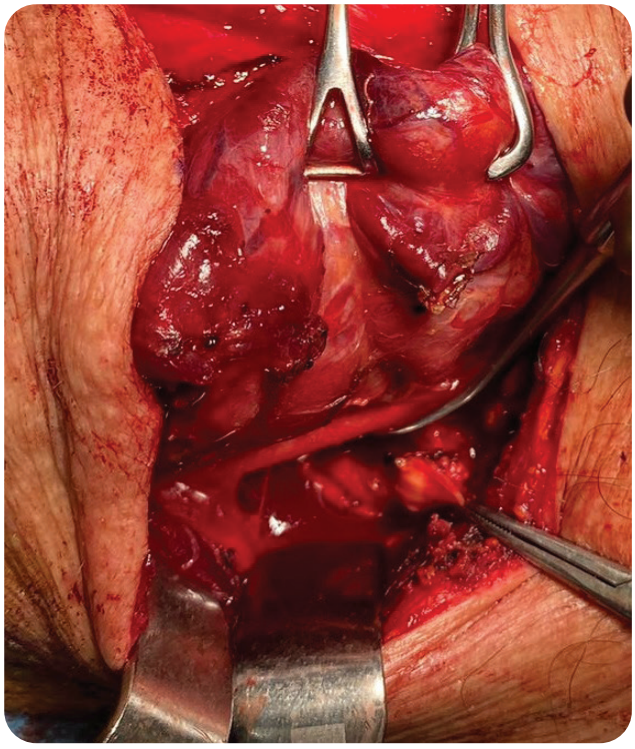

Figure 2
Image of the same patient's thyroid lobe taken before right CND after the lobe has been displaced upwards and towards the midline.

Patient records have been reviewed by evaluating data related to various factors such as age, sex, tumor histopathology, tumor size, type, differentiation, thyroid capsule invasion (CI), lymph vascular invasion (LVI), extrathyroidal extension, number of metastatic lymph nodes, the diameter of the largest metastatic lymph node, and the presence of incidental parathyroidectomy (IP). In the follow-up assessments whether a second operation was performed because of lymph node metastasis or residual tissue has also been checked. Patients with normalized serum PTH levels at 1-year postoperative follow-up were considered to have transient hypoparathyroidism and patients without normalized serum PTH levels were considered to have permanent hypoparathyroidism.

Statistical Analysis
The Shapiro-Wilk test is used to determine whether the data show a normal distribution between the groups subjected to different surgical techniques. For the analysis of categorical variables, the Pearson Chi-Square test is used to assess the significance of differences between groups, whereas, for non-categorical data, the Mann-Whitney U test is used to assess the significance of differences between groups. To evaluate the relationship between the presence of CI, LVI, and tumor size, a ROC curve was constructed, and the cut-off value was determined.

Results

Of the patients included in the study, 35.7% (41) are male and 64.3% (74) are female. The median age of the patients in group 1 is 47 years (minimum: 18, IQR: 28, maximum: 80), and the median age of the patients in group 2 is 35 years (minimum: 29, IQR: 52, maximum: 81). In terms of the presence of CLNM and the gender of the patient, it has been observed that there is no significant relation (p=0.446). Similarly, between the presence of CLNM and the age of the patient less than 45 years, there has also been no significant relation (p=0.774). There has been no significant difference between the operation times in terms of both surgical techniques (time spent for LND was excluded) (p = 0.129). The mean operative time is 158.75±30.69 minutes. Of the patients, 1.7% (2) had poorly differentiated thyroid carcinoma, 1.7% (2) had Hürthle cell carcinoma, 1.7% (2) had NIFTP, 10.4% (12) had medullary carcinoma, and 83.5% (97) had PTC. In the pathology of PTC cases, 4.1% (4) were microcarcinomas, 64.9% (63) were classic, 8.2% (8) were follicular, 8.2% (8) were mixed, 2.1% (2) were hobnail, 4.1% (4) were oncocytic, 4.1% (4) were tall cell, and 4.1% (4) were Warthin tumor-like variants (Table 1). The mean tumor size of thyroid cancer was 2.17±1.8 cm (minimum: 0.1, median: 1.5, maximum: 9 cm). The median tumour size in men was 2 cm (minimum: 0.1, IQR: 3.1 cm, and maximum: 9 cm), and 1.4 cm in women (minimum: 0.6, IQR: 0.8 cm, and maximum: 6.8 cm). A ROC curve was generated to determine the significance of the relationship between LVI and tumor diameter. Tumor size was significantly higher in men than in women (p < 0.001). The ROC curve was used to show the relationship between LVI and tumor diameter and the cut-off value for tumor diameter was determined to be 1.55 cm. While no significant association has been found between the tumor size and CI, a significant association has been observed with multifocality and LVI (Tables 2-3). The median size of metastatic lymph nodes was 1.5 cm (minimum: 0, IQR: 4.2 and maximum: 8) in men

Table 1 The pathology of patients who underwent neck lymph node dissection

N	%	Histopathology
2	1.7	Poorly Differentiated
2	1.7	Hurtle
2	1.7	NIFTP
12	10.4	Medullary
4	3.5	PTC Microcarcinoma
8	7	PTC Follicular variant
8	7	PTC Mixt variant
2	1.7	PTC Hobnail variant
4	3.5	PTC Oncocytic variant
4	3.5	PTC Tall cell variant
4	3.5	PTC Warthin variant
63	54.8	PTC Classic

Table 2 The comparison of tumor diameter and some pathological parameters

		N	Mean Rank	Test Value	p value
LVI	No	46	38.1	670	< 0.001
	Yes	69	71.3		
CI	No	37	22.9	1018	0.011
	Yes	78	63.4		
MC	No	58	66.9	1132	0.003
	Yes	57	48.9		

LVI: lymphovascular invasion, CI: capsule invasion, MC:multisentricity, N: number. Mann-Whitney U test was used.

and 1 cm (minimum: 0, IQR: 0.8 and maximum: 3.6) in women. The size of metastatic lymph nodes was significantly higher in men than in women ($p < 0.001$). It has been observed that there is a significant relationship between metastatic lymph nodal size and LVI and CI (Table 4). Besides, no significant relationship has been found between multifocality and metastatic lymph node size ($p = 0.063$). In the group that underwent BTT followed by CND, 12.2% (14) of the patients underwent prophylactic central lymph node dissection, and no lymph node metastases were detected in these patients. The pathological examination of these patients revealed that there has been multicentricity in 6 cases, capsule invasion in 6 cases, and lymph vascular invasion in 2 cases. In patients with MTC (8), 67% had a serum calcitonin level >200 ng/L and underwent bilateral CND and bilateral LND in addition to BTT. Two other patients received bilateral CND and unilateral LND in addition to BTT. Patients with lymph node metastases had an average of 6.89±8.38 lymph nodes with metastases. There is no significant relationship detected between Hashimoto's disease and the number of lymph nodes removed in CLNM and CND ($p = 0.835$, $p = 0.318$,

Table 3 The comparison of pathological parameters according to the cut-off value determined for tumor diameter after taking the ROC curve

			Tumor Diameter		Total	p value
			<1.55 cm	≥ 1.55		
LVI	No	N	40	6	46	<0.001
		%	87	13	100	
	Yes	N	28	41	69	
		%	40.6	59.4	100	
CI	No	N	28	9	37	0.013
		%	75.7	24.3	100	
	Yes	N	40	38	78	
		%	51.3	48.7	100	
MC	No	N	26	32	58	0.002
		%	44.8	55.2	100	
	Yes	N	42	15	57	
		%	73.7	26.3	100	

LVI: lymphovascular invasion, CI: capsule invasion, MC:multisentricity, N: number. Mann-Whitney U test was used.

Table 4 The comparison of metastatic tumor diameter and some pathological parameters

		N	Mean Rank	Test Value	p value
LVI	No	46	40.9	804	<0.001
	Yes	69	69.4		
CI	No	37	45.1	964	0.004
	Yes	78	64.1		

LVI: lymphovascular invasion, CI: capsule invasion, N: number. Mann-Whitney U test was used.

Table 5 The effect of neck lymph node dissection surgical approaches on IP

Neck Lymph Node Dissection Approach		N	Mean Rank	p value
Incidental Parathyroidectomy	BTT after CND	94	63.5	<0.001
	LND and/or CND after BTT	21	33.3	
	Total	115		

BTT: bilateral total thyroidectomy, CND: central neck lymph node dissection, LND: lateral neck lymph node dissection, N: number. Mann-Whitney U test was used.

Table 6 The need for re-operation after surgical approaches to neck lymph node dissection

Neck Lymph Node Dissection Approach		Reoperation		p value
		No	Yes	
BTT after CND	N	74	20	0.104
	%	78.7	21.3	
LND and/or CND after BTT	N	13	8	
	%	61.9	38.1	
Total	N	87	28	
	%	75.7	24.3	

BTT: bilateral total thyroidectomy, CND: central neck lymph node dissection, LND: lateral neck lymph node dissection, N: number. Pearson Chi-Squared test was used.

respectively). Incidental parathyroidectomy (IP) was found in pathology specimens in 47.4% (54) of patients. Among these patients, 29.8% (34) had 1 parathyroid gland removed, 14% (16) had 2 glands removed, and 3.5% (4) had 3 parathyroid glands removed. The median number of IPs was 1 (minimum: 0, IQR: 1, maximum: 3) in patients who underwent BTT followed by CND and/or LND, whereas only 1 IP was detected in patients who underwent LND and/or CND followed by BTT. It has been observed that there has been a significant reduction in the number of IPs in patients who underwent LND and/or CND followed by BTT ($p < 0.001$) (Table 5). No hypoparathyroidism occurred in patients who underwent LND and/or SND followed by BTT, whereas in patients who initially underwent BTT+CND, 12.3% (14) had transient hypoparathyroidism and 3.5% (4) had permanent hypoparathyroidism. When the need for re-operation due to the detection of central lymph node metastasis at follow-up has been evaluated, it is detected that there is no significant difference between surgical techniques ($p = 0.104$) (Table 6). While 11.55±5.15 lymph nodes were dissected in CND in patients who underwent thyroidectomy first and then thyroidectomy, 12.8±4.1 lymph nodes were dissected in patients who underwent CND first and then BTT. It has been revealed that there is no statistically significant difference between the two methods ($p = 0.104$).

Discussion

The primary result of our study was that patients who underwent thyroidectomy after lateral neck dissection or central lymph node dissection were at a lower risk of developing incidental parathyroidectomy and hypoparathyroidism than those who underwent thyroidectomy first. The secondary results of our study are that as tumor diameter increases, LVI and CI increase, while MC decreases. As the metastatic lymph node diameter increases, LVI and CI positivity increases.

Overall, CLNM in PTC typically occurs first in the central compartment, and then it spreads to the lateral compartment (29-32). Although positive CLNM is associated with a higher regional recurrence rate, it has little significance in terms of mortality. There is a consensus that therapeutic central neck lymph node dissection (CND) should be performed in CLNM-positive PTC patients, but the question of whether prophylactic CND should be performed in clinically node-negative PTC patients remains controversial (33, 34). Therefore, routine prophylactic CND has not been performed in our clinic. Prophylactic dissections performed in suspicious cases did not reveal metastatic lymph nodes. In addition, in the group that underwent LND and/or CND before BTT, all patients had lymph node metastasis in the lateral compartment, and these patients included those with lymph node metastasis detected by fine-needle aspiration biopsy before surgery. Of the patients who underwent BTT followed by CND, 12.3% (7) underwent prophylactic CND. While the impact of CND on reducing locoregional recurrence rates remains uncertain, it does increase the risk of recurrent laryngeal nerve (RLN) paralysis and hypoparathyroidism (35, 36). Permanent hypoparathyroidism is more common in patients who undergo bilateral CND than in those undergoing unilateral CND (37). Incidental parathyroidectomy is mainly due to prophylactic and therapeutic CND. However, performing more lymph node dissections during CND does not increase the incidence of IP (38).

Although IP is less common when CND is performed by experienced surgeons, it is important to carefully identify and protect the parathyroid glands during surgery (39).

Thyroidectomy performed by ligating the main trunk of the inferior thyroid artery results in a significantly higher rate of hypoparathyroidism than when the branches of the inferior thyroid artery are ligated near the thyroid capsule (40). The close connection of the thyroid arteries and veins to the thyroid capsule preserves the supply to the parathyroid gland and reduces the development of hypoparathyroidism, even in patients undergoing SND (41). In previous studies, it is thought that IP occurs as a result of the mixing of parathyroid glands with central region lymph nodes due to CND after thyroidectomy. Our study suggests that performing CND only after ligating the middle thyroid vein before BTT, together with careful identification and preservation of the parathyroid glands during BTT, can prevent IP. In this study, it has been detected that there is no statistically significant difference between the two techniques in terms of the need for a re-operation due to the detection of lymph node metastases during the postoperative follow-up period.

The retrospective design of the study can be represented as its weakness. On the other hand, the strengths of the study include its conduct in a center specializing in thyroid surgery, well-maintained records, and regular patient follow-up and monitoring.

In conclusion, similar to previous studies, in this study, it has been found that the size of the largest metastatic lymph node is associated with CI and LVI, and performing CND before BTT might prevent IP. In this area, further studies in larger series are required.

Conflict of Interest Statement
There is no potential conflict of interest.

Ethical Approval
The study has been conducted following the approval by the Ankara City Hospital Clinical Research Ethics Committee Research Ethics Committee on 21 June 2023 under decision number 3746. The study was conducted in accordance with the principles set forth in the Declaration of Helsinki.

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Availability of Data and Materials
Data availability: The datasets generated and/or analysed during the current study are available in Article metadata, and in the zenodo site, 10.5281/zenodo.10369423.

Authors Contributions
FS: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Validation; Visualization; Writing-original draft.

SK: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Validation; Visualization; Writing-original draft; Writing-review & editing.

MOY: Formal analysis; Investigation; Writing-original draft; Writing-review & editing.

AFB: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Validation; Visualization; Writing-original draft.

BK: Writing-original draft; Writing-review & editing.

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ORIGINAL RESEARCH

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Evaluation of Systemic and Hematological Inflammatory Markers in Patients with Vitamin D Deficiency

Tugba ALISIK[1]

[1] Bolu Abant Izzet Baysal University, Faculty of Medicine, Department of Physical Medicine and Rehabilitation, Bolu, Türkiye

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Abstract

Objective
This study aims to comprehensively examine the effects of vitamin D (VD) on systemic and hematological inflammatory markers.

Material and Method
A total of 2889 patients with albumin, C-reactive protein (CRP), creatinine and leukocyte values within the reference ranges were included in this retrospective study. Patients were divided into three groups based on their 25-hydroxy VD levels: group-1 (VD deficiency, VD<12 ng/mL (30 nmol/L)), group-2 (VD insufficiency, VD=12-20 ng/mL (30-50 nmol/L)) and group-3 (sufficient VD status, VD>20 ng/mL (50 nmol/L)) groups. CRP-albumin ratio (CAR), neutrophil–lymphocyte ratio (NLR), platelet–lymphocyte ratio (PLR), lymphocyte-monocyte ratio (LMR), and prognostic nutritional index (PNI) were calculated.

Results
The age of the groups did not differ significantly (p=0.094), while the percentage of females was significantly higher in group-1 than in group-2 and group-3 (p<0.05). CRP, CAR, and PLR values were significantly lower in group-2 and group-3 compared to group-1 (p<0.05 for all). Albumin and PNI values were significantly higher in group-2 and group-3 compared to group-1 (p<0.05 for all). In the multinomial multivariate logistic regression analysis, conducted using sex, CAR, PNI, NLR, PLR, and LMR parameters with group-3 as the reference category, significant odds ratios (OR) were observed for female sex, CAR, and PNI in relation to group-1.

Conclusions
These results show that there were increases in CAR and PNI, markers of inflammation and nutritional status, in the VD-deficient population, although routine laboratory parameters were normal.

Keywords: CRP-albumin ratio, inflammatory markers, prognostic nutritional index, Vitamin D deficiency

Correspondence: T.A. / md.tugbaa@gmail.com
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ORCID IDs of the Authors: T.A: 0000-0003-1856-9513

Introduction

VD plays a key role in bone metabolism and maintenance of calcium and phosphorus balance by creating negative feedback with parathyroid hormone ts deficiency has been clearly demonstrated to be associated with rickets in children and with osteomalacia and osteoporosis in adults (3, 4). Besides bone metabolism, studies provide evidence for the protective role of VD in autoimmune conditions (e.g. multiple sclerosis (MS) and type 1 diabetes mellitus) and infectious diseases (e.g. respiratory tract infections) (5). Furthermore, VDD shows an increased risk of developing multiple malignancies and inflammatory diseases (e.g. MS, rheumatoid arthritis (RA) (6). Vitamin D deficiency (VDD) has become the most common micronutrient disorder worldwide, surpassing iron deficiency, and its frequency may exceed 50% in some countries (1). Vitamin D deficiency (VDD) is the most prevalent micronutrient disorder globally, surpassing iron deficiency, with a prevalence exceeding 50% in certain countries (1). The National Institutes of Health recommends using the 25-hydroxy (25-OH) vitamin D (VD) level to assess VD status (2).

The biological effects of VD are mediated by the nuclear VD receptor (VDR), which is present in various cell types including intestinal cells, osteoblasts, muscle cells, kidney cells, parathyroid epithelial cells, immune cells, adipocytes, nonparenchymal hepatic cells, and pancreatic ß-cells (7, 8). VDR is widely expressed in all immune cellular subsets, and binding of VD and VDR results in the activation of essential innate immune cells such as neutrophils, monocytes, and macrophages, leading to increased chemotactic, phagocytic, and bactericidal activities (9). Anti-inflammatory effects of VD also occur through upregulation of interleukin-10 (IL-10) (10).

The markers of the systemic inflammatory response are circulating white blood cells originating from lymphoid/myeloid tissues and acute phase proteins such as C-reactive protein (CRP) and albumin originating from the liver. CRP/Albumin ratio (CAR), determined by serum CRP and albumin levels, is a commonly used liver-related parameter in the follow-up of systemic inflammation. It has been shown that CAR is significantly higher in cancer (11), infection (12), inflammatory diseases (13) and DM with complications (14). In addition, markers such as neutrophil/lymphocyte ratio (NLR), lymphocyte/monocyte ratio (LMR), and platelet/lymphocyte ratio (PLR) obtained from hematological parameters have also been demonstrated as inflammatory markers (15). The prognostic nutritional index (PNI) serves as an indicator of both the nutritional and immune status of patients. It is calculated based on the serum albumin level and the peripheral blood lymphocyte count (16). Recently, it has been shown that it can be an important marker in predicting clinical outcomes in many diseases, such as COVID-19 disease (16), nasopharyngeal cancer (17), and gastric cancer(18).

Some studies demonstrate the protective effect of vitamin D (VD) in conditions associated with inflammation. To achieve a more comprehensive understanding, it is important to examine how systemic and hematological inflammatory parameters vary across different VD levels in patients without inflammatory diseases or infections. The aim of this study was to evaluate the impact of VD levels on systemic and hematological inflammatory markers in patients without inflammatory and infectious diseases.

Material and Method

In this retrospective study, we reviewed and recorded data from patients who had their 25-OH VD levels measured at AIBU Izzet Baysal Physical Treatment and Rehabilitation Training and Research Hospital Hospital between January 2021 and January 2022 during a general health check-up. Due to seasonal variations in vitamin D levels, data for an entire year were used to minimize the impact of these variations. The data included age, sex, 25-OH VD levels, whole blood results, and biochemistry test results (CRP, albumin, creatinine). Patients over 18 years of age were included in the study. Patients were excluded from the study if their CRP, albumin, hemoglobin, or white blood cell (WBC) values were outside the normal range, as abnormal levels of these biomarkers could indicate infection, inflammation, or malnutrition, which are factors associated with the inflammatory markers being evaluated in this study. Additionally, to exclude patients with renal failure, which can affect the kidneys' role in VD production, only those with creatinine levels within the reference range were included in the study. Patient files were analyzed, and patients with diabetes mellitus, hypertension, cancer, liver disease, renal disease and inflammatory diseases (ankylosing spondylitis, RA, MS, Behçet's disease, etc.) were excluded. Also, CAR, NLR, PLR, LMR, and PNI values were calculated using the obtained data. PNI values were calculated as albumin(gr/L) + 5xlymphocyte (109/L), and other parameters were calculated as ratios: CAR (CRP/Albumin), NLR (Neutrophil/Lymphocyte), PLR (Platelet/Lymphocyte), and LMR (Lymphocyte/Monocyte).

Patients were divided into three groups according

to their 25-OH VD levels (2): group 1 (VDD group, patients with VD level <12 ng/mL (30 nmol/L)), group 2 (VD insufficiency group, patients with a VD level of 12-20 ng/mL (30 - 50 nmol/L)) and group 3 (sufficient VD status group, patients with VD level >20 ng/mL (50 nmol/L)).

Statistical Analysis

Statistical analysis was performed using the SPSS statistical software for Windows (version 21, released in 2012, IBM, Armonk, NY, USA).

The distribution of continuous variables was assessed for normality using the Kolmogorov–Smirnov test and visual inspection of distribution histograms. For normally distributed variables, three-group comparisons were conducted using one-way analysis of variance (ANOVA) followed by Bonferroni post-hoc tests. Descriptive statistics for these variables are presented as mean ± standard deviation. Non-normally distributed variables were analyzed using the Kruskal-Wallis test, followed by post-hoc pairwise comparisons using the Dunn-Bonferroni method. For these variables, the descriptive statistics were shown as the median (1st-3rd quartile value). Categorical variables were compared using Pearson's Chi-square test. Correlation analysis was performed with Pearson's correlation test for parametric variables or Spearman's rank correlation test for non-parametric variables. Multivariate regression analysis was used to evaluate predictive factors, with results presented as odds ratios (OR) and 95% confidence intervals (CI). A p-value of less than 0.05 was considered statistically significant.

Results

A total of 2889 patients were included in the study. When compared in terms of sex distribution, there were more females in group 1 (N=875 (73.0%)), group

Table 1 Comparison of demographic and laboratory parameters of patients

	Group 1 (n=1199)	Group 2 (n=898)	Group 3 (n=869)	p
Sex, females	875 (73.0%)	523 (58.2%)†	427(53.9%)†	<0.001
Age	48 (36-58)	48 (36-58)	50 (40-58)	0.094
25-Hidroksi Vitamin D	8.2 (6.5-9.9)	15.7 (13.6-17.7)†	25.5 (22.8-30)†,‡	<0.001
WBC	6.99 (6.04-8.16)	7.09 (6.08-8.17)	6.83 (5.88-8)†,‡	0.008
CRP	1.4 (0.6-2.6)	1.2 (0.5-2.4)†	1.1 (0.4-2.5)†	<0.001
ALB	46 (43.0-48.0)	46.7 (44.0-49.0)†	47.0 (44.3-49.0)†	<0.001
LYM	2.03 (1.64-2.48)	2.05 (1.7-2.5)	2.08.(1.66-2.52)	0.377
MONO	0.61 (0.48-0.76)	0.62 (0.51-0.76)	0.59 (0.49-0.74)‡	0.024
NEU	4.04 (3.36-4.93)	4.06 (3.31-4.96)	3.88 (3.19-4.7)†,‡	0.001
PLT	274 (236-318)	265.5 (229-306)†	263 (224.3-308.0)†	0.001
CAR	0.031 (0.014-0.058)	0.026 (0.011-0.052)†	0.023 (0.010-0.054)†	<0.001
PNI	56.3 (53.1-59.4)	57.1 (54.1-60.5)†	57.5 (54.3-60.7)†	<0.001
NLR	2.031 (1.552-2.648)	1.938 (1.501-2.561)	1.862 (1.443-2.486)†	<0.001
PLR	134.0 (107.2-168.0)	129.2 (103.7-158.6)†	128.1 (101.2-164.3)†	0.002
LMR	3.259 (2.473-4.481)	3.294 (2.513-4.37)	3.443 (2.571-4.478)	0.139

Values are given as median (1st-3rd quartile) values and compared with the Kruskal-Wallis Test. Sex parameter was given as number (%) and compared with Pearson Chi-Square test. †: Significantly different from Group 1 based on the Dunn-Bonferroni pairwise comparison test (adjusted p-value < 0.05) ‡: Significantly different from Group 2 based on the Dunn-Bonferroni pairwise comparison test (adjusted p-value < 0.05), WBC: white blood cells, CRP: C-reactive protein, ALB: Albumin, LYM: Lymphocyte, MONO: Monocyte, NEU: Neutrophil, PLT: Platelet, CAR: C-reactive protein-albumin ratio, PNI: Prognostic nutritional index, NLR: Neutrophil-lymphocyte ratio, PLR: Platelet-lymphocyte ratio, LMR: Lymphocyte-monocyte ratio. The "Sex, females" parameter represents the proportion of female participants in each group, with all other participants being male. All parameters are reported as follows: Age in years; 25-Hydroxy Vitamin D in ng/mL; WBC, LYM, MONO, NEU, PLT in 10^9/L; CRP in mg/L; ALB in g/L.

Table 2 Multinominal multivariate logistic regression analysis of parameters.

Model 1: Reference category = Group 3; Nagelkerke R^2 = 0.052					
	Parameters	**B (SE)**	**Wald**	**p value**	**OR (95% CI)**
Group 1 vs Group 3	CAR	3.693 ± 1.602	5.312	0.021	40.178 (1.738-928.914)
	PNI	-0.039 ± 0.012	11.485	0.001	0.962 (0.94-0.984)
	NLR	0.082 ± 0.064	1.642	0.200	1.086 (0.957-1.231)
	PLR	-0.002 ± 0.001	1.724	0.189	0.998 (0.996-1.001)
	LMR	-0.021 ± 0.035	0.351	0.554	0.979 (0.914-1.049)
	Sex (Female vs. Male)	0.828 ± 0.101	66.808	<0.001	2.288 (1.876-2.79)
Group 2 vs Group 3	CAR	0.587 ± 1.717	0.117	0.733	1.798 (0.062-52.072)
	PNI	0.007 ± 0.012	0.379	0.538	1.007 (0.984-1.032)
	NLR	0.145 ± 0.067	4.657	0.031	1.156 (1.013-1.318)
	PLR	-0.003 ± 0.001	4.825	0.028	0.997 (0.995-1.000)
	LMR	-0.079 ± 0.038	4.171	0.041	0.924 (0.857-0.997)
	Sex (Female vs. Male)	0.263 ± 0.103	6.495	0.011	1.301 (1.063-1.593)
Model 2: Reference category = Group 1; Nagelkerke R^2 = 0.052					
Group 2 vs Group 1	CAR	-3.107 (1.525)	4.152	0.042	0.045 (0.002-0.888)
	PNI	0.047 (0.011)	17.624	<0.001	1.048 (1.025-1.071)
	NLR	0.063 (0.058)	1.167	0.280	1.065 (0.95-1.193)
	PLR	-0.001 (0.001)	1.185	0.276	0.999 (0.996-1.001)
	LMR	-0.058 (0.036)	2.639	0.104	0.944 (0.88-1.012)
	Sex (Female vs. Male)	-0.565 (0.098)	33.092	<0.001	0.569 (0.469-0.689)
Group 3 vs Group 1	CAR	-3.693 (1.602)	5.312	0.021	0.025 (0.001-0.575)
	PNI	0.039 (0.012)	11.485	0.001	1.04 (1.017-1.064)
	NLR	-0.082 (0.064)	1.642	0.200	0.921 (0.813-1.044)
	PLR	0.002 (0.001)	1.724	0.189	1.002 (0.999-1.004)
	LMR	0.021 (0.035)	0.351	0.554	1.021 (0.953-1.094)
	Sex (Female vs. Male)	-0.828 (0.101)	66.808	<0.001	0.437 (0.358-0.533)

B: Beta, SE: Standard error, OR: odd ratio, CI: Confidence Interval, CAR: C-reactive protein-albumin ratio, PNI: Prognostic nutritional index, NLR: Neutrophil-lymphocyte ratio, PLR: Platelet-lymphocyte ratio, LMR: Lymphocyte-monocyte ratio.

2 (N=523 (58.2%)) and group 3 (N=427 (53.9%)) (p<0.05 for both). There was no significant difference between the groups in terms of age (p=0.094).

Table 1 shows the comparison of CRP, albumin, hemoglobin, WBC, neutrophil, lymphocyte, platelet, CAR, PNI, NLR, PLR, and LMR values between groups. CRP, CAR, and PLR values were significantly lower in group 2 and group 3 compared to Group 1, and NLR values were significantly lower in group 3 compared to group 1 (p<0.05 for all). Albumin and PNI values were significantly higher in group 2 and group 3 compared to group 1 (p<0.05 for all). When neutrophil and WBC parameters were evaluated, no significant difference was found between group 1 and group 2 (p>0.05); they were significantly lower in group 3 than both group 2 and group 1 (p<0.05 for both).

The multinominal multivariate logistic regression analysis was performed with using the sex (female vs

Table 3 Correlation analysis of parameters with vitamin D levels.

		CAR	PNI	NLR	PLR	LMR
25-hidroxy vitamin D	rho	-0.082	0.122	-0.081	-0.070	0.032
	p value	<0.001	<0.001	<0.001	<0.001	0.089

rho: Spearman's correlation coefficient, CAR: C-reactive protein-albumin ratio, PNI: Prognostic nutritional index, NLR: Neutrophil-lymphocyte ratio, PLR: Platelet-lymphocyte ratio, LMR: Lymphocyte-monocyte ratio.

male), CAR, PNI, NLR, PLR and LMR parameters, with group 3 as the reference category. OR of female sex (OR: 2.288 (95% CI:1.876-2.790)), CAR (40.178 (95% CI:1.738-928.914)) and PNI (0.962 (95% CI:0.964-0.984)) were significant for group 1. For group 2, NLR, PLR, LMR, and female sex were significant ($p<0.05$ for all, Table 2). In the second model, group 1 was used as the reference category, OR of the female sex, CAR, and PNI for group 2 and 3 were significant ($p<0.05$ for all, Table 2). Other parameters were not statistically significant ($p>0.05$ for all, Table 2). Overall, female individuals, with elevated CAR levels, and lower PNI values were more inclined to belong to group 1 compared to group 2 and group 3. Table 3 shows the correlation analysis between VD and inflammatory markers.

Discussion

In the present study, VDD patients showed higher CRP, CAR, PLR, and NLR values and lower albumin and PNI values. Furthermore, in the multinomial multivariate logistic regression analysis, conducted with sex, CAR, PNI, NLR, PLR, and LMR parameters, significant OR were observed for female sex, CAR, and PNI in relation to VDD.

A review reported that some studies found a negative association between VD and inflammatory markers, while others found no significant association (19). Kruit et al. observed a negative correlation between serum VD and CRP levels in elderly patients with both inflammatory and non-inflammatory diseases, with a stronger correlation observed in the former group (20). Similarly, Lopez-Munoz et al. reported a negative correlation between serum VD and CRP levels in patients with ulcerative colitis but not those with Chron's disease (21). In a study conducted in patients with COVID-19, significant inverse correlations were found between 25-OH VD levels and interleukin-6 (IL-6), CRP, tumor necrosis factor-α (TNF-α), D-dimer, and IL-10 levels. Among these patients, it was shown that those with hypovitaminosis D (25-OH VD ≤ 20 ng/ml) had higher IL-6, CRP, TNF-α, D-dimer, and IL-10 values than those without hypovitaminosis D (22). In a Mendelian randomization analyses study, it was found that genetically predicted serum 25-OH VD had an L-shaped association with serum CRP (23). The relationship between VD and CRP levels has been extensively researched; however, some studies have failed to demonstrate a correlation (24, 25). While some studies have not established a connection between VD and CRP levels, the majority of literature, including our study, indicates that inflammatory parameters are generally elevated in individuals with VDD, thereby reinforcing the association between VD and inflammatory markers. The possible explanations for these differences may be variations in sample size, population demographics, or methodological approaches.

Albumin, is synthesized by the liver and acts as a negative acute phase reactant. A study has shown a linear relationship between albumin and VD levels in patients with COVID-19. (26). Along with its many other functions, albumin also plays a role in the transport of VD along with the VD binding protein. Albumin-bound vitamin D and free vitamin D yield the bioavailable form of vitamin D. However, it is free VD that plays a more direct role in biological processes (26). Hence, assessing variations in albumin levels along with CRP may allow the evaluation of the anti-inflammatory effects of VD and its bioavailable levels together. Furthermore, the CRP and albumin ratio, referred to as the CAR ratio, serves as a systemic inflammatory marker associated with the severity of inflammation and prognosis across various diseases such as malignancies, chronic inflammatory conditions, and sepsis (27). This ratio could potentially be utilized as an indicator of inflammation linked to VDD in patients exhibiting normal CRP and albumin values. For instance, a study on cerebral venous sinus thrombosis in pregnant women found that the CAR ratio was significantly elevated in those with venous

sinus thrombosis and severe vitamin D deficiency, highlighting its potential as a marker for inflammation related to VDD (28). In our study, higher CAR values were obtained in the VDD group (group 1) compared to the VD insufficient (group 2) and sufficient VD status (group 3) groups. Also, despite CRP and albumin values falling within the reference range, CAR values, serving as a systemic inflammatory marker, were elevated in patients with VDD compared to those with insufficient and sufficient VD status. This observation suggests that CAR can serve as an indicator of inflammation associated with VDD even in patients with normal CRP and albumin values. Furthermore, being female, having a higher CAR and lower PNI were found to be a predictor of being in the VDD group in our study, suggesting that there may be a cause-effect relationship between VD and inflammatory markers.

NLR, LMR, and PLR were hemogram-derived, easily available non-specific markers of inflammation (29). Studies have shown that patients with VDD exhibit significantly higher NLR levels (30) and increased PLR values, with decreased LMR in children with VDD (31).

Likewise, our study revealed higher levels of NLR and PLR in the VDD group compared to those with sufficient VD status. Furthermore, PLR values, obtained from the hemogram and serving as another inflammatory marker, were also elevated in the VDD group compared to both insufficient and sufficient VD status groups. In addition, NLR, PLR, and LMR values were found as significant predictors in the insufficient VD group compared to the sufficient VD status in our study (Table 2), which indicates that these parameters were more affected by the decrease in the VD level rather than CAR or PNI. The relationship between VDD and non-specific inflammation markers such as the NLR, LMR, and PLR has garnered significant attention in recent research. VD is known to play a crucial role in modulating the immune response, influencing both innate and adaptive immunity. It has been observed that VDD correlates with increased levels of inflammatory markers such as IL-6, TNF-α, and CRP (32, 33). For instance, reported that lower levels of 25-OH VD were associated with higher NLR and PLR, suggesting that VD may exert an anti-inflammatory effect that could help in regulating these ratios (32).Similarly, found that patients with VDD exhibited significantly higher NLR and PLR, indicating that chronic systemic inflammation might adversely affect VD metabolism, potentially leading to conditions such as osteoporosis (33). The mechanisms by which VD influences inflammatory markers are multifaceted. VD is believed to modulate the expression of various cytokines and inflammatory pathways. For example, it has been shown to inhibit the NF-kB and p38 MAPK pathways, which are critical in the inflammatory response (34). This inhibition can lead to a reduction in the production of pro-inflammatory cytokines, thereby lowering the levels of inflammatory markers such as IL-6 and TNF-α (34, 35). Furthermore, VD's role in macrophage polarization is significant; it promotes the M2 (anti-inflammatory) phenotype while inhibiting the M1 (pro-inflammatory) phenotype, thereby contributing to a balanced immune response (36). In addition to its direct effects on immune cells, VD may also influence the inflammatory milieu indirectly through its impact on metabolic processes. For instance, VDD has been linked to insulin resistance and obesity, both of which are associated with chronic low-grade inflammation (37). This connection suggests that VD may help mitigate inflammation by improving metabolic health, which in turn could influence NLR, LMR, and PLR. While there is a correlation between vitamin D deficiency and elevated non-specific inflammation markers such as NLR, LMR, and PLR, the direct and indirect mechanisms through which vitamin D exerts its effects are complex and multifactorial. Future research should focus on elucidating these mechanisms to better understand the therapeutic potential of vitamin D in managing inflammation.

PNI is both an inflammatory and nutritional status biomarker calculated from albumin and lymphocyte counts (38). It was shown that VDD and PNI were significantly associated with all-cause mortality, and there was a relationship between VDD and PNI (39). Similarly, we found that PNI levels were lower in patients with VDD than in patients with insufficient VD and sufficient VD status, and PNI was a significant predictor of VDD. Also, there was a weak but significant positive correlation between VD and PNI levels. Our study indicates that the PNI, serving as both an inflammatory and nutritional biomarker, was reduced in cases of VDD and exhibited a correlation with VD levels. This suggests that VD levels might contribute to heightened inflammation or be linked to a decline in nutritional status. Moreover, PNI values, which have been shown to be an indicator of the nutritional and immune status of individuals, were found to be lower in patients with VDD than in other groups, suggesting that it may be a marker that can be used in the evaluation of increased risk such as infectious and inflammatory diseases in patients with VDD.

This study has some limitations. First, the retrospective design of the study limits the ability to establish causality between vitamin D levels and the observed clinical outcomes. Second, the reliance on electronic health records introduces the possibility of

incomplete data. Also, we did not measure vitamin D supplementation or sun exposure, both of which could have influenced the results. Lastly, we only included patients whose routine laboratory parameters, except for VD, were within reference values. Therefore, any potential inflammatory effects of VD levels exceeding the reference range may not have been demonstrated.

Conclusion

Results of current study shows that there were increases in CAR and decrease in PNI, markers of inflammation and nutritional status, in the VD-deficient population, although routine laboratory parameters were normal. It is suggested that maintaining adequate VD levels may help improve inflammatory profiles. Further longitudinal prospective studies with patients with VDD may better isolate the cause-and-effect relationship between VD and the inflammatory markers CAR and PNI.

Conflict of Interest Statement
The authors have no conflicts of interest to declare.

Ethical Approval
The study was approved by the Bolu Abant Izzet Baysal University Clinical Researches Ethics Committee (No: 2021/295, date: 21/12/2021) and the Declaration of Helsinki for research on humans was followed.

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Availability of Data and Materials
Data available on reasonable request from the authors.

Authors Contributions
TA: Conceptualization; Data curation, Formal analysis; Investigation; Methodology; Resources; Validation; Writing- original draft, review & editing.

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ORIGINAL RESEARCH

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Relationship Between p53 and Recurrence in Endometrial Cancer

Duriye OZTURK[1], Cem YAGMUR OZDEMIR[2], Nayif CICEKLI[2], Cigdem OZDEMIR[3], Beyza UNLU[4], Hacer DEMIR[4], Dagistan Tolga ARIOZ[2]

[1] Afyonkarahisar Health Sciences University Hospital, Faculty of medicine, Department of Radiation Oncology, Afyonkarahisar, Türkiye
[2] Afyonkarahisar Health Sciences University Hospital, Faculty of medicine, Department of Gynecological Oncology, Afyonkarahisar, Türkiye
[3] Afyonkarahisar Health Sciences University Hospital, Faculty of medicine, Department of Pathology, Afyonkarahisar, Türkiye
[4] Afyonkarahisar Health Sciences University Hospital, Faculty of medicine, Department of Medical Oncology, Afyonkarahisar, Türkiye

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Abstract

Objective
Tumor protein 53 (p53), were included in the new FIGO 2023 staging system. Tumor protein 53 (p53) was incorporated into the new FIGO 2023 staging system. This study aimed to assess recurrence rates, overall survival (OS), and progression-free survival (PFS) in endometrial cancer patients with p53 mutations treated in the radiation oncology clinic.

Material and Method
260 patients were included in the study. The patients were divided into 2 groups according to the p53 mutation: p53 abnormal (p53 mutant) and p53 wild type. The Kaplan-Meier method was used to evaluate OS and PFS. Survival rates; were compared in terms of p53 mutations. Patients who underwent surgery for EC between January 1, 2008, and January 1, 2023, were included if their postoperative pathology reports evaluated p53 mutations, and they were referred to the radiation oncology clinic.

Results
In our study; OS of EC was 84.2%, PFS was 88.8%. Total of 29 patients (%11.2) with recurrence were detected in the follow-up of the patients. The OS of p53 wild type patients was 88.6% and p53 mutant patients was 61% (p<0.001). The PFS of p53 wild type patients was 91.8% and p53 mutant patients was 73.2% (p<0.001). When risk calculation was made, we found a 4.094-fold increased risk of recurrence in cases with p53 mutation (95% CI: 1.763-9.508). Based on p53 status, 41 patients (15.8%) were classified as the p53 mutant group, while 219 patients (84.2%) were categorized as the p53 wild-type group.

Conclusion
As a result, the most important point we want to emphasize in the study is that vaginal cuff recurrence was observed in 3 patients with p53 mutation despite brachytherapy. We found that endometrial cancer with p53 mutation were associated with increased recurrence rate and decreased OS and PFS.

Keywords: Endometrial carcinoma, p53, recurrence, survival

Correspondence: C.Y.O / cyozdemir@hotmail.com
Received: 08.05.2024 • Accepted: 04.11.2024
ORCID IDs of the Authors: D.O: 0000-0002-3265-2797; C.Y.O: 0000-0001-5560-2162; N.C: 0000-0001-9480-7769; C.O: 0000-0001-8500-0744; B.U: 0000-0002-8178-0277; H.D: 0000-0003-1235-9363; D.T.A: 0000-0003-3616-0789

Introduction

The American Cancer Society reported that an estimated 61,880 new cases will be diagnosed in the United States in 2022, and 12,550 women will die from EC (2). As obesity increases worldwide and life expectancy increases, the incidence of EC is also increasing. (3). The Cancer Genome Atlas Research Network (TCGA) has made a new classification of EC based on sequence and sequencing technologies. POLE-ultramutated (POLEmut), mismatch repair defcient (MMRd), p53-abnormal (p53abn), and No Specific Molecular Profile Subgroup (NSMP) are the four subgroups of this molecular classifcation (4). Molecular markers were included in the staging system. Current changes to the endometrial staging system by FIGO have been made to further define the reported differences in prognosis and survival since the 2009 system was published. Patients with tumor protein 53 (p53) mutation are now considered stage 2c, regardless of myometrial invasion (5). There are studies on poor prognosis of endometrial cancer in p53 mutation. A better understanding of the molecular changes in the p53abn subgroup is necessary to identify better treatments for these most aggressive endometrial cancers (6,7) In our study, we aimed to evaluate recurrence rates, overall survival (OS) and progression free survival (PFS) in endometrial cancer patients with p53 mutations.Endometrial carcinoma (EC) is the sixth most commonly diagnosed cancer in women worldwide (1). Endometrial carcinoma (EC) ranks as the sixth most frequently diagnosed cancer among women worldwide (1). The FIGO (Fédération Internationale de Gynécologie et d'Obstétrique) introduced a revised staging system for EC in 2023.

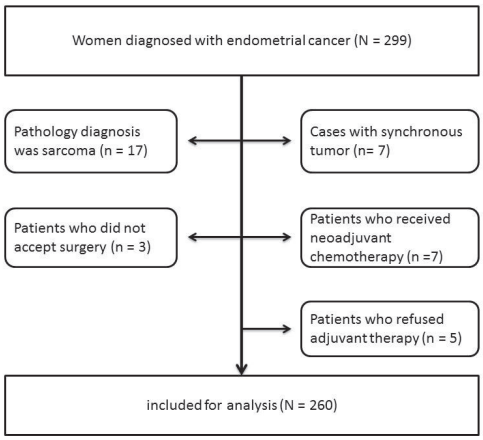
Material and Method

Patients

Cases with synchronous tumor or sarcoma detected in postoperative pathology diagnosis, patients who received neoadjuvant chemotherapy, those who did not accept the operation, and those who refused to receive adjuvant treatment were excluded from the study. As a result, 260 patients were included in the study (Supplemental Figure 1). Patients who underwent surgery for EC between January 1, 2008, and January 1, 2023, were included if their postoperative pathology reports evaluated p53 mutations, and they were referred to the radiation oncology clinic.

Sample Evaluation

Data related with age, body mass index (BMI), grade, CA125 levels, histopathological type, stage, and survival were derived from hospital files.



Supplemental Figure 1
Flowchart

Vaginal examination, CA125 levels, transvaginal ultrasonography and imaging methods (MRI, CT, PET-CT) were used to detect recurrence. Staging was done according to FIGO 2009 criteria. P53 mutational status dichotomized as "wild type" vs "abnormal (mutated)". Patients were divided into 2 groups according to the p53 mutation: p53 abnormal (p53 mutant) and p53 wild type. In the evaluation of p53 immunohistochemical staining, diffuse strong staining in >80% tumor cell nuclei (overexpression) or complete loss of expression ("null" expression) or 25 diffuse cytoplasmic staining were evaluated as abnormal expression, other heterogeneous intensity staining was accepted as normal expression.

Statistical Analysis

SPSS Version 26.0 was used for statistical analysis. Mean, median, and standard deviation were calculated for continuous variables. The relationship between qualitative variables was examined with Fisher Exact and Continuity Correction (Yates) Chi Square analysis. Two-tailed p values <0.05 were accepted to be statistically. Time-to-event analyses were conducted using the KaplanMeier method and log-rank test.

Results

The mean age of the patients was 61.9±10.1 years. The mean BMI was calculated as 32.5±5.6 kg/m². The median CA 125 value was 17 IU/mL (3 IU/mL - 10655 IU/mL). According to the postoperative pathology results, the mean tumor size was 4.21 ± 2.13 cm. The most common stage was stage1b and endometrioid carcinoma was the most common histological type. The grades, myometrial invasion, histological type, lymphovascular space invasion (LVSI), cytology results, p53 mutations, staging of the postoperative

Table 1 Postoperative pathology findings

	No. of Patients	%
Surgical stage		
IA	85	32.7
IB	98	37.6
II	19	7.3
IIIA	18	6.9
IIIB	0	0
IIIC1	16	6.2
IIIC2	3	1.2
IVA	0	0
IVB	21	8.1
Histology		
Endometrioid adenocarcinoma	223	85.7
Clear cell carcinoma	3	1.2
Serous carcinoma	26	10
Malignant Mix Mullerian Tumor	6	2.3
Mucinous carcinoma	2	0.8
Grade		
1	76	29.2
2	108	41.6
3	76	29.2
Lymphovascular space invasion		
Negative	183	70.4
Positive	77	29.6
Myometrial invasion		
<1/2	103	39.6
≥1/2	157	60.4
Cytology		
Positive	38	14.6
Negative	222	85.4
P53		
Wild type	219	84.2
Abnormal (mutant)	41	15.8

pathology results of the patients are summarized in Table 1. Based on p53 status, 41 patients (15.8%) were classified as the p53 mutant group, while 219 patients (84.2%) were categorized as the p53 wild-type group.

106 patients received only adjuvan radiotherapy, 30 patients received only adjuvan chemotherapy, and 54 patients received adjuvan chemo-radiotherapy. 70 patients are under follow-up with no treatment. A total of 160 patients received adjuvan radiotherapy. Of these, 109 patients received brachytherapy (BRT) alone, 20 patients received external beam radiotherapy (ERT) alone, and 31 patients received both BRT and ERT. Total of 29 patients (%11.2) with recurrence were detected in the follow-up (Table 2). The median time to recurrence was 30 months (range: 2-125 months). Three patients with vaginal cuff recurrence were p53 wild type. These patients are stage 1b grade 3 (adjuvant BRT), stage 2 grade 3 (adjuvant chemotherapy + ERT + BRT), stage 4b grade 2 (adjuvant chemotherapy + ERT + BRT), respectively. When the relationship between p53 and recurrence was evaluated, the p value was found to be significant ($p<0.001$). When risk calculation was made, we found a 4.094-fold increased risk of recurrence in cases with p53 mutation (95% CI: 1.763-9.508). A notable finding of this study is that vaginal cuff recurrence was observed in three patients with p53 mutations despite undergoing brachytherapy. In our study; OS of EC was 84.2%, PFS was 88.8%.

Table 2 Recurrence location and no of patients with recurrence

Recurrence site	No of Patients
Pelvic lymph node	4
Vaginal cuff	3
Adrenal gland	2
Pulmonary	8
Para-aortic lymph nodes	3
Hepatic	4
Bone (vertebral)	3
Bowel (sigmoid colon)	2
Total	29

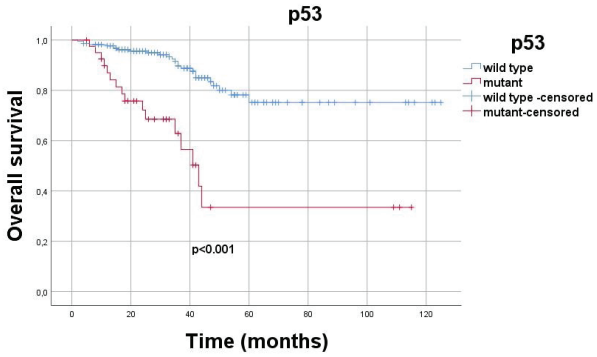


Figure 1
Overall survival rates with respect to p53 mutation

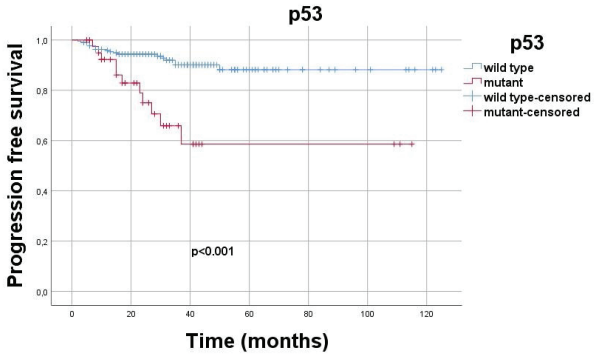


Figure 2
Progression-free survival rates with respect to p53 mutation

OS rates; were compared in terms of p53 mutation (Figure 1). Also PFS rates were compared in terms of p53 mutation (Figure 2). The OS of p53 wild type patients was 88.6% and p53 mutant patients was 61% (p<0.001). The PFS of p53 wild type patients was 91.8% and p53 mutant patients was 73.2% (p<0.001).

Discussion

EC is usually detected between the ages of 50 and 65 years, with mean age of 60 years during diagnosis (8). Accordingly, the mean age of patients with endometrial cancer in this study was 61.9 years old.

A Turkish study reported that the OS of EC was 85% (9). In a study we conducted in Turkey and a region close to ours, the OS of EC was found to be 91.2% (10). Eltabbakh at al. showed that the 5-year PFS and OS rates of these patients were denoted as 95.2 and 96.4%, respectively (11). Chen at al. reported that 5-year relative survival rate was 81.0% in Germany (12). Although the 5-year survival rate changed between 74 and 91% for stage 1 and stage 2 tumors, this number decreased to 20 to 26% for stage 4 EC (13). Crosbie, E. J. et al. reported survival according to stages as 92% for stage 1, 74% for stage 2, 48% for stage 3, and 15% for stage 4, respectively (14). In line with previous studies, our study observed an OS rate of 84.2%.

The World Health Organization classification of obesity is non-obese (<30.0) and obese (≥30.0) (15).

Nowadays, obesity is known to be a risk factor for many types of cancer (16). In our study, we found the mean BMI value in the obese group.

Mutation of the p53 gene increases the uncontrolled proliferation of cells, causing aggressive tumor behavior. (17). In a study by Raffone et al. they found that the p53 mutant group had a prognosis approximately 2 times worse than the control group (18). PORTEC 3 trial showed that molecular classifcation of EC has a strong prognostic value in high-risk uterine cancer, and adjuvant chemotherapy and radiation signifcantly improved recurrence in p53abn tumors, regardless of histologic subgroup (19). Vermij, L et al. reported that abnormal p53 expression was observed in 131/408 (32%) tumors (20). Tresa, A et al. found that the 2-year OS of the p53 wild type and p53 mutant type was 97.2% and 91.7%, respectively (21). Shivkumar, V et al. concluded that p53 overexpression was associated with more aggressive behavior and poor survival outcome in EC cases (22). Consistent with these data in the literature, we found that it was associated with a 4,094-fold increased recurrence rate and decreased OS and PFS in those with p53 mutation. As it is known, FIGO published a new staging system for EC in 2023. p53 mutation has become part of the new staging system. Patients with p53 mutation are now considered stage 2c, regardless of myometrial invasion (5).

Most of patients has early stage endometrial cancer and our study is retrospective. Exclusion criteria for sarcoma or synchronous tumor in the postoperative pathology report results and exclusion of cases who did not receive postoperative adjuvant therapy may have led to differences in OS and PFS values. In addition, the fact that the study was conducted on radiation oncology data may have caused the low number of stage 1a cases who did not receive adjuvan treatment. The relatively small number of cases can be considered as another limitation of our study. This study is subject to several limitations.

Conclusion

In conclusion, p53 mutation must be investigated in the postoperative pathological evaluation of endometrial cancer. We found that endometrial cancer with p53 mutation were associated with increased recurrence rate and decreased OS and PFS. A notable finding of this study is that vaginal cuff recurrence was observed in three patients with p53 mutations despite undergoing brachytherapy.

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Conflict of Interest Statement
The authors declare no conflict of interest.

Ethical Approval
The present study was approved by the Ethical Committee of Afyonkarahisar Health Sciences University Hospital (grant no: 2011-KAEK-2, 02/06/2023). The study was conducted in accordance with the Declaration of Helsinki. Consent was obtained from all patients during their hospitalization

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Availability of Data and Materials
All data are available from the corresponding author upon reasonable request.

Authors Contributions
DO: Conceptualization, Formal analysis, Project administration

CYO: Writing - Original Draft, Data Curation, Formal analysis

NC: Investigation, Validation, Review & Editing

CO: Data Curation, Visualization

BU: Validation, Formal analysis, Investigation

HD: Validation, Formal analysis, Investigation

DTA: Methodology, Supervision, Review & Editing

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ORIGINAL RESEARCH

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Inflammation and Apoptosis-Related Damage in Lung, Liver, and Kidney Tissues due to Subarachnoidal Hemorrhage

Ali Serdar OGUZOGLU[1], Halil ASCI[2], Musa CANAN[1], Nilgün SENOL[1], Ozlem OZMEN[3]

[1] Department of Brain Surgery, Faculty of Medicine, Suleyman Demirel University, Isparta, Türkiye
[2] Department of Pharmacology, Faculty of Medicine, Suleyman Demirel University, Isparta, Türkiye
[3] Burdur Mehmet Akif Ersoy University, Faculty of Veterinary Medicine, Department of Pathology, Burdur, Türkiye

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Abstract

Objective
Oxidant and inflammatory substances released into the blood due to subarachnoidal hemorrhage (SAH) can pass into the peripheral compartment, causing distant organ damage due to blood-brain barrier permeability caused by oxidative stress, inflammation, and apoptosis. This study aimed to demonstrate the secondary damage to peripheral organs, including the lung, kidney, and liver, resulting from SAH.

Material and Method
Twenty rats were divided into sham and SAH groups, each consisting of ten animals. In the SAH group animals, 0.3 mL autologous blood taken from the tail artery was injected into the cisterna magna for 2 minutes. Seven days after SAH formation, all animals were euthanized under anesthesia. Following decapitation, brain tissues, lung, liver, and kidney tissues were placed in 10% formaldehyde for histopathological and immunohistochemical analysis.

Results
In the SAH group, neuronal degeneration in the cerebral cortex, and hyperemia and hemorrhage in the lung, kidney, and liver were observed histopathologically. In immunohistochemical examinations, decreased expression of brain-derived neurotrophic factor (BDNF) and neurofilament (NF) in the cerebral cortex, cerebellum, and hippocampus sections; In lung tissues, enhanced caspase (Cas)-3, hypoxia-inducible factor 1 alpha (Hif-1α) and nuclear factor kappa beta (NF-κB) expressions in the lung, Cas-5, cyclooxygenase-1 (Cox-1) and interleukin (IL)-1 expressions in the liver, Cas-3, Cox-1 and IL-3 expressions in the kidney were observed.

Conclusion
Following SAH, in addition to damage to brain tissue, peripheral tissues such as the lung, kidney, and liver can also be damaged through inflammation and apoptosis.

Keywords: Subarachnoid hemorrhage, liver, lung, kidney, inflammation, apoptosis

Correspondence: A.S.O / a.serdaroguzoglu@yahoo.co.uk
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ORCID IDs of the Authors: A.S.O: 0000-0002-1735-4062; H.A: 0000-0002-1545-035X; M.C: 0009-0005-7279-0026; N.S: 0000-0002-1714-3150; O.O: 0000-0002-1835-1082

Introduction

Subarachnoidal hemorrhage (SAH) is a highly progressive clinical condition that occurs secondary to damage to the vessels supplying brain tissue due to various causes, such as acquired or hereditary factors (1,2). Subarachnoidal hemorrhage (SAH) is a severe clinical condition resulting from damage to the blood vessels supplying brain tissue due to various acquired or hereditary factors (1,2). Bleeding is observed due to structural deterioration in the vessel wall, especially secondary to aneurysmal events in the vascular layer. Bleeding occurs due to structural deterioration in the vessel wall, often caused by aneurysmal events in the vascular layer. Increased levels of oxidant and inflammatory substances in the blood and brain tissue, triggered by the hypoxic environment distal to the bleeding area, are known to exacerbate the damage (3,4).

These substances can trigger mechanisms such as oxidative stress, inflammation, and apoptosis by binding to their receptors in the tissue they come into contact with (6). To investigate such damage mechanisms, oxidative stress index (OSI), total antioxidant status (TAS), total antioxidant status (TOS), levels for oxidative stress; interleukin 1 (IL-1β), caspase-5 (Cas-5), interleukin-3 (IL-3), hypoxia-induced factor 1 alpha (Hif-1α), cyclooxygenase-1 (Cox-1) and nuclear factor kappa beta (NFkβ) expressions to show inflammation and caspase-3 (Cas-3) expressions to show apoptosis. Oxidant and inflammatory molecules released into the bloodstream can penetrate the peripheral compartment, causing distant organ damage by increasing blood-brain barrier permeability (5).

This study aimed to demonstrate the damage in the brain and some peripheral tissues of SAH-induced rats by histopathologic and immunohistochemical methods.

Material and Method

Experimental Animals

Twenty adult male Wistar albino rats (250-350 g) were obtained from the Burdur Mehmet Akif Ersoy University Experimental Animal Laboratory. They were housed under controlled conditions (60% ± 5% humidity, 21-22 °C, and a 12-hour dark/light cycle) and provided with standard commercial water and feed.

Experimental Procedure

Twenty rats were divided into sham and SAH groups containing ten animals. In the SAH group animals, after aspiration of 0.3 mL CSF into the cisterna magna reached following neck dissection, 0.3 mL autologous blood taken from the tail artery was injected into the cisterna magna for 2 minutes (7). In the sham group, 0.3 mL of physiologic saline solution was applied to the cisterna magna after the aspiration of 0.3 mL of cerebrospinal fluid (CSF), to match the stress levels of the SAH group.

Seven days after SAH formation, all animals were euthanized under 8-10mg/kg xylazine (Xylazinbio 2%, Bioveta, Czech Republic), 90 mg/kg ketamine (Ketacontrol, Doğa İlaç, Turkey)anesthesia by surgical bleeding method by taking blood samples from the inferior vena cava through an abdominal incision. Following decapitation, brain tissues, lung, liver, and kidney tissues were placed in 10% formaldehyde for histopathological and immunohistochemical analysis.

Histopathologic Evaluation

Brain, lung, liver, and kidney tissues were collected and fixed in 10% buffered formalin for histopathological analysis at sacrifice. Following routine processing of tissues with a fully mechanized tissue processor, 5-µm-thick paraffin block pieces were cut using fully automatic rotary microtomes (Leica RM2155, Leica Microsystems, Wetzlar, Germany). Deparaffinization, rehydration with decreasing amounts of graded ethanol, staining with hematoxylin-eosin (HE), clearing in xylene, and sealing sections were the next steps. Histologic changes were evaluated under light microscopy.

Brain sections were stained with brain-derived neurotrophic factor (BDNF) and neurofilament (NF); lung sections with cas-3, Hif-1α, and NF-κB; kidney sections with Cas-3, Cox-1, and IL-3; and liver sections with Cas-5, Cox-1, and IL-3. Sections were mounted on polylysine-coated slides and stained with BDNF (Recombinant Anti-BDNF antibody [EPR1292] (ab108319)), NF (Anti-160 kD Neurofilament Medium antibody [EPR23510-76)) (ab254348)); Cas-3 (Anti-Cas-3 antibody [EPR18297] (ab184787)); Cas-5 (Recombinant Anti-Cas-5 antibody [EP876Y] (ab40887)); Hif-1α (Anti-HIF-1α) alpha antibody [mgc3] (ab16066)); NF-κB (Anti-NF-kB p65 antibody (ab16502)): Cox-1 (Anti-cox-1 antibody [EPR5866] (ab109025)); IL-1 (Anti-cox-1 antibody/[EPR5866] (ab109025)); -IL-3 antibody (ab190941); streptavidin was performed using the biotin technique. Primary and secondary antibodies were purchased from Abcam (Cambridge, UK), and primary antibodies were used at 1/100 dilution. After incubating the sections with primary antibodies for 60 minutes, they were stained using biotinylated secondary antibodies

and streptavidin-alkaline phosphatase conjugate through immunohistochemistry. The results were obtained using the EXPOSE Mouse and Rabbit Specific HRP/DAB Detection IHC kit (ab80436) as the secondary antibody, and diaminobenzidine was used as the chromogen (DAB). Negative controls were run with a dilution solution instead of primary antiserum. The slides were analyzed for immunopositivity for each marker, and the number of positive cells was determined by manually counting 100 cells for each rat under 20X magnification using ImageJ (National Institutes of Health, Bethesda MD). The microphotography was taken using the Database Guide Cell Sens Life Sciences Imaging Software System (Olympus Co., Tokyo, Japan).

Statistical Analysis

The variables were presented in the form of mean ± standard deviations. The Mann-Whitney U test was used to compare the histopathological and immunohistochemical scores between the groups. The statistical calculations were performed using the Graphpad Prism 8.0 program pack (Graphpad Software Inc., USA). A significance value of $p<0.05$ was set.

Results

Histopathologic Findings

Microscopic examinations showed no lesions in the control group. Nevertheless, subarachnoid hemorrhage led to neuronal degeneration characterized by SAH in the brain. Microscopic examinations showed no lesions in the control group. However, subarachnoid hemorrhage caused neuronal degeneration in the brain. SAH also caused hyperemia and hemorrhage in the lung, kidney, and liver (Figure 1).

Immunohistochemical Findings

Immunohistochemical examinations of the brain, cerebellum, and hippocampus sections showed decreased expression of BDNF and NF in the SAH group compared to the control group (Figures 2-3). Cas-3, Hif-1α, and NF-κB expressions were increased in lung tissues, which are generally localized in inflammatory and alveolar epithelial cells (Figure 4). In the liver of the SAH group, Cas-5, Cox-1, and IL-1 expressions were increased significantly in hepatocytes (Figure 5). Increased Cas-3, Cox-1, and IL-3 expressions localized in tubular epithelial cells in the renal cavities of the SAH group were observed (Figure 6)

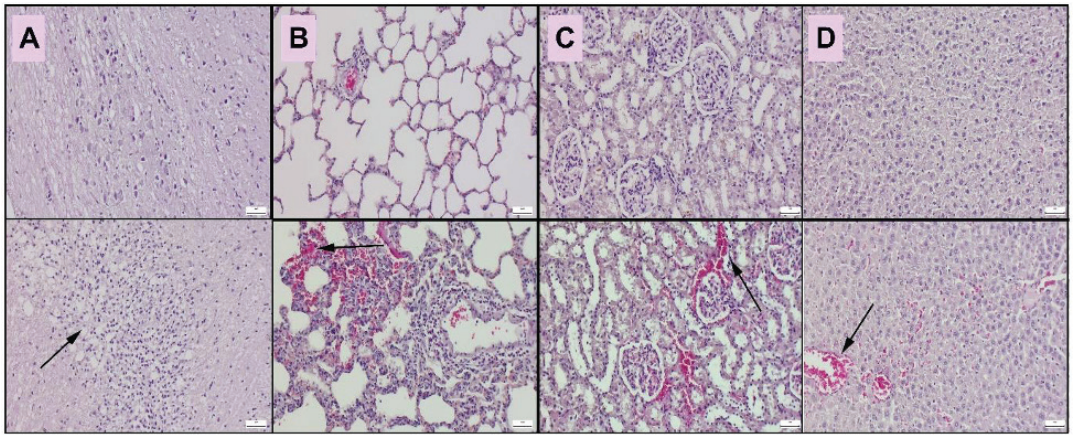

Figure 1

Histopathological findings of organs in control (upper row) and SAH (below row) between the groups. **(**A) Normal brain in normal brain and inflammatory reaction (arrow) brain in SAH group, (B) normal lung tissue in the lung in the control group and inflammatory cell infiltrations and hemorrhage (arrow) in SAH group, (C) normal kidney histology in control group and cortical hemorrhage (arrow) in SAH group, (D) normal heart tissue in control group and slight hemorrhage in (arrow) in SAH group, (E) normal liver histology in control group and hemorrhage (arrow) in SAH group HE, scale bars=50µm.

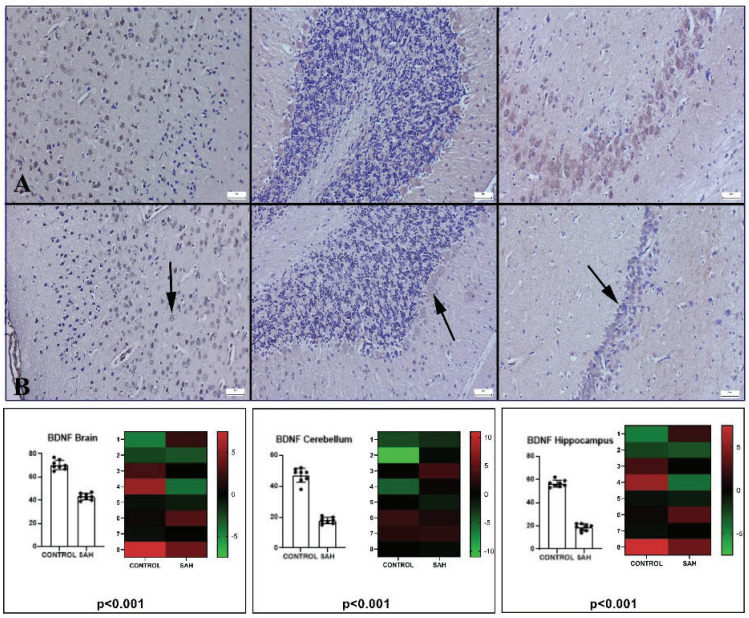

Figure 2

BDNF immunoexpression in the brain (first column), cerebellum (second column), and hippocampus (third column)

(A) BDNF expression in control and (B) SAH groups, arrows indicate decreased expression, Streptavidin biotin peroxidase method, Scale bars= 50µm

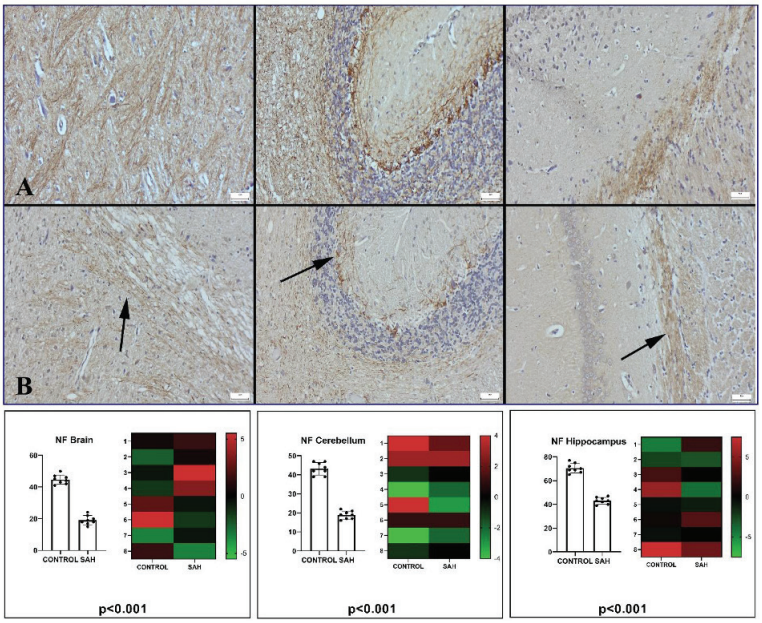

Figure 3

NF immunoexpression in the brain (first column), cerebellum (second column), and hippocampus (third column)

(A) Control and (B) SAH groups, arrows indicate reduced expression, Streptavidin biotin peroxidase method, Scale bars=50µm.

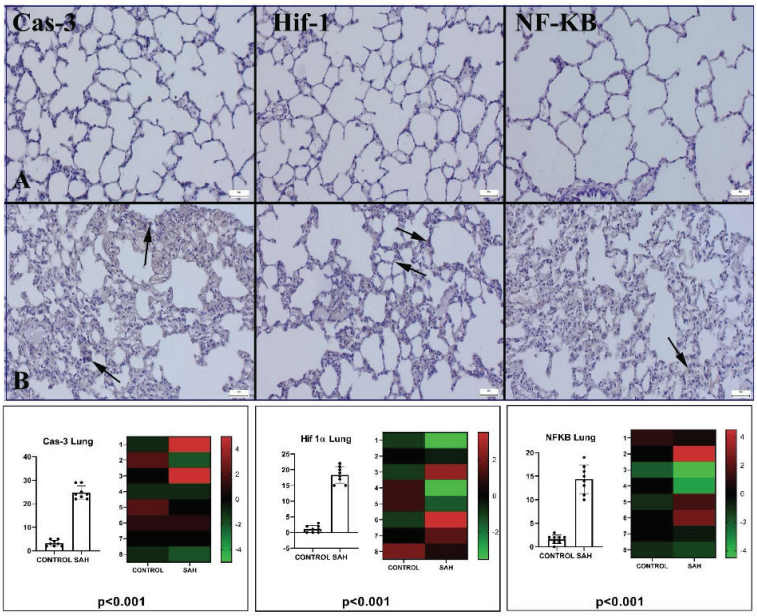

Figure 4

Cas-3, Hif-1α, NF-κB immunoexpressions in lung tissue

A) Control group, (B) SAH group, arrows indicate immunopositive cells, Streptavidin biotin peroxidase method, Scale bars=50μm.

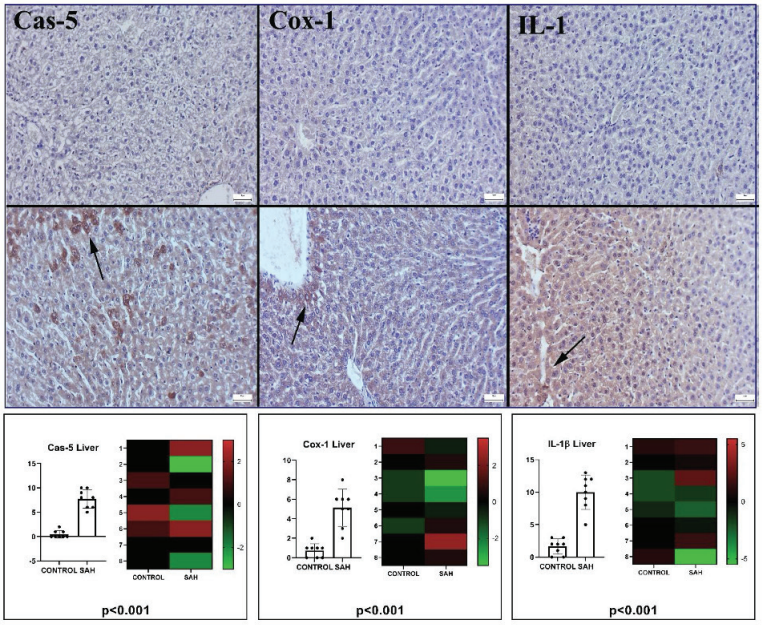

Figure 5

Cas-5, Cox-1, IL-1β immunoexpressions in liver tissue

(A) Control group, (B) SAH group, arrows indicate immunopositive cells, Streptavidin biotin peroxidase method, Scale bars=50μm.

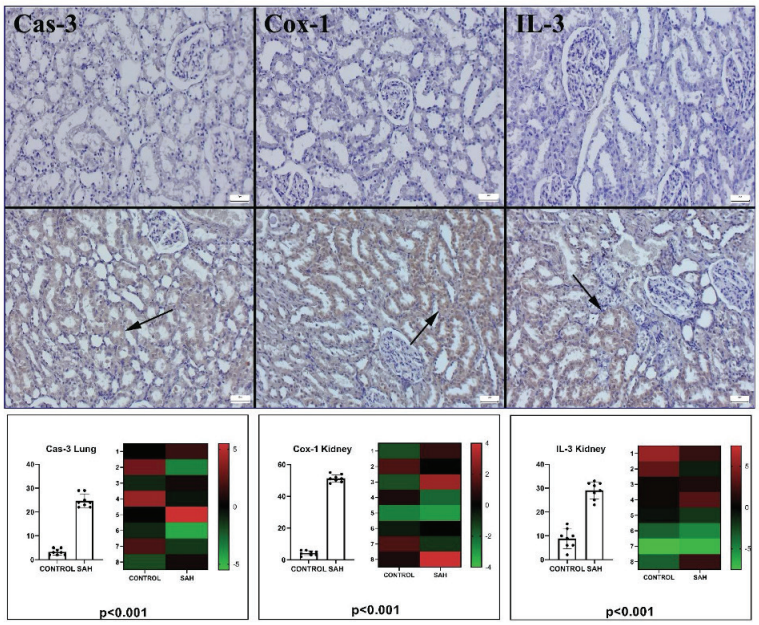

Figure 6

Cas-3, Cox-1, IL-3 immunoexpressions in kidney tissue

(A) Control group, (B) SAH group, arrows indicate immunopositive cells, Streptavidin biotin peroxidase method, Scale bars=50µm.

Discussion

Hypoxia-induced inflammatory reactions in the brain tissues distal to the hemorrhage area may cause local diffuse damage to the brain tissue. They may also cause damage to peripheral organs by passing into the peripheral blood due to increased blood-brain barrier permeability caused by cytokines released into the blood (9, 10). The histopathological findings from the brain tissue analyzed in the study indicate occurrences of SAH, evidenced by neuronal degeneration, gliosis, varying degrees of hyperemia, mild hemorrhage, and neuronal shrinkage. Hyperemia and hemorrhage findings in the lung, kidney, and liver tissues damaged by inflammatory cytokines passing to the periphery also indicate the development of peripheral organ damage. Consistent with the findings, Han et al. demonstrated that increased NF-κB levels following SAH lead to damage in the lungs (11).

In an observational study by Tujjar et al., it was reported that acute kidney injury developed in more than 10% of patients admitted to the intensive care unit following SAH. Additionally, Li et al. emphasized the development of fibrosis due to liver damage in some patients following SAH (12). SAH is a life-threatening disease with a severe prognosis due to its secondary effects (8).

For example, studies have shown that nephrotoxicity developing in patients with SAH increases mortality in the intensive care unit (2). It was also found that creatinine and BUN levels increased in these patients with SAH-dependent or independent mechanisms. Similarly, liver damage may also be observed in some patients following SAH (13). For example, a clinical study has shown that liver damage in patients with SAH is associated with rebleeding, intracranial infection, pneumonia, and acute kidney injury (13). There is also clinical data on the lung, one of the organs damaged after SAH, and it has been reported to increase over the years. For example, Veeravagu et al. showed in a study that the incidence of acute respiratory distress after SAH has increased since 1993 (14). Therefore, elucidating the cause-and-effect relationship of peripheral organ damages that develop after SAH will provide support for clinical studies to reduce mortality and morbidity in hundreds of patients with various complications. Clinical responses in patients with these organ damages, potentially linked to SAH, also corroborate these findings.

The expression of neurogenesis indicator BDNF and intermediate filaments that comprise the neuronal cytoskeleton indicator NF, whose expression was examined by immunohistochemical analysis to show the damage in brain tissue, decreased in the

cerebral cortex, cerebellum, and hippocampus (13). These damages may cause leukomotor dysfunctions, decreased memory and memory capacity, and balance disorders secondary to SAH (14, 15). In support of this, Chen et al. demonstrated in their study that the administration of exogenous BDNF reduced neurological deficits associated with SAH (16).

Immunohistochemical analyses were performed to determine the expression of various proinflammatory and apoptotic substances in lung, kidney, and liver tissues. In lung tissues, Cas-3 levels increased in inflammatory and alveolar epithelial cells as a common junction of many apoptotic pathways, indicating the development of apoptosis in the tissue. Also, NF-κB expression, which has a central role in cytokine production, and Hif-1α expression, which plays a role in hypoxia-induced inflammation, increased (17). Another study by Suresh et al. demonstrates that inflammatory damage occurring in the lungs is mediated through Hif-1α and NF-κB (18). Due to such cellular damage mechanisms in the lung tissue, the expansion capacity of the lung gradually decreases, and hypoxia-induced damage may be even more profound (19).

In this study, tissue responses in liver and kidney tissues, which are the organs of elimination, were also examined. Increased Cas-5, Cox-1, and IL-1 expressions in liver tissue of the SAH group were associated with increased prostaglandin synthesis due to arachidonic acid metabolism and activation of the nod-like receptor protein pathway (20). In their study, Galea et al. supported these findings by demonstrating that administering an IL-1 antagonist reduced inflammation associated with SAH (21). Increased apoptotic Cas-3 and proinflammatory IL-3 and Cox-1 expression in kidney tissue suggest that apoptosis and inflammation are also triggered in this tissue. Hvas et al. demonstrated that the damage caused by brain injury leads to an increase in COX-1 and COX-3 levels in the renal medulla (22). Damage to these organs, which are the organs of elimination, indicates that there may be problems in the excretion of the harmful substances produced in the disease and the drugs used in treatment (23, 24).

The critical aspect of this study, which includes the expression of very different markers in different tissues, is to help scientists who want to research these tissues in the future. In addition to these expressions, the lack of molecular genetic and biochemical analyses constitutes the study's limitation.

As a result, in SAH, which is a hazardous disorder, peripheral organ damage occurs in addition to brain tissue. The damage mechanisms induced by proinflammatory substances circulating in the blood by binding to their receptors in these organs may cause loss of function in tissues and aggravate the clinical response. More detailed molecular investigations including the expressions, must be conducted in future studies.

Conflict of Interest Statement
There is no conflict of interest between the authors.

Ethical Approval
In this study, all experiments were performed under the guidelines for animal research from the National Institutes of Health and were approved by the Committee on Animal Research of Mehmet Akıf Ersoy University, Burdur (Ethic No:24.04.2024/122-1298).

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Availability of Data and Materials
A data availability statement should include.

Authors Contributions
ASO: Conceptualization; Data curation; Investigation; Methodology; Validation; Visualization; Writing-original draft; Writing- review & editing.

HA: Methodology; Validation; Visualization; Writing-original draft; Writing- review & editing.

MC: Methodology; Validation; Visualization; Writing-original draft

NS: Methodology; Validation; Visualization; Writing-original draft, Writing- review & editing.

OO: Formal analysis; Validation; Visualization

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ORIGINAL RESEARCH

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Impact of COVID-19 History on Hospital Stay Duration and Postoperative Complication Rates in Patients Undergoing VATS Lobectomy for Stage I and II Lung Cancer: A Retrospective Cohort Study

Onur DERDIYOK[1]

[1] Sisli Hamidiye Etfal Training and Research Hospital, Department Of Thoracic Surgery, Istanbul, Türkiye

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Abstract

Objective

This study investigates how a prior COVID-19 history influences hospital stay durations and postoperative complication rates among patients undergoing VATS lobectomy for stage I and II lung cancer between 2020 and 2024.

Material and Method

A retrospective cohort study was conducted, including 145 patients who underwent VATS lobectomy at Şişli Etfal Hospital. Patients were divided into two groups: those with a history of COVID-19 (n=59) and those without (n=86). Data on demographics, clinical characteristics, hospital stay duration, and postoperative complications were collected and analyzed. Additionally, perioperative blood loss and the duration of chest tube placement were recorded for each patient. Patients who required more than 300 mL of drainage within the first 24 hours were carefully monitored, and further interventions were noted if necessary.

Results

Additionally, the postoperative complication rate was higher in the COVID-19 group (36.5%) compared to the non-COVID-19 group (22.1%) ($P < 0.05$). Common complications included pneumonia, fever, and wound infection. Patients with a history of COVID-19 experienced a notably longer mean hospital stay (8.5 ± 3.7 days) compared to those without (5.2 ± 2.8 days). The perioperative blood loss was higher in patients with a history of COVID-19, with an average of 150 mL compared to 100 mL in the control group. Additionally, the chest tube duration was significantly prolonged in the COVID-19 group (6.2 ± 1.5 days) compared to the non-COVID-19 group (4.3 ± 1.2 days).

Conclusion

These findings highlight the need for careful perioperative management and monitoring in this patient population. Patients with a prior history of COVID-19 undergoing VATS lobectomy exhibit prolonged hospital stays and increased postoperative complication rates.

Keywords: COVID-19, VATS, lung cancer

Correspondence: O.D. / onur_derdiyok@hotmail.com
Received: 05.07.2024 • Accepted: 19.11.2024
ORCID IDs of the Authors: O.D: 0000-0001-9994-8501

Introduction

The COVID-19 pandemic, caused by the novel coronavirus SARS-CoV-2, has had a significant and far-reaching impact on global health systems. Since its emergence in late 2019, COVID-19 has led to unprecedented challenges in the diagnosis, treatment, and management of various diseases, including those requiring surgical intervention. Thoracic surgery, particularly Video-Assisted Thoracoscopic Surgery (VATS) lobectomy, is a crucial treatment modality for early-stage lung cancer. VATS lobectomy offers several advantages over traditional open surgery, including reduced postoperative pain, shorter hospital stays, and quicker recovery times. However, the advent of COVID-19 has introduced new complexities into the perioperative management of patients undergoing this procedure (1-3).

The respiratory and systemic effects of COVID-19, particularly in individuals who have recovered from the infection, pose significant concerns for thoracic surgeons. Additionally, the systemic inflammatory response induced by COVID-19 can affect various organs and systems, potentially complicating the perioperative and postoperative course of surgical patients (4-6). COVID-19 often results in chronic pulmonary sequelae, including interstitial lung disease, pulmonary fibrosis, and diminished lung function.

These patients are at higher risk for postoperative complications, including respiratory complications, infections, thromboembolic events, and prolonged hospital stays. The immune dysregulation and inflammatory response associated with COVID-19 can also impair wound healing and increase the susceptibility to infections (7,8). Given these potential complications, it is imperative to understand how a history of COVID-19 influences the outcomes of patients undergoing VATS lobectomy for lung cancer (9). Existing evidence highlights that a history of COVID-19 is associated with elevated perioperative morbidity and mortality.

This study aims to fill a critical gap in the literature by examining the impact of a history of COVID-19 on the duration of hospital stay and postoperative complication rates in patients undergoing VATS lobectomy for stage I and stage II lung cancer. By comparing these outcomes between patients with and without a history of COVID-19, we seek to provide valuable insights that can inform clinical practice and improve perioperative management strategies for this unique patient population.

Understanding these differences is crucial for optimizing perioperative care and improving surgical outcomes in this patient population. The study hypothesizes that patients with a history of COVID-19 will have longer hospital stays and higher postoperative complication rates compared to those without such a history.

Material and Method

This retrospective cohort study was conducted at Şişli Etfal Hospital, analyzing data from patients who underwent VATS lobectomy for stage I and stage II lung cancer between January 2020 and December 2024. Patients who underwent VATS lobectomy for stage I or stage II lung cancer were included in the study, while patients with incomplete medical records or without a definitive diagnosis of stage I or stage II lung cancer were excluded. Patients were divided into two groups: Group 1 (COVID-19 History): Patients with a documented history of COVID-19 (n=59). Group 2 (No COVID-19 History): Patients without a history of COVID-19 (n=86).

Patient selection was randomized based on their COVID-19 history. However, additional factors such as age, comorbidities, and smoking status were also considered to ensure balanced groups.

Data including demographic information (age, gender), clinical features (comorbidities, smoking

Data including demographic information (age, gender), clinical features (comorbidities, smoking history), details of surgical procedure, length of hospital stay and postoperative complications (type and frequency) were collected from hospital medical records.

Statistical analysis was performed using SPSS software. Continuous variables were compared using the t-test, while categorical variables were analyzed using the chi-square test. A P-value of less than 0.05 was considered statistically significant.

Results

The mean age of patients with a history of COVID-19 was 65.7 (±8.2) years, while that of patients without COVID-19 history was 63.4 (±7.9) years. Both groups had a similar gender distribution, with a higher proportion of male patients (Group 1: 62%, Group 2: 58%). Common comorbidities included hypertension, diabetes, and chronic obstructive pulmonary disease (COPD) (Table 1). The mean hospital stay for patients with a history of COVID-19

Table 1 Patient Demographics and Clinical Characteristics

Variable	COVID-19 History (n=59)	No COVID-19 History (n=86)	P-value
Age (years)	65.7 ± 8.2	63.4 ± 7.9	0.12
Male (%)	62	58	0.34
Hypertension (%)	36	32	0.45
Diabetes (%)	28	26	0.50
COPD (%)	24	22	0.52
Smoking History (%)	60	58	0.37

Table 2 Comparison of Hospital Stay and Postoperative Complications

Variable	COVID-19 History (n=59)	No COVID-19 History (n=86)	P-value
Mean Hospital Stay (days)	8.5 ± 3.7	5.2 ± 2.8	< 0.01
Complication Rate (%)	36.5	22.1	< 0.05
Pneumonia (%)	12.3	6.5	< 0.05
Fever (%)	8.4	4.3	< 0.05
Wound Infection (%)	6.9	3.5	< 0.05

was 8.5 (±3.7) days, compared to 5.2 (±2.8) days for patients without a history of COVID-19 ($p< 0.01$). The postoperative complication rate was significantly higher in the COVID-19 group (36.5%) compared to the non-COVID-19 group (22.1%) ($p< 0.05$). Common complications included pneumonia, fever, and wound infection (Table 2).

Patient selection was randomized based on their COVID-19 history. Patients with a history of COVID-19 showed a significantly longer hospital stay (8.5 ± 3.7 days) compared to those without a history (5.2 ± 2.8 days). This difference underlines the additional challenges faced by COVID-19 survivors undergoing thoracic surgery. However, additional factors such as age, comorbidities, and smoking status were also considered to ensure balanced groups.

Discussion

These results are consistent with existing literature, suggesting that the residual effects of COVID-19 can have a lasting impact on surgical outcomes. The prolonged hospital stay and increased complication rates can be attributed to several factors, including compromised lung function, persistent inflammation, and immune dysregulation in COVID-19 survivors (10,11). This study demonstrates that a prior COVID-19 history significantly extends hospital stays and increases postoperative complication rates for patients undergoing VATS lobectomy for stage I and II lung cancer.

Studies have shown that survivors often suffer from interstitial lung disease, pulmonary fibrosis, and reduced lung function long after recovery from the acute phase of the disease. These pulmonary sequelae can severely impact the outcomes of thoracic surgeries such as VATS lobectomy, where optimal lung function is crucial for recovery (12,13). For instance, a study by Mo et al. reported that a significant proportion of COVID-19 survivors experienced persistent respiratory symptoms and abnormal lung function tests several months after recovery (10) . This study's findings align with our results, where patients with a history of COVID-19 experienced higher rates of pneumonia and other respiratory complications, which likely contributed to

their prolonged hospital stays and increased morbidity. COVID-19 is recognized for inducing substantial and prolonged respiratory system damage.

The systemic inflammatory response induced by COVID-19 can persist for months, leading to a hyperinflammatory state that affects various organs and systems . This chronic inflammation can impair wound healing and increase the susceptibility to infections, which are common postoperative complications (12-14). Huang et al. observed that COVID-19 survivors exhibited elevated levels of inflammatory markers and immune dysregulation for several months post-recovery. This chronic inflammation can impair wound healing and increase susceptibility to infections, which are common postoperative complications (12). Our findings support this, as patients with a history of COVID-19 had higher rates of wound infections and fever compared to those without such a history.

COVID-19 can cause significant immune dysregulation, characterized by lymphopenia, reduced immune cell function, and altered cytokine profiles. This immune impairment can weaken the body's ability to fight off infections and recover from surgical trauma. In the context of thoracic surgery, where the immune system plays a critical role in healing and defense against pathogens, such dysregulation can lead to increased postoperative complications (13-15). Our study found that patients with a history of COVID-19 had higher overall complication rates, supporting the hypothesis that immune dysregulation contributes to poorer surgical outcomes.

Another important factor to consider is the increased risk of thromboembolic events in COVID-19 survivors. The virus has been shown to cause endothelial damage, increased platelet activation, and a hypercoagulable state. These changes can increase the risk of deep vein thrombosis (DVT) and pulmonary embolism (PE) postoperatively. A study by Lee and Connors highlighted the heightened risk of thromboembolic events in COVID-19 patients, which can complicate postoperative recovery (16). While our study did not specifically track thromboembolic events, the higher complication rates observed in the COVID-19 group may partially be due to such events. The management of surgical patients with a history of COVID-19 presents unique challenges. Del Rio et al. emphasized the importance of comprehensive perioperative care for COVID-19 survivors, including thorough preoperative assessment and enhanced postoperative monitoring (11). This aligns with our findings, which underscore the need for tailored perioperative care protocols to mitigate the increased risks associated with a history of COVID-19.

The findings of this study have significant clinical implications for the management of patients undergoing VATS lobectomy for lung cancer. Given the increased risks associated with a history of COVID-19, it is essential to implement tailored perioperative care protocols for these patients. Preoperative assessment should include comprehensive evaluations of lung function and inflammatory markers to identify patients at higher risk of complications. Enhanced perioperative monitoring, including regular assessments of respiratory function and vigilant monitoring for signs of infection, is crucial for early detection and management of complications.

Perioperative Care Strategies

To mitigate the risks associated with COVID-19, several perioperative care strategies should be considered:

1.Preoperative Optimization: Thorough preoperative evaluation and optimization of pulmonary and overall health status are critical. This includes managing comorbidities such as hypertension, diabetes, and COPD, which were common in our study population.

2.Enhanced Monitoring: Continuous monitoring of respiratory function and early intervention for signs of respiratory distress or infection can help reduce the incidence of severe complications. This may involve the use of advanced monitoring technologies and protocols.

3.Anti-inflammatory and Antimicrobial Prophylaxis: Given the persistent inflammatory state in COVID-19 survivors, the use of anti-inflammatory medications and prophylactic antibiotics may be beneficial in reducing postoperative complications.

4.Thromboprophylaxis: Considering the increased risk of thromboembolic events, the use of anticoagulants in the perioperative period should be carefully managed and monitored to balance the risk of thrombosis against the potential for bleeding complications.

5.Postoperative Rehabilitation: Early and aggressive postoperative rehabilitation can help improve respiratory function and overall recovery. This includes respiratory physiotherapy, mobilization, and nutritional support to enhance healing and immune function.

Limitations of the Study

This study has several limitations that should be

acknowledged. Firstly, the retrospective design may introduce selection bias, and the relatively small sample size limits the generalizability of the findings. Additionally, the study was conducted at a single center, which may limit the external validity of the results. Another limitation is the lack of detailed data on the severity and treatment of COVID-19 in the study cohort, which precludes an analysis of how these factors may influence surgical outcomes. Further prospective, multicenter studies with larger sample sizes are needed to confirm these findings and explore the underlying mechanisms in greater detail.

Future Research Directions

Given the significant impact of COVID-19 on surgical outcomes, future research should focus on several key areas:

1.Prospective Studies: Large-scale prospective studies are needed to validate the findings of this retrospective analysis and provide more robust evidence on the impact of COVID-19 on surgical outcomes.

2.Mechanistic Studies: Research into the specific mechanisms by which COVID-19 affects lung function, immune response, and overall recovery in surgical patients will help to develop targeted interventions.

3.Perioperative Care Protocols: Studies examining the effectiveness of different perioperative care protocols in reducing complications and improving outcomes in COVID-19 survivors undergoing thoracic surgery are essential.

4.Long-term Outcomes: Investigating the long-term outcomes of COVID-19 survivors undergoing thoracic surgery, including survival rates and quality of life, will provide valuable insights into the broader implications of the pandemic on cancer care.

Conclusion

In conclusion, this study demonstrates that patients with a history of COVID-19 undergoing VATS lobectomy for stage I and stage II lung cancer experience longer hospital stays and higher postoperative complication rates compared to those without such a history. Enhanced preoperative assessment, vigilant perioperative monitoring, and aggressive management of inflammation and infections are crucial for improving outcomes in this vulnerable patient population. Further research is needed to fully understand the long-term impact of COVID-19 on surgical outcomes and to develop effective strategies for optimizing perioperative care. Our findings underscore the critical need for individualized perioperative management strategies to mitigate the increased risks in this patient cohort.

Conflict of Interest Statement

The author has no conflicts of interest to declare.

Ethical Approval

This study was conducted in line with the principles of the "Helsinki Declaration". Ethics committee approval has been granted from University of Health Sciences on 25/06/2024 with protocol number 4451, and informed consent has been obtained from all participants.

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Availability of Data and Materials

Data available on request from the authors.

Authors Contributions

OD: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Validation; Visualization; Writing-original draft.

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ORIGINAL RESEARCH

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Relationship Between Placenta Previa and Premature Preterm Rupture of Membranes: Case-Cohort Study

Seval YILMAZ ERGANI[1], Büşra SAHIN[1], Yıldız AKDAS REIS[1], Sadun SUCU[1], Can Ozan ULUSOY[1], Mevlüt BUCAK[1], Zeynep SEYHANLI[1], Gulsan KARABAY[1], Betul TOKGOZ CAKIR[1], Gizem AKTEMUR[1], Izzet OZGURLUK[2], Can Tekin ISKENDER[1]

[1] Department of Perinatology, Etlik Zubeyde Hanım Women's Health Training and Research Hospital, Ankara, Türkiye

[2] Department of Obstetrics and Gynecology, Ankara Etlik City Hospital, Ankara, Türkiye

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Abstract

Objective

This study aimed to evaluate whether the prevalence of premature preterm rupture of membranes (PPROM) is higher among patients with placenta previa.

Material And Method

A retrospective screening was conducted on a total of 59,567 pregnant women who delivered at our hospital between 2016 and 2021. Among the patients, 1,721 pregnant women meeting the inclusion criteria with PPROM were identified. The participants were divided into two groups: PPROM without placenta previa (control group, n=1,698) and PPROM with placenta previa (n=23). The data were analyzed subsequently.

Results

The birth week of PPROMs with placenta previa was found to be earlier (p = 0.028). The time between diagnosis and birth was shorter in PPROMs with placenta previa than in the second group (p<0.001), and there was a higher frequency of clinical chorioamnionitis in these patients (p=0.037). The prevalence of PPROM with placenta previa was 8.4%, compared to 2.88% for PPROM without placenta previa, demonstrating a statistically significant difference (p<0.001).

Conclusion

Our study found a significantly higher prevalence of PPROM in patients with placenta previa compared to those without placenta previa. Moreover, the interval from diagnosis to delivery was shorter, and clinical chorioamnionitis was more common in patients with PPROM and placenta previa.

Keywords: Chorioamnionitis, placenta previa, premature preterm rupture of membranes, PPROM

Correspondence: S.Y.E. / dr.svl7@gmail.com
Received: 10.07.2024 • Accepted: 23.09.2024
ORCID IDs of the Authors: S.Y.E: 0000-0002-7017-8854; B.S: 0000-0001-9800-6838; YAR: 0000-0001-9345-6899; S.S: 0000-0003-3758-0136; C.O.U: 0009-0005-7931-5172; M.B: 0000-0002-5035-8727; Z.S: 0000-0003-3924-3723; G.K: 0000-0003-2567-2850; B.T.C:0000-0003-0202-4981; G.A: 0000-0001-6824-881X; I.O: 0000-0002-9553-9265; C.T.I: 0000-0003-1376-5734

Introduction

Placenta previa is a placental implantation disorder that occurs in approximately 0.5% of all pregnancies and can cause peripartum hemorrhage (1, 2). This condition, diagnosed by second trimester ultrasound findings and vaginal bleeding, is associated with maternal and fetal morbidities. These include maternal morbidities such as cesarean section, sepsis, postpartum hysterectomy, blood transfusion, placenta acreata, and fetal morbidities such as preterm birth, low birth weight, and infections (3, 4).

PPROM can lead to fetal morbidity and mortality, prematurity, hemorrhage, abruptio placenta, fetal infections, oligohydramnios, fetal cord complications, which occur in approximately 3% of all pregnancies (5). This condition is known to occur secondary to inflammation due to ascending infection (6). Premature births have also been known to occur due to inflammation and infection at placenta previa (7). However, it is not known whether fetal and neonatal outcomes differ in PPROM in the presence of placenta previa. Premature preterm rupture of membranes (PPROM) refers to the rupture of membranes before 37 weeks of gestation in the absence of active labor.

PPROM has been established as an exclusion criterion in many studies. The association between PPROM and placenta previa. is not clear in the literature. The association between placenta previa. and pregnancy diseases such as chorioamnionitis or PPROM has been reported in very few studies (8). Some authors suggest that ascending infection is less common in the presence of a placenta located in the lower segment (8), and some studies have reported that histological chorioamnionitis increases in the presence of placenta previa (9). Considering that PPROM is not a local infection but the result of subclinical inflammation of the entire placental unit, the association between a low-lying placenta and PPROM is significant.

This study's primary goal is to ascertain whether placenta previa has a greater prevalence of PPROM. Our secondary objective is to investigate whether low-lying placentas negatively affect fetal and neonatal outcomes compared with normally located placentas.

Material and Method

We designed our study as follows: In the presence of placenta previa, ascending infection may penetrate more easily into the choriodecidua, the chorionic plate, amnion, and/or umbilical cord respectively, due to the pathological location of the placenta, and the likelihood of chorioamnionitis and PPROM may increase. PPROM is not a local infection. It is a more subclinical process affecting the entire placental unit. Evidence of increased PPROM in women with placenta previa supports this hypothesis. Placental placement in placenta previa may increase susceptibility to all of this condition.

Pregnant women diagnosed with PPROM between 2016 and 2021 who delivered at our hospital were divided into two groups: those with and those without placenta previa. Nonviable gestational weeks (< 22 weeks), multiple pregnancies, and pregnancies with major fetal anomalies were excluded from the study. During the years in question, a total of 59.567 pregnant women were delivered at our hospital, and a total of 1721 pregnant women with PPROM who met our criteria were found. Of these, 1698 were PPROM without placenta previa (control group) and 23 were PPROM with placenta previa.

The diagnosis of PPROM was made on the basis of clinical history and vaginal amniotic drainage by the physician and/or the AmniSure ROM test (Qiagen Sciences LLC, Germantown, MD, USA) (based on the determination of placental alpha-microglobulin-1 in vaginal fluid) performed in patients with suspected PPROM. In our clinic, all pregnant women with PPROM receive a single dose of betamethasone (6 mg intramuscularly every 24 hours for two doses, Celestone Chronodoses®, Schering Corp) and antibiotics (1 g azithromycin (Azro®) orally and an additional 2 g ampicillin (Penbisin®) intravenously four times daily for two days, followed by 2 g ampicillin orally four times daily for five days) as a treatment protocol. Many signs are used to diagnose clinical chorioamnionitis, including fever, maternal or fetal tachycardia, foul/purulent amniotic fluid, uterine discomfort, and maternal leukocytosis.

Data were obtained from individual medical and laboratory records via the hospital's digital registration system. Maternal demographic data and maternal and perinatal outcomes were examined.

Statistical Analysis

The statistical analyses of the investigation were conducted utilizing the SPSS 23.0 software. Mean and standard deviation are provided as descriptive statistics for the categorical variables in the data set, while median, minimum, and maximum values are provided as descriptive statistics for the continuous variables. The Shapiro-Wilk test was used to determine if the continuous variables agreed with

the normal distribution. For two-group comparisons of variables that were not normally distributed, the Mann-Whitney U test was employed. p-values less than 0.05 were deemed statistically significant.

Results

Between January 2022 and December 2022, a total of 59567 deliveries were performed in our hospital. 23 of the deliveries were placenta previa + PPROM and 1698 were placenta previa without PPROM. While the prevalence of PPROM+ placenta previa was 8.4%, the prevalence of PPROM without placenta previa was 2.88%, and there was a significant difference between them (p< 0.001).

Table 1 shows the demographic characteristics of the two groups. In either group, there was no significant difference in interventional procedures, BMI, smoking, or assisted reproductive techniques during pregnancy. The age difference between the two groups was significant (p= 0.003) and group 1 patients were older. Significant differences were observed between the two groups in the frequency of more than two births (p = 0.001), previous cesarean deliveries (p = 0.001), early pregnancy bleeding lasting longer than one week (p = 0.005), and more than two miscarriages in prior pregnancies (p = 0.026).

Table 2 shows that there was no significant difference in weeks of gestation at the time of diagnosis (p=

Table 1 Demographic and clinical characteristics of the study population

	PPROM and placenta previa (n:23)	PPROM without placenta previa (n:1698)	p
Age(year)	31.8 ± 3.7	27.4 ± 7.2	**0.003**
Parity			
0	5 (21.7 %)	951 (56 %)	**0.002**
1	5 (21.7 %)	439 (25.9 %)	0.835
>2	13 (56.5 %)	308 (18.1 %)	**< 0.001**
Miscarriage			
0	13 (56.5 %)	1193 (70.3 %)	0.230
1	6 (26.1 %)	422 (24.9 %)	1
>2	4 (17.4 %)	83 (4.9 %)	**0.026**
Previous cesarean			
0	8 (34.8 %)	1275 (75.1 %)	**< 0.001**
>1	15 (65.2 %)	423 (24.9 %)	
BMI (kg/m²)	28.3 ± 5.5	26.6 ± 6.1	0.257
Assisted reproduction	1 (4.3 %)	141 (8.3 %)	1
Smoking	6 (26.1 %)	635 (37.3 %)	0.370
Bleeding in early pregnancy lasting longer than one week	5 (22.7 %)	83 (4.9 %)	**0.005**
Invasive testing in the current pregnancy	1 (4.3 %)	47 (2.8 %)	0.480

Mann Whitney U Test was performed.
BMI, body mass index; kg/m2, kilograms per square meter.
Data are expressed as mean± standard deviation, or frequency (percentage) where appropriate. A p-value of <0.05 indicates a significant difference. Statistically significant p-values are in bold.

0.257). The first group had a shorter week of birth, and there was a significant difference in weeks of delivery between the two groups (p=0.028). The duration of the delivery interval was shorter in the first group of patients than in the second group (p 0.001). Whereas there were 5 (21.7%) patients with prenatal clinical chorioamnionitis in group 1, there were 138 (8.1%) in group 2, with a significant difference (p= 0.037).

Significant differences were found between the two groups in terms of hysterectomy at the time of delivery (p= 0.002), and the number of patients undergoing hysterectomy was higher in the second group. In addition, 4 (17.3%) of our PPROM+ placenta previa patients were confirmed to have PAS after surgery. There was no significant difference in fetal outcomes (birth weight, fetal loss, sepsis, retinopathy of prematurity, respiratory distress syndrome, bronchopulmonary dysplasia) between the two groups.

Discussion

In this study, the prevalance of PPROM was found to be significantly higher in patients with placenta previa than in patients without placenta previa. The interval from diagnosis to delivery was shorter, and clinical chorioamnionitis occurred more frequently

Table 2 Comparison of maternal and fetal outcomes between groups

	PPROM and placenta previa (n:23)	PPROM without placenta previa (n:1698)	p
Prevalence of disease in the cohort	23 (8.4 %)	1698 (2.88 %)	**< 0.001**
Maternal outcomes			
Gestational age at diagnosis (weeks)	29.5 ± 4.1	30.3 ± 3.4	0.257
Gestational age at delivery (weeks)	31.0 ± 4.1	32.5 ± 3.4	**0.028**
Interval from diagnosis to delivery (days)	10.1 ± 18.2	15.4 ± 12.6	**<0.001**
Anhydramnios diagnosed during the follow-up	3 (13 %)	566 (33.3 %)	0.067
Clinical chorioamnionitis diagnosed before delivery	5 (21.7 %)	138 (8.1 %)	**0.037**
Surgically confirmed PAS	4 (17.3 %)	-	**-**
Hysterectomy	3 (14.3 %)	17 (1.0 %)	**0.002**
Fetal outcomes			
Neonatal birth weight			
Mean ± SD	1700 ± 726	2070 ± 672	**0.023**
< 10th percentile	2 (8.7 %)	84 (4.9 %)	0.321
NICU admission	14 (60.9 %)	935 (55.1%)	0.730
Perinatal death	1 (4.3 %)	13 (0.8 %)	0.172
Sepsis	2 (8.7 %)	68 (4.0 %)	0.240
ROP	5 (21.7 %)	241 (14.2 %)	0.361
BPD	3 (13 %)	189 (11.1 %)	0.736
RDS	5 (21.7 %)	164 (9.7 %)	0.067

Mann Whitney U Test was performed.
PAS, placenta accreta spectrum; NICU, neonatal intensive care unit; ROP, Retinopathy of prematurity; BPD, bronchopulmonary dysplasia; RDS, respiratory distress syndrome.
Data are expressed as mean±standard deviation or frequency (percentage) where appropriate. A p-value of <0.05 indicates a significant difference. Statistically significant p-values are in bold.

in patients with PPROM and placenta previa. In addition, anhydramnios was less frequent in patients with PPROM and placenta previa.

Placenta previa is one of the most common causes of third trimester hemorrhage and is associated with severe maternal complications, including bleeding requiring blood transfusions, disseminated intravascular coagulation, and emergency hysterectomy (10). There are many mechanisms for the transmission of microorganisms in the occurrence of intrauterine infections in pregnancy. The ascending infection route is the most common from the vagina to the uterus (11). Romero et al. found that histologic chorioamnionitis in patients with twin births was more common in the firstborn twin than in the second twin because the membranes of the first twin were generally adjacent to the cervix, suggesting ascending intrauterine infection associated with preterm labor (11). We think that the higher prevalence of chorioamnionitis in the firstborn twin in this study is similar to the higher prevalence of chorioamnionitis in women with PPROM and placenta previa found in our study compared to PPROM without placenta previa due to the proximity of the placental membranes.

PPROM and intra-amniotic infection/inflammation were discussed at most studies but the cases with with placenta previa were excluded because of heterogeneous clinical features compared with cases without placenta previa. Therefore, the number of studies that have investigated the presence of intrauterine infection and inflammation in patients with placenta previa is very limited in the literature, and there is some inconsistency between the results of these few studies. Yonn et al showed that, the incidance of intraamniotic infection was 10%, intraamniotic inflammation was found in 32% of cases, and 53% of placentas available for analysis had histologic chorioamnionitis in patients with spontaneous preterm birth and intact membranes without placenta previa (7). Park et al. found that intra-amniotic inflammation was present in 16.7%, proven amniotic fluid infection in 4.9%, and histologic chorioamnionitis in 19.0% of patients with placenta previa and preterm labor and intact membranes. Also, all cases with histological chorioamnionitis had inflammation in the choriodecidua exposed to the cervical canal at placenta previa, but inflammation of the chorionic plaque was present in 63%. Compared to the results of the study by Yonn et al, these authors interpreted the low rates in patients with placenta previa in their study to mean that the placenta may play a protective role against ascending infections in contrast to our current study. On the other hand ,in their study, the time interval to delivery was shorter associated with intraamniotic inflammation in patients with placenta previa and preterm labor with intact membranes, similar to our study (9).

Idiopathic vaginal bleeding is associated with intra-amniotic infection in 14% of patients (12). Additionally, antepartum decidual hemorrhage is an important risk factor for PPROM, which can occur even in the presence of placenta previa. Madan et al showed that intraamniotic infection was present in 5.7% and intraamniotic infection and/or inflammation in 17.9% in patients with placenta previa who had vaginal bleeding. In addition, they found that intra-amniotic infection and/or inflammation was a risk factor for preterm delivery within 48 hours in the group (8). This finding suggests that vaginal bleeding in a subset of patients with placenta previa may also reflect microbial invasion. In this study, we found that vaginal bleeding which had longer than 1 week in early pregnancy was higher and the interval from diagnosis to delivery was shorter, and clinical chorioamnionitis occurred more frequently in patients with PPROM and placenta previa.

In the study by Kim et al, defective placentation, defined as failure of physiological transformation of the myometrial segment of the spiral artery, was frequently found in PPROM and the mean number of spiral arteries with failed physiological transformation of the myometrial segment was significantly higher in patients with PPROM and preeclampsia than in normal pregnant women (13). The finding that patients with PPROM also have a higher rate of vascular lesions in the villous tree and basal plate suggests that vascular disease may play a role in the pathogenesis of PPROM (14, 15). We also think that the increase in the prevalance of PPROM development in placenta previa patients in our study is due to this pathogenesis. Several explanations can be suggested for the shorter interval from diagnosis to delivery in patients with placenta previa and PPROM. First of all, active bleeding in placenta previa is an obstetric emergency, if vaginal bleeding occurs in the presence of PPROM with placenta previa, in this case, it may not be possible to distinguish whether the bleeding is due to previa or placental abruption and it may be considered bleeding requiring delivery. Another important situation is clinical chorioamnionitis was more common in PPROM with placenta previa, and the interval from diagnosis to delivery may have been shorter because chorioamnionitis is one of the conditions under which delivery should be performed. Lastly, considering the close correlation between placenta previa and placenta accreta spectrum (PAS), it is advisable to

rule out PAS in any patient with placenta previa. In the presence of PAS spectrum suspicion, the timing of delivery is earlier or due to a lower threshold for the decision to deliver.

Because the clinical approach was standardized, all patients received the same care from the same clinical personnel and according to the same protocol, which is the strength of our study. The weaknesses of the study are that it was obtained from a relatively small cohort over a period of only 4 years and that all data were obtained from an electronic database because of the retrospective design. In addition, we based the study on the evaluation of clinical chorioamnionitis, not histological chorioamnionitis for the same reason.

Conclusion

Our study showed the prevalence of PPROM was found to be significantly higher in patients with placenta previa than in patients without placenta previa and a higher incidance of chorioamnionitis in women with PPROM and placenta previa than in PPROM without placenta previa. The interval from diagnosis to delivery was shorter in these patients. In this regard, more data is needed and further pathological, morphological investigations will be necessary to clarify placenta previa, especially in the presence of PPROM. Our findings suggest that in patients with placenta previa, the extension of the placenta beyond the internal os may heighten the risk of ascending infection and PPROM development.

Conflict of Interest Statement
The authors declare no conflicts of interest.

Ethical Approval
The study was approved by the Etlik Zubeyde Hanım Women's Health Training and Research Hospital's ethics committee (Approval number: 19/11/2021/13). All procedures were performed in accordance with the Declaration of Helsinki.

Funding
The study did not receive funding.

Availability of Data and Materials
Individual level data (excluding identifiers) will be made available on request.

Authors Contributions
SYE: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Validation; Visualization; Writing-original draft, Project administration;

BS: Data curation; Formal analysis

YAR: Investigation

SS: Formal analysis

COU: Resources

MB: Investigation

ZS: Visualization

GK: Writing-original draft

BTC: Data curation

GA: Validation

IO: Writing-original draft

CTI: Supervision; Methodology; Writing-original draft. Writing-review & editing.

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ORIGINAL RESEARCH

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The Protective Effects of Cannabidiol on Chest Trauma-Induced Brain Injury by its Antioxidant and Anti-Inflammatory Effects

Adem MILLETSEVER[1], Abdurrahman GULAL[2], Halil ASCI[3]

[1] Department of Pathology, Faculty of Veterinary Medicine, Burdur Mehmet Akif Ersoy University, Burdur, Türkiye
[2] Department of Pharmacology, Institute of Health Sciences, Suleyman Demirel University, Isparta, Türkiye
[3] Department of Pharmacology, Faculty of Medicine, Suleyman Demirel University, Isparta, Türkiye

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Abstract

Objective
Chest trauma-induced brain injury (CTBI) is caused by the formation of inflammatory cytokines in the lungs and blood. Cannabidiol (CBD), a non-psychoactive agent, has antioxidant, anti-inflammatory, and antiapoptotic properties. In this study, we aimed to investigate the protective effects of CBD on CTBI.

Material and Method
Forty male Wistar Albino rats were divided into four groups: control, CTBI (200 g weight drop on the anterior chest wall from a height of 1 meter), CTBI+CBD (5 mg/kg, single dose intraperitoneally), and CBD. After 48 hours, rats were sacrificed under anesthesia, and brain tissues were placed in a 10% formaldehyde solution for histopathological and immunohistochemical examination.

Results
In the CTBI group, hemorrhagic areas, tumor necrosis factor-alpha, caspase-3, and malondialdehyde expressions increased in histological and immunohistochemical examinations compared to the control group. CBD treatment reduced hemorrhagic areas and reversed immune expressions.

Conclusion
Inflammation, apoptosis, and oxidative stress in brain tissue may develop in CTBI. These damages can be corrected with CBD treatment.

Keywords: Blunt chest trauma, brain, cannabidiol, apoptosis, malondialdehyde.

Introduction

Vehicle accidents and physical assaults are among the most common causes of chest trauma (CT) (1). Damage to the interstitial and vascular structures due to the pressure in the lung tissue as a result of trauma can cause local or systemic damage (2). Local damage mechanisms such as oxidative stress and inflammation can trigger more serious mechanisms such as apoptosis and necrosis. Oxidant and inflammatory substances produced as a result of such tissue damage and increased vascular permeability

Correspondence: A.M. / adem.milletsever@gmail.com
Received: 11.07.2024 • Accepted: 25.11.2024
ORCID IDs of the Authors: A.M.: 0000-0002-3614-7798; A.G.: 0009-0007-1743-412X; H.A.: 0000-0002-1545-035X

can enter the bloodstream and initiate distant organ damage, including brain tissue (3).

Peroxidation of lipids, especially those formed in cell membranes, plays an important role in the development of oxidative stress caused by free oxygen radicals in lung tissue. Increased levels of malondialdehyde (MDA), which is an indicator of lipid peroxidation at very low levels in healthy tissues, can be used as an indicator of oxidative stress. In addition to oxidative stress, tumor necrosis factor-alpha (TNF-α) can be used to show inflammation, and caspase 3 (Cas-3) can be used to show apoptosis (4).

CBD is a non-psychoactive phytocannabinoid derived from the Cannabis sativa plant. CBD has many beneficial effects, including anti-inflammatory and antioxidant properties. The lipophilicity of CBD and its ability to cross the blood-brain barrier make it a new candidate for central nervous system diseases (5). In an ischemia-reperfusion study, it was found that CBD supplementation decreased MDA levels in mouse hippocampal (HT22) neuron cells and thus reduced oxidative stress (6).

This study aimed to investigate the protective effect of CBD, which has known antioxidant and anti-inflammatory properties, on CT-induced brain injury (CTBI).

Material and Method

Animals and Ethical Approval

The design of all experimental procedures was carried out following the animal research guidelines of the National Institutes of Health and approved by the Local Ethics Committee for Animal Experiments of Suleyman Demirel University (Approval No. 2023-01/116). Animals were obtained from the Suleyman Demirel University Experimental Animal Laboratory and housed at 21-22 °C and 60% ± 5% humidity with a 12 h light:12 h dark cycle and fed ad libitum with standard commercial feed and water during the experiments.

Chemicals

Suleyman Demirel University, Natural Products Application and Research Center. The source of the CBD was the extract of Cannabis sativa L. (Cannabaceae). CBD content is >99.9 and the tetrahydrocannabinol content is <0.01. Limits of residual alcohol and heavy metals comply with USP and EU pharmacopeias. Ketamine HCl (Keta-Control 10%) and Xylazin HCl (Control 10%) were purchased from Doğa İlaç Turkey.

Chest Trauma Procedure

A bilateral CTBI model was developed as a trauma model by modifying the isolated bilateral pulmonary contusion model described by Raghavendran et al. (7). Lung contusion was induced by dropping a 200 g ball from a height of 1 meter onto the anterior thoracic wall of rats. The energy transferred to the chest wall was calculated as 1.96 joules using the formula E = mgh (E: energy, g: gravitational acceleration; 9.8 m/s², h: height; 100 cm, and m: dropped weight; 0.2 kg).

Anesthesia Procedure

Rats were anesthetized with 10 mg/kg Xylazine HCl + 50 mg/kg Ketamine HCl anesthesia before the thoracic trauma model. Before sacrification, 8-10 mg/kg Xylazine HCl + 90 mg/kg Ketamine HCl intraperitoneal (i.p.) anesthesia was administered.

Experimental Design

In the experimental project, 40 male Wistar Albino rats weighing 300-350 g were used. The animals were divided into four main groups of 10 animals each (Fig. 1). Groups;

Control group: Anesthesia was applied but no trauma was induced. Afterward, 0.5-1 mL saline injection was given i.p.

CTBI group: Thoracic trauma was applied under anesthesia. Afterward, rats were injected with 0.5-1 mL i.p. saline injection (7).

CTBI+CBD group: Thoracic trauma was applied under anesthesia. Afterwards, rats were given 5 mg/kg CBD i.p.

CBD group: Anesthesia was applied but no trauma was induced. Afterward, 5 mg/kg CBD was given to the rats i.p. (8).

48 hours after the beginning of the experiment, the rats were sacrificed under the anesthesia protocol. Brain tissues were removed and placed in a 10% formaldehyde solution for histopathologic and immunohistochemical examination.

Histopathological Method

The rat brains were removed from the skull by carefully opened with great care to prevent damage to the brain tissue during necropsy. All groups' brain samples underwent carefully gross examination. They were fixed in a 10% buffered formalin solution after this examination. Brain tissues were embedded in paraffin after undergoing standard tissue processing. Using

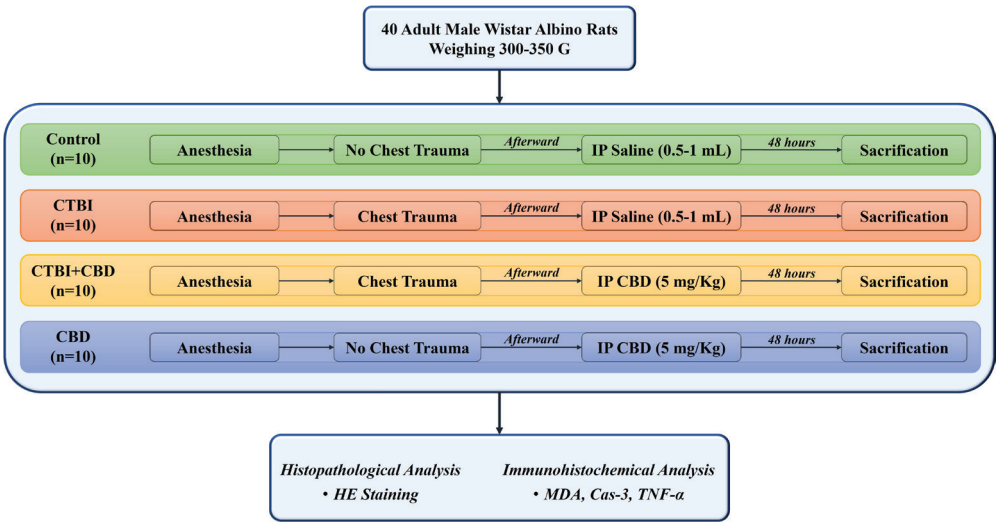

Figure 1
Experimental design

an automated rotary microtome, serial sections with a thickness of 5 microns were extracted from each tissue. Routine hematoxylin and eosin (HE) staining was used to stain sections and examined under a light microscope.

As in the previous studies (9), histopathological results were assessed using the standards given in Table 1. The scores ranged from 0 to 4.

Immunohistochemical Method

Sections generated on slides coated with poly-L-lysine were subjected to streptavidin-biotin peroxidase immunohistochemical staining. Brain sections were subjected to immunohistochemical investigation using primary antibodies against MDA (Anti-malondialdehyde antibody [11E3] (ab243066)), cas-3 (Anti-Caspase-3 antibody [EPR18297] (ab184787)) and TNF-α (Recombinant Anti-TNF alpha antibody [TNFA/1172] (ab220210)). All of the primary antibodies used in the immunohistochemistry analyses were obtained from Abcam and diluted using antibody dilution solutions at a ratio of 1/100. The immunohistochemical procedure was performed according to the manufacturer's instructions. The secondary kit used in this study was the Mouse and Rabbit Specific HRP/DAB Detection Kit - Micropolymer (ab236466) from Abcam, Cambridge, UK. Other procedures were followed as directed; however, for the negative controls, antibody dilution solutions were applied to the sections at the primary antibody stage rather than primary antibodies.

Immunohistochemical analysis was conducted by counting the percentage of positive cells, and the results were compared and evaluated among the groups. Special attention was paid to evaluating cells in the same areas of the brain where CT was induced and in other groups. For this purpose, 100 cells were counted per area, with 20 cells randomly selected from each of five areas of the same brain region for each rat, using a 40X objective. The Image J 1.46r software (National Institutes of Health, Bethesda, MD) was used to determine the number of cells

Table 1 Histopathological scores of subarachnoid hemorrhages

0	Normal meningeal and parenchymal structure
1	No blood in the subarachnoid space, ventricles, or brain parenchyma.
2	No localized or diffuse thin subarachnoid hemorrhage, intraventricular, or intraparenchymal hemorrhage.
3	No diffuse or localized thick subarachnoid blood layers, intraventricular, or intraparenchymal hemorrhage.
4	Intraventricular or intraparenchymal hemorrhage in association with subarachnoid hemorrhage, regardless of thickness or location.

showing a positive immunohistochemical reaction. An Olympus CX41 model microscope was used for photographing the results, and the Database Manual Cell Sens Life Science Imaging Software System (Olympus Corporation, Tokyo, Japan) was used for microphotography.

Statistical Analysis

For the statistical analysis of histopathological scores and the number of immunohistochemically positive cells, the GraphPad Prism 8.0.2 software package was utilized. Group differences were determined using the One-way ANOVA test. Values with p≤0.001 were considered statistically significant.

Results

The sham group showed signs of mild meningeal hemorrhage during the histological evaluation. Rats in the CTBI group showed extensive and noticeable hemorrhage. The administration of CBD was found to significantly reduce hemorrhagic regions in the CTBI+CBD group. In the CBD group, normal brain histology was noted (Fig. 2). Histological evaluation was scored and presented graphically (Fig. 3).

Immunohistochemical Findings

Upon immunohistochemical investigation, the control and CBD groups exhibited mild to negative expressions of TNF-α, Cas-3, and MDA in their

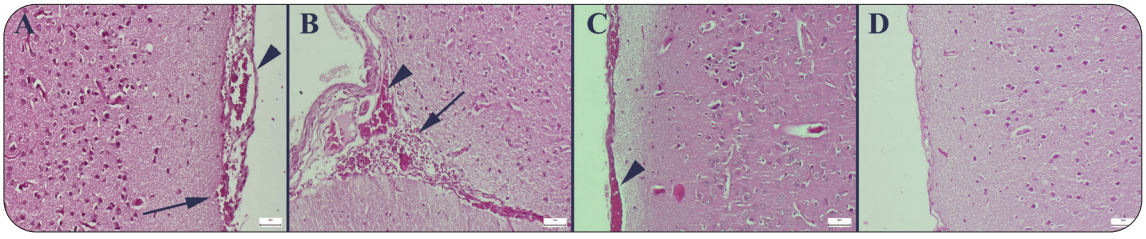

Figure 2

Histopathological appearance of brains between the groups

(A) Mild hyperemia (arrowhead) and slight hemorrhage (arrow) in meningeal vessels in the control group. (B) Marked hyperemia (arrowhead) and hemorrhage foci (arrow) in the brain of a rat in the CTBI group. (C) Markedly reduced hemorrhage area (arrowhead) in the CTBI+CBD group. (D) Normal brain and meningeal structure in a rat in the CBD group, HE, Scale bars=50μm.

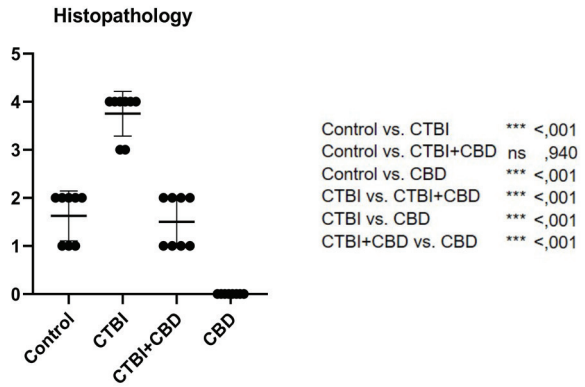

Figure 3

Statistical analyses of histopathological findings

CTBI: Chest trauma-induced brain injury, CBD: Cannabidiol. Values are presented as means ± standard deviation. A one-way ANOVA test was used. * p≤0.05, ** p≤0.01, *** p≤0.001.

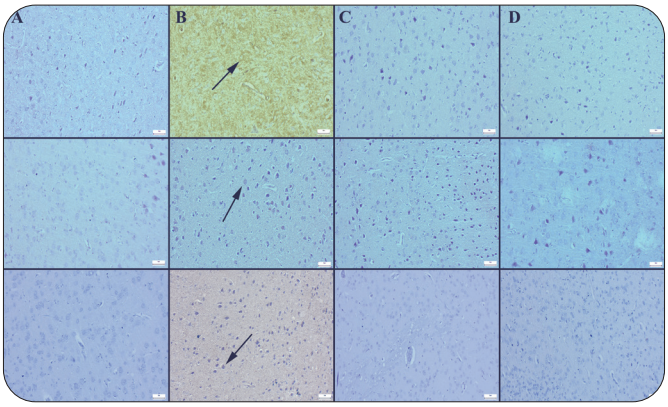

Figure 4

Expression of MDA (top row), Cas-3 (middle row), and TNF-α (bottom row) in the brain

(A) Negative MDA, Cas-3, and TNF-α expression in the control group. (B) Significant increase in MDA, Cas-3, and TNF-α expressions (arrows) in the CTBI group. (C) A marked decrease in MDA, Cas-3, and TNF-α expressions in the CTBI+CBD group. (D) No MDA, Cas-3, and TNF-α expressions in the CBD group, streptavidin-biotin peroxidase method, scale bars=50μm.

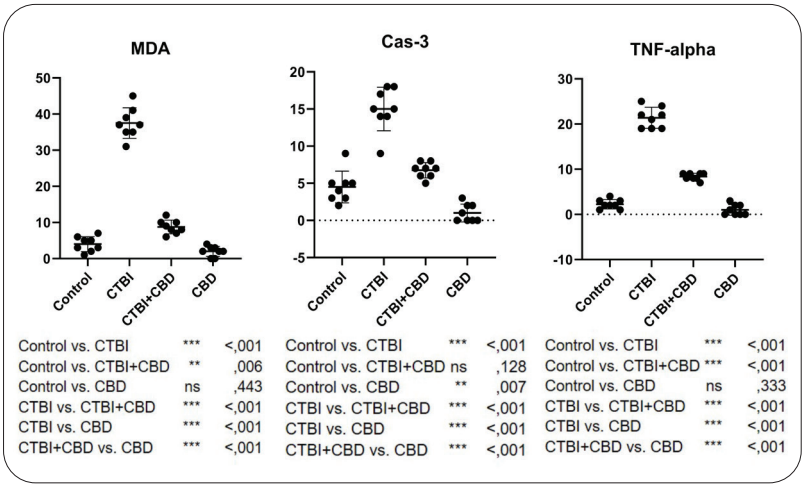

Figure 5

Statistical analyses of immunohistochemical findings

CTBI: Chest trauma-induced brain injury, CBD: Cannabidiol Values are presented as means ± standard deviation. A one-way ANOVA test was used. * p≤0.05, ** p≤0.01, *** p≤0.001

brains. Following the administration of CTBI, there was an increase in the expressions of TNF-α, Cas-3, and MDA. However, it was observed that CBD therapy caused all marker expressions to revert to normal (Fig. 4). Immunohistochemical evaluation was scored and presented graphically (Fig. 5).

Discussion

The lung is a vital organ that meets the body's need for oxygen, is rich in blood vessels, and therefore ensures the continuity of the functions of other organs. When this tissue is damaged, hypoxia, which is a picture of oxygen deficiency, can trigger inflammatory reactions in many tissues (10). The inflammatory picture in lung tissue damage allows oxidant substances and proinflammatory cytokines, whose synthesis increases with increased capillary membrane permeability, to enter the blood. These substances passing into the bloodstream are known to bind to their receptors in distant organs and activate various intracellular pathways (3). Inflammatory cytokines circulating

freely in the blood may also increase the permeability of the blood-brain barrier. These substances passing into the brain may cause damage (11). In this study, the presence of intense hemorrhagic areas in the CTBI group detected histologically, and the increase in TNF-α expression detected in immunostaining indicates that the experimental model is established, inflammation is triggered, and brain damage develops. Intracellular mechanisms play an important role in the cellular response to various stimuli. It has been found that these pathways, either on their own or by activating other pathways, cause the response to grow. As the number of affected cells increases, clinical progression worsens and loss of tissue function occurs. Scientists are trying to reverse cell damage mechanisms such as oxidative stress, inflammation, and apoptosis by trying many different active substances (12). The decrease in hemorrhagic areas in the brain tissue, which is an indicator of the inflammatory reaction histologically of CBD used in this study shows that the drug can both pass into the brain tissue and provide anti-inflammatory activity. The decrease in TNF-α expressions detected in immunohistochemical analysis is another indicator of CBD's anti-inflammatory activity.

As known, oxidative stress and inflammation can trigger each other and cause a rapid and aggressive course. The increase in MDA levels in parallel with the increase in the expression of inflammatory cytokines indicates the development of oxidative stress caused by lipid peroxidation (4). The decrease in MDA levels with the use of CBD may be interpreted as the drug providing antioxidant activity by increasing antioxidant enzyme levels or may reduce secondary oxidative stress by regressing inflammation by another mechanism (6). To clarify this situation, studies in which CBD alone is used and antioxidant enzyme levels are examined in such models are needed.

As a result of the occurrence of apoptosis, which has more severe consequences than the mechanisms mentioned above and is also stimulated by these mechanisms, death occurs in cells (12). It is very important to prevent this irreversible process. The reduction of apoptotic responses observed in cells in the CTBI model with CBD treatment also shows that the active substance has an antiapoptotic effect.

In conclusion, oxidative stress, inflammation, and apoptosis develop in brain tissue as a result of chest trauma, resulting in neuronal damage. It is possible that this damage can be reversed with CBD treatment, but more detailed studies investigating molecular mechanisms are needed.

Conflict of Interest Statement
Milletsever A and the co-authors have no conflicts of interest to declare in association with this study.

Ethical Approval
The study was carried out at Süleyman Demirel University Experimental Animal Production and Experimental Research Laboratory. Ethical approval was obtained from the National Institutes of Health and approved by the Local Ethics Committee for Animal Experiments of Suleyman Demirel University (Approval No. 2023-01/116).

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Availability of Data and Materials
The datasets generated during and/or analyzed during the current study are available from the corresponding author upon reasonable request.

Authors Contributions
A.M: Conceptualization; Data curation; Investigation; Formal Analysis; Methodology; Validation; Visualization; Writing review & editing; Writing original draft.

A.G: Conceptualization; Data curation; Investigation; Formal Analysis; Methodology; Validation; Visualization; Writing original draft

H.A: Conceptualization; Data curation; Investigation; Formal Analysis; Methodology; Validation; Visualization; Writing review & editing; Writing original draft

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ORIGINAL RESEARCH

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Comparing the Effects of Infusion and Bolus Doses of Bupivacaine Applied with Infraclavicular Catheter on the Duration and Need of Postoperative Analgesia

Umit FIDANCICEK[1], Ali KOCMAN[2], Filiz ALKAYA SOLMAZ[3], Mustafa Soner OZCAN[3], Pakize KIRDEMIR[3]

[1] Opera Yasam Hospital, Department of Anesthesiology and Reanimation, Antalya, Türkiye
[2] Oztan Hospital, Department of Anesthesiology and Reanimation, Usak, Türkiye
[3] Suleyman Demirel University, Faculty of Medicine, Department of Anesthesiology and Reanimation, Isparta, Türkiye

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Abstract

Objective

This study aimed to evaluate postoperative analgesia duration, analgesic requirements, and patient satisfaction between continuous infusion and bolus injection techniques using an infraclavicular catheter in patients undergoing forearm surgery.

Material and Method

We examined 100 patients which were divided into 2 groups to evaluate the data retrospectively. Bolus Injection Group (B): Patients who received 4 mL of bupivacaine (0.5%) from the catheter if the VAS value was > 3. Continuous Infusion Group (C): Patients who received 20 mg bupivacaine (0.02%) infusion via catheter using an infusion pump in 24 hours. Demographic data, American Society of Anesthesiologists (ASA) score, intraoperative and postoperative hemodynamic data, sensory and motor block onset times, postoperative Visual Analogue Scale (VAS) (1-2-6-12-24th hour), postoperative 24th and 48th hour satisfaction score, obtained from anesthesia and algology follow-up forms, were evaluated.

Results

When both groups were compared, VAS6 and VAS24 values of Group C were found to be statistically significantly lower than Group B. Satisfaction scores revealed that significantly more patients in Group C reported being very satisfied compared to Group B.

Conclusion

Our findings suggest that continuous local anesthetic infusion via catheter offers more sustainable analgesia compared to bolus administration.

Keywords: Plexus Blockade, Brachial, Catheterization, Peripheral, Analgesia

Correspondence: F.A.S. / filizalkaya@hotmail.com
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ORCID IDs of the Authors: Ü.F: 0000-0001-6375-7746; A.K: 0000-0002-9452-7633; F.A.S: 0000-0001-5772-6708; M.S.Ö: 0000-0003-0385-2308; P.K: 0000-0001-7784-1818

Introduction

The use of opioids is limited due to side effects such as sedation, dizziness, respiratory depression, nausea and vomiting (1). Serious complications such as perioperative acute kidney injury, coagulation disorders and anaphylactic reactions are observed in the use of nonsteroidal anti-inflammatory drugs (2,3). Postoperative pain management, which aims to prevent complications, often incorporates multimodal analgesia methods such as intravenous (IV), oral analgesics, and regional techniques.

Infraclavicular block constitutes a good alternative to general anesthesia, especially in patients undergoing forearm surgery and that it can be applied as a single injection or continuous infusion (4). Especially the use of ultrasound (US) has resulted in a significant increase in block success rate. In addition, there has been a reduction in the doses of local anesthetics used (5-10). Peripheral nerve block catheters allow for the administration of local anesthetics and adjuvant drugs to manage postoperative analgesia.

In this study, it was aimed to retrospectively examine the postoperative analgesia duration, analgesic requirement and patient satisfaction of continuous infusion and bolus injection technique performed via infraclavicular catheter in patients who underwent forearm surgery.

Material and Method

After the approval of Suleyman Demirel University Faculty of Medicine Clinical Research Ethics Committee (Date: 31.05.2017, protocol no: 113), the files of 140 patients who underwent elective surgery by Orthopedics and Plastic Surgery between 2016 and 2017 were examined. This study was conducted in accordance with the principles outlined in the Declaration of Helsinki.

Patients who underwent forearm surgery with infraclavicular brachial plexus block, between the ages of 18-75, ASA score I-II, who received continuous infusion or bolus injection of bupivacaine with a catheter for postoperative analgesia were included in the study.

Bolus Injection Group (B): Patients who received 4 mL of bupivacaine (0.5%) from the catheter if the visual analogue scale (VAS) value is > 3.

Continuous Infusion Group (C): Patients who received total 20 mg bupivacaine (0.02%)/ 100ml isotonic infusion via catheter using an infusion pump (Perfusor® Space, B. Braun, Germany) in 24 hours.

Demographic data (age, height, weight), ASA score, intraoperative and postoperative hemodynamic data [(systolic arterial pressure (SAP), diastolic arterial pressure (DAP), oxygen saturation (SpO2), heart rate (HR)] sensory and motor block onset times, postoperative VAS value (1th-2nd-6th-12th-24th hour), postoperative 24th and 48th hour satisfaction score, obtained from anesthesia and postoperative follow-up forms, were evaluated. Patients who expressed no satisfaction were stated as 1 point, those who expressed dissatisfaction 2 points, those who were satisfied 3 points, and those who were very pleased were stated as 4 points.

Data analysis was performed using SPSS (Statistical Package for Social Sciences INC; Chicago, IL, USA) version 15.0 software. Kolmogorov-Smirnov test was used for distribution of data. While parametric tests were performed on data with normal distribution, non-parametric tests were performed on data that did not show normal distribution. Qualitative data were presented as numbers and percentages, and quantitative data as mean and standard deviation. Chi-square test was used for qualitative analysis and Mann-Whitney U test was used for quantitative analysis. A value of $p<0.05$ was considered statistically significant.

Results

Patients who performed general anesthesia after infraclavicular block and who used postoperative oral or IV analgesics were excluded from the study. Ten patients with missing files, 17 patients who were operated under general anesthesia and 13 patients who used postoperative oral or IV analgesics were excluded from the study. The remaining 100 patients were divided into 2 groups to evaluate the data (Figure 1)

There was no statistically significant difference between the groups in terms of demographic data (Table 1). In the comparison of ASA scores, there was no significant difference between the groups ($p> 0.05$). When the type and duration of the operation performed were compared, no significant difference was observed between the groups ($p> 0.05$) (Table 2). Mean operation time was 93.2 minutes in Group B and 106.4 minutes in Group C.

No significant difference was found between the groups, when the motor and sensory block onset

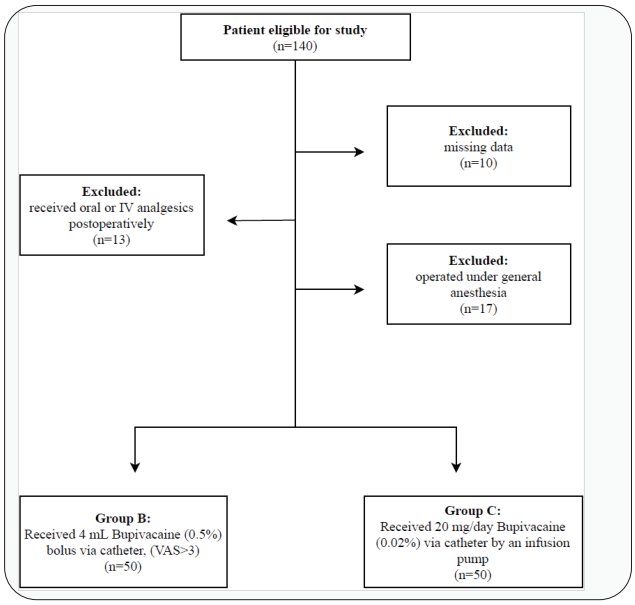

Figure 1
Patient selection flow chart.

times recorded after the block were compared. Motor block onset time was 10.9 minutes on average in Group B, while it was 9.8 minutes in Group C. While the mean sensory block initiation time was 21.24 minutes in Group B, it was found to be 19.3 minutes in Group C (p> 0.05) (Table 3). No statistically significant differences were observed in SAP, DAP, SpO2, and HR measurements recorded before surgery and at various intraoperative intervals (1st, 30th, 60th, 90th, and 120th minutes).

In the algology follow-up forms, no statistically significant difference was found between the SAB, DAB, SpO2, HR values measured at the postoperative 1st-12th-24th hours (p> 0.05). It was observed that when both groups were compared, 6th hour VAS (VAS6) (p <0.001) and 24th hour VAS (VAS24) (p <0.001) values of Group C were found to be statistically significantly lower than Group B (Table 4). Patients who stated that they were very satisfied (4 points) at the 24th hour and the 48th hour were found to be significantly higher in Group C compared to Group B (p = 0.002, p = 0.009 respectively) (Tables 5 and 6). Postoperative VAS scores were consistently lower in Group C compared to Group B across the 1st, 2nd, 6th, 12th, and 24th hours.

Table 1 Demographic Data

	GROUP B (n=50)	GROUP C (n=50)	p value
Age (year)	40,80±16,75	37,92±14,24	0,430
Height (cm)	170,16±6,49	172,86±7,85	0,054
Weight (kg)	73,9±13,76	78,18±12,01	0,097

Mean±SD (p>0.05)

Table 2 Operation Types

	GROUP B	GROUP C
	n (%)	n (%)
Hand injury	29 (58%)	32 (64%)
Tenolysis	6 (12%)	5 (10%)
Tendon injury	2 (4%)	4 (8%)
Amputation	2 (4%)	4 (8%)
Others	11 (22%)	5 (10%)
Total	50 (100%)	50 (100%)

Data are shown as n (%), p>0.05

Table 3 Motor and Sensory Block Onset Times

	GROUP B	GROUP C	p value
MB onset time (min.)	10.9±4,48	9.8±4,73	0.162
SB onset time (min.)	21.24±9,41	19.3±7,21	0.347

MB: Motor block, SB: Sensory block min: Minutes. Mean±SD (p>0.05)

Table 4 VAS values

	GRUP B	GRUP C	p value
VAS1	2.58±0,81	2.32±0,71	0.120
VAS2	2.3±0,61	2.14±0,70	0.237
VAS6	2.68±0,84	1.96±0,63	<0.001*
VAS12	2.18±0,74	1.78±0,67	0.007
VAS24	1.96±0,66	1.46±0,54	<0.001*

Mean±SD (p<0.05)*

Table 5 Satisfaction Scores After 24 Hours

	Satisfaction/ 24th			TOTAL
	2 points	3 points	4 points	
Group B	10	33	7	50
Group C	1	31	18*	50
TOTAL	11	64	25	100

*p=0,002

Table 6 Satisfaction Scores After 48 Hours

	Satisfaction/ 48th			TOTAL
	2 points	3 points	4 points	
Group B	2	35	13	50
Group C	0	23	27*	50
TOTAL	2	58	40	100

*p=0,009

Discussion

In this study, we found that adequate analgesia can be achieved with both methods, since VAS values were below 3 in bolus injection and continuous infusion groups in patients who underwent forearm surgery under infraclavicular brachial plexus block with a perineural catheter for postoperative analgesia. Although it was observed that sufficient analgesia was obtained in both groups, higher satisfaction scores were achieved especially in the Group C (0.02% bupivacaine) ($p < 0.05$). In addition, while a steady decrease was observed in VAS values of Group C, a statistically significant increase was observed in the Group B, especially at the 6th hour.

Klein et al. compared two different infusion protocols (0.2% ropivacaine 10 mL/hour and placebo) in interscalene block applications for rotatory cuff operation and achieved lower pain scores in the ropivacaine group compared to the placebo group, and thus it was shown that the continuous infusion method can be used in pain management (11). Ilfeld et al. showed that 0.2% ropivacaine infusion administered for 3 days to patients via infraclavicular catheters significantly reduced postoperative pain, oral opioid use and related side effects. Overall satisfaction was also found to be significantly higher in the ropivacaine group (12). In both studies mentioned above, it has been shown that continuous local anesthetic infusion applied through an infraclavicular catheter can be used effectively in reducing pain. In the present study, the effects of bolus injection or continuous infusion techniques on patient satisfaction and pain scores were compared in patients who were administered bupivacaine via infraclavicular perineural catheter for postoperative analgesia.

In studies comparing supraclavicular and infraclavicular brachial plexus block approaches by Gürkan et al, Koscielniak et al, Sandhu et al. and Sauter et al, the mean time to occurrence of infraclavicular block was reported to be 12.5, 19.0, 9.7, and 13.9 minutes, respectively (13-16). In this study, the mean time to occurrence of sensory block was 21.24 minutes in the Group B and 19.3 minutes in the Group C, the mean time to occurrence of motor block was 10.9 minutes in the Group B and 9.8 minutes in the Group C. These values are consistent with the times found in the literature and no statistically significant difference was found between the two groups.

It is unclear whether local anesthetic concentration or total drug dose is the main determinant of continuous peripheral nerve block effects. For this purpose, in a multi-center study conducted by Ilfeld et al, ropivacaine infusion in two different concentrations was applied to the patients for postoperative analgesia via the infraclavicular catheter. The presence of numbness in the extremities, pain scores, opioid needs and satisfaction scores of the patients were evaluated after 24 hours. High concentration (0.4%) ropivacaine was administered to 27 of 50 patients included in their study, and limb numbness was observed more frequently in these patients compared to the other group (0.2% ropivacaine). While 67% of the high concentration group described numbness in the extremities, this rate was 37% in the low concentration group. Although there was no difference between the pain scores of both groups, patient satisfaction was found to be significantly higher in the patients in the low concentration group. Consequently, in continuous infusion protocols, it has been stated that side effects such as numbness in the extremities will decrease and patient satisfaction will increase by decreasing the local anesthetic drug concentrations (17). In spite of the total amount of local anesthetic administered in the present study was less in the infusion group, we think that the reason for the higher patient satisfaction was the lower bupivacaine concentration we gave.

Ilfield et al. compared 3 different dosing regimens in patients who were administered infraclavicular perineural local anesthetic to provide postoperative analgesia. They concluded that the addition of patient-controlled bolus administration to continuous infusion is more ideal than bolus or infusion regimen alone in terms of providing postoperative analgesia and patient satisfaction, and minimizing the need for oral analgesic (18). In the present study, the VAS values of both groups at the 1st-2nd-6th-12th-24th hour and their satisfaction after 24-48 hours were compared. The VAS values of the Group C tended to decrease continuously during the day and were lower than the Group B, especially the difference in VAS6 and VAS24 values between the groups was found to be statistically significant. In addition, in the end-of-day satisfaction questionnaires, patients in the Group C had statistically significantly higher satisfaction scores compared to the Group B. These results are consistent with the studies in the literature, and accordingly, we think that local anesthetic infusion application via catheter provides a more sustainable analgesia than bolus application.

The safety and efficacy of peripheral nerve blocks have been proven in many prospective studies (19-21). However, the local anesthesia concentrations (0.1-0.5%, ropivacaine, 0.1-0.2% bupivacaine) and volumes (up to 10 mL/hour) used in these studies

are relatively high and this has been associated with an increase in muscle weakness due to motor block (22-23). It has been stated that muscle weakness, which is the result of motor block, prevents patients from starting physical therapy, but also increases the duration of hospital stay. In the study conducted by Qing Liu et al, 1768 patients who underwent knee arthroplasty between 2010 and 2012 were evaluated. One group (n = 439) received postoperative hydromorphone infusion and non-opioid analgesic without block, while the other group (n = 1329) received 0.03% bupivacaine infusion (3 ml / hour) via sciatica or femoral nerve catheters after the block. Patients with perineural catheters also received 3 ml bolus 0.03% bupivacaine per hour when needed. Resting VAS values and opioid use on postoperative first and second days were found to be significantly lower in those who received local aneshetic via catheter compared to the other group. There was no difference between the groups in VAS values after the pain caused by activity. While 96.41% of the patients with perineural catheter on the postoperative first day started physical therapy, this rate remained at 57.14% in the group without catheter, and this difference between the two groups was found to be statistically significant (24). In the present study, the concentration of bupivacaine (0.02%) used in the infusion group was lower than in the previous study. Despite the lower concentration, we found results consistent with the resting VAS values determined by Qing et al. Therefore, we think that more studies should be conducted on the concentration of local anesthetic administered for postoperative analgesia.

The present study has some limitations. The first of these is that the study is retrospective. In addition, the deficiencies in the postoperative nurse observation form and the epicrisis forms in the service and the complexity in accessing archive documents caused difficulties. Apart from this, we could not obtain results related to the postoperative physical therapy participation process and motor block evaluation of the patients were not documented.

In conclusion, although analgesia and patient satisfaction were found to be sufficient in both bolus and infusion methods, statistically significant low VAS values and high satisfaction scores were found in the lower concentration infusion group. The application of continuous local anesthetic infusion via catheter provides a more sustainable analgesia than bolus application.

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Conflict of Interest Statement
The authors have no conflicts of interest to declare.

Ethical Approval
Suleyman Demirel University Faculty of Medicine Clinical Research Ethics Committee, (Date: 31.05.2017, protocol no: 113). This study was conducted in line with the principles of the "Helsinki Declaration".

Consent to Participate and Publish
Studies involving human subjects should include a statement that: Written informed consent to participate and publish was obtained from all individual participants or legal guardians included in the study.

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Availability of Data and Materials
The data that support the findings of this study are available from the corresponding author upon reasonable request.

Authors Contributions
ÜF: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Validation; Visualization; Writing-original draft.

AK: Investigation; Validation; Writing-original draft

FAS: Data curation; Formal analysis; Resources; Writing- review & editing

MSÖ: Formal analysis; Visualisation; Writing- review & editing

PK: Supervision; Writing- review & editing

Editorial Statement
Although one of the authors of the article in question, PK, is one of the journal's sectional editorial board members, he was not involved in any stage of the article's publication process.

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ORIGINAL RESEARCH

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Knowledge Levels and Attitudes of Pediatric Physicians Regarding Meningococcal Infections and Vaccines: A Cross-Sectional Study

Hicran ALTIN[1]

[1] University of Health Sciences, Antalya Health Practice and Research Hospital, Department of Paediatrics, Antalya, Türkiye

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Abstract

Objective

Invasive meningococcal disease (IMD) clinically manifests as meningitis, meningococcemia, or a combination of both. Globally, approximately 1.2 million meningococcal cases are reported annually, resulting in 135,000 deaths. Despite meningococcal infections being a significant public health concern, widespread vaccination is not commonly practiced. The aim of this study is to evaluate the knowledge levels and attitudes of pediatric specialists and assistant doctors regarding meningococcal infections and associated vaccines.

Material and Method

This descriptive cross-sectional study included pediatric specialists and assistant doctors working in Antalya. During the study period, 170 pediatricians were identified, and it was aimed to reach at least 80% of this population. A total of 150 participants, selected via snowball sampling, participated in the survey. Data collection was carried out through face-to-face interviews and online questionnaires.

Results

Of the participants, 72.7% were pediatric specialists and 27.3% were assistant doctors. The conjugate meningococcal vaccine was recommended by 68% of the participants for all patients, by 20% for high-risk patients only, while 12% did not recommend the vaccine. The primary reasons for this hesitancy included the cost of the vaccine, concerns about adverse effects, perceptions of low vaccine efficacy, and the rarity of the disease. Additionally, 47.4% of the participants suggested that the conjugate meningococcal vaccine should be included in the national immunization schedule, followed by 39.3% who recommended the rotavirus vaccine and 13.3% who recommended the human papillomavirus (HPV) vaccine.

Conclusion

Our study found that pediatric specialists and assistant doctors had sufficient knowledge and generally positive attitudes towards Neisseria meningitidis infections and vaccines. However, the most significant barriers to vaccine administration were identified as cost, concerns about adverse effects, and the perception of low vaccine efficacy. It is anticipated that improving the knowledge and awareness of pediatricians will positively influence vaccine acceptance.

Keywords: Meningococcal vaccine, pediatricians, vaccine hesitancy, attitudes

Correspondence: H.A. / hicranaltindr@gmail.com
Received: 29.09.2023 • Accepted: 02.12.2024
ORCID IDs of the Authors: H.A: 0000-0002-6416-8510

Introduction

Invasive meningococcal disease (IMD) progresses clinically as meningitis, meningococcemia, or both. Approximately 1.2 million meningococcal cases are seen annually worldwide, and 135 thousand of them lose their lives (1). Meningitis is a globally prevalent disease, with Neisseria meningitidis serotypes and epidemiological trends varying significantly across geographic regions. The disease burden is largely due to serogroups A, B, C, W, and Y. Serogroups B (MenB) and serogroup C (MenC) account for the majority of meningitis cases in Europe and the United States (1, 2). Except for the neonatal period, the most common cause of bacterial meningitis in the 3-month-10 age group is Streptococcus pneumoniae, while the most common cause in the 10-18 age group is Neisseria meningitidis. (4). Although the agents of acute bacterial-purulent meningitis vary by geographical region and year, Streptococcus pneumoniae and Neisseria meningitidis are among the most common meningitis agents worldwide (5). For treatment, there are five licensed conjugate meningococcal vaccines in Turkey: MenACWY-TT (NimenrixTM), MenACWY-DT (MenactraTM), MenACWY-CRM (MenveoTM), MenQuadfi (MenACWY-TT) containing the ACYW serotype and MenB-4C (Bexero) containing the meningococcal B serotype (3, 4). Although menin-gococcal infections are a significant public health problem, vaccination is not widely practiced (5). Studies have shown that there is a lack of knowledge among parents about IMD vaccines and the vaccination status of their children (6-11). When parents are given accurate information, they are generally willing to have their children vaccinated (6, 10, 12, 13). Often, however, inadequate recommendations from family physicians and pediatricians contributes to many parents not getting their children vaccinated (6). Previous studies have shown a strong relationship between healthcare professionals' knowledge and attitudes about vaccines, their frequency of recommending vaccines to their patients, and the rate at which their patients get vaccinated (14).

In order to understand inadequate vaccination rates and to find solutions, it is of great importance to identify the barriers to vaccination at the level of healthcare professionals and healthcare institutions. In this context, the evaluation of the knowledge and attitudes of healthcare professionals, especially pediatricians regarding IMD and vaccines, plays a critical role in preventing and reducing the spread of meningococcal infections. This study aimed to determine the knowledge and opinions of pediatricians and pediatric residents regarding meningococcal infections and related vaccines.

Material and Method

This cross-sectional-descriptive study consists of pediatric specialists and pediatric residents working in various hospitals in Antalya. During the period when the study was conducted, it was determined that a total of 170 pediatric specialists and pediatric residents were working in Antalya. We aimed to reach at least 80% of the healthcare providers in the area, which provides an acceptable representative power in terms of epidemiological studies. Snowball sampling technique, one of the non-probability sampling methods, was used to determine the participants. The data collection process was carried out online through face-to-face interviews and Google Forms between September 10, 2022 and May 10, 2023. A total of 150 participants were enrolled in the study.

Statistical Analysis

Statistical analysis was analyzed using SPSS 22 program. Descriptive statistics were presented as numbers and percentages. Chi-square tests were used to compare categorical variables in independent groups. A p-value of less than 0.05 was considered statistically significant.

Results

The sociodemographic characteristics of the participants are presented in Table 1. Twenty-eight percent (n=42) of the participants were less than 30 years old, 58.7% (n=88) were female, 78% (n=117) were married, and 62.7% (n=94) had children. Seventy-two-point seven percent (n=109) of the participants were pediatricians and 34% (n=51) worked in a private hospital.

Among the most common causes of bacterial meningitis, 52% of the participants stated meningococcus (N. meningitidis) and 39.3% stated pneumococcus (S. pneumoniae). Regarding the transmission route of meningococcal meningitis, 97.3% of the participants stated that transmission occurs via droplets, and 79.3% stated that the only reservoir is humans. When asked about risk factors for meningococcal infection, immunodeficiency was stated as 97.3%, travel to risky areas as 96%, splenectomy as 94.7%, under the age of two as 86.7%, overcrowding as 69.3%, having had a viral upper respiratory tract infection as 22.7%, smoking as 22.7% and adolescent age group as 22% (Table 2).

Table 1 Sociodemographic characteristics of participants.

Sociodemographic Characteristics	n	%
Age		
≥ 30 years	42	28.0
31-40 Years	34	22.7
41-50 Years	35	23.3
51-60 Years	25	16.7
≤ 61 years	14	9.3
Gender		
Male	62	41.3
Female	88	58.7
Marital Status		
Married	117	78.0
Single	33	22.0
Having Children		
Has Children	94	62.7
No Children	56	37.3
Hospital Where Worked		
State Hospital	30	20.0
University	69	46.0
Private Hospital/Examination	51	34.0
Experience		
Specialist	109	72.7
Resident	41	27.3

When asked which strains cause IMD in our country, 94% of the participants responded as serotype W, 78% as serotype A, 77.3% as serotype B and Y, and 22.7% as serotype X. When asked about the age of administration of conjugate pneumococcal vaccines, 80.7% of the participants responded as 2 months, and 10.7% as 2 years. When asked whether polysaccharide meningococcal vaccines should be administered to children under the age of 2, 59.3% of the participants responded as no. When asked about the contents of conjugate meningococcal vaccines, 98.7% of the participants stated that the vaccine had 4 components (A, C, Y, W), and 80% stated that it had a single component (B). When asked about protection methods against IMD, 98.7% of the participants responded with vaccination and 67.3% responded with post-exposure chemoprophylaxis. When asked whether lifelong immunity is formed after meningococcal infection, 80% of the participants responded with no. When asked about the most common sequela after IMD, 89.3% of the participants responded with hearing loss (Table 2).

Sixty-eight percent of participants recommended conjugated meningococcal vaccine to all patients and 20.0% to risky patients, while 12.0% stated that they did not recommend meningococcal vaccine. The most frequently stated reason for not recommending meningococcal vaccine was cost (27.3%). The rate of participants wanting their own children to have conjugated meningococcal vaccine was 94.6%. When the actual vaccination status was evaluated, 60.7% of the participants stated that they had their children vaccinated, while 10.0% stated that they had not. It was determined that 98.7% of the participants viewed meningococcal vaccine positively, and recommended

Table 2 Knowledge levels about meningococcal vaccine

	n	%
The Most Common Cause of Bacterial Meningitis		
Meningococcus	78	52.0
Pneumococcus*	59	39.3
Hib	13	8.7
Meningococcus Meningitis Transmission Modes		
Droplet*	146	97.3
Contact Route	3	2.0
Contaminated Food and Water	1	0.7
Meningococcal Infection Host		
Only Human*	119	79.3
Human. mammals	30	20.0
Human. poultry	1	0.7
Human. cold-blooded animals	0	0.0
Meningococcal Infection Risk Factors**		
Splenectomy*	142	94.7
Immunodeficiency*	145	97.3
Travel to Risky Areas*	144	96.0
Under 2 Years of Age*	130	86.7
Overcrowding*	104	69.3
Having Viral Upper Respiratory Tract Infection*	34	22.7
Smoking*	34	22.7
Adolescent Age Group*	33	22.0
Meningococcal Strains That Cause Invasive Meningococcal Disease**		
A*	117	78.0
X	34	22.7
B*	116	77.3
C*	108	72.0
Y*	116	77.3
W*	141	94.0
Age at which conjugated meningococcal vaccines can be administered		
2 months*	121	80.7
1 year	5	3.3
9 months	8	5.3
2 years	16	10.7
Can polysaccharide meningococcal vaccines be administered to children under 2 years of age?		
Yes	61	40.7
No*	89	59.3
Invasive Meningococcal Infection Agents**		
4 Components (A, C, Y, W)*	148	98.7
Single Component (B)*	120	80.0
Single Component (C)	5	3.3
Single Component (X)	1	0.7
Invasive Meningococcal Disease Prevention Methods**		
Vaccination*	148	98.7
Droplet Isolation*	78	52.0
Contact Isolation	63	42.0
Post-Contact Chemoprophylaxis*	101	67.3
Provides Lifelong Immunity After Meningococcal Infection		
Yes	30	20.0
No*	120	80.0
The Most Common Sequelae After Invasive Meningococcal Infection		
Hearing Loss*	134	89.3
Convulsion	5	3.3
Extremity Amputation	8	5.3
Mental Retardation	3	2.0

* Correct Answer; ** More than one option is marked

Table 3 Participants' attitudes about meningococcal vaccine

	n	%
Recommend Meningococcal Vaccine		
Recommend to All Patients	102	68.0
Recommend to Risky Patients	30	20.0
Do Not Recommend18 12.0	18	12.0
Reasons for Not Recommending Meningococcal Vaccine*		
Cost	41	27.3
Infrequency of Disease	11	7.3
Unwanted Effect	13	8.7
Low Effectiveness of Vaccine	13	8.7
Wanting to Have Meningococcal Vaccine for Your Child		
Yes	142	94.6
No	8	5.3
Status of Having Meningococcal Vaccine for Your Child		
Yes	91	60.7
No	15	10.0
I Don't Have a Child	44	29.3
Wanting Meningococcal Vaccine to Be Included in the National Vaccination Scheme		
Yes	148	98.7
No	2	1.3
Vaccine Desired to Be Included in the National Immunization Scheme as a Priority		
Conjugated Meningococcal Vaccines	71	47.4
Rota Virus Vaccine	59	39.3
Human Papilloma Virus Vaccine	20	13.3

* More than one option is marked

it to be included in the national vaccination schedule. Among the vaccines that the participants wanted to be included in the national immunization schedule as a priority, 47.4% responded as conjugated meningococcal vaccines and 39.3% as rotavirus vaccine (Table 3).

The comparison of the participants' attitudes towards meningococcal vaccine with the institution they work for is given in Table 4. Of the physicians working in private hospitals, 76.4% recommend the vaccine to all patients, while 26.1% of the physicians working in university recommend it only to risky patients. Those working in state hospitals (16.7%) did not recommend the vaccine. There was no significant difference in the status of recommending the vaccine according to the institution they work in (p=0.335). Among the reasons for not recommending the meningococcal vaccine, 39.1% of the physicians working in university cited the cost, while 16.7% of the physicians working in private and state hospitals put forward the undesirable effects (p=0.011). All the physicians working in public hospitals and 96.1% of the physicians working in private hospitals responded positively to the request to have their children vaccinated with conjugated meningococcal vaccine (p=0.179). Of physicians working in private hospitals, 88.9% had their children vaccinated against meningococcus, while university and state hospital physicians were 82.8% 80%, respectively (p=0.593). There was no significant difference in terms of the

Table 4 Evaluation of participants' attitudes towards meningococcal vaccine according to the institution they work for.

	State Hospital n (%)	University Hospital n (%)	Private Hospital n (%)	p*
Recommend Meningococcal Vaccine				
To All Patients	19 (63.3)	44 (63.8)	39 (76.4)	0.335
To Risk Patients	6 (20.0)	18 (26.1)	6 (11.8)	
I Do Not Recommend	5 (16.7)	7 (10.1)	6 (11.8)	
Reasons for Not Recommending Meningococcal Vaccine				
Cost	5 (16.7)	27 (39.1)	9 (16.7)	**0.011**
Infrequency of Disease	1 (3.3)	8 (11.6)	2 (3.9)	0.181
Unwanted Effect	5 (16.7)	8 (11.6)	0 (0.0)	**0.018**
Low Effectiveness of Vaccine	2 (6.7)	9 (13.0)	2 (3.9)	0.195
Willingness to Have Meningococcal Vaccine for Your Child				
Yes	30 (100.0)	63 (91.3)	49 (96.1)	0.179
No	0 (0.0)	6 (8.7)	2 (3.9)	
Status of Having Meningococcal Vaccine for Your Child				
Yes	16 (80.0)	24 (82.8)	40 (88.9)	0.593
No	4 (20.0)	5 (17.2)	5 (11.1)	
Willing Meningococcal Vaccine to Be Included in the National Immunization Scheme				
Yes	30 (100.0)	68 (98.6)	50 (98.0)	0.754
No	0 (0.0)	1 (1.4)	1 (2.0)	
Vaccine Desired to Be Included in the National Immunization Scheme as a Priority				
Conjugated Meningococcal Vaccines	17 (56.7)	33 (47.8)	21 (41.2)	0.218
Rota Virus Vaccine	9 (30.0)	24 (34.8)	26 (51.0)	
Human Papilloma Virus Vaccine	4 (13.3)	12 (17.4)	4 (7.8)	

institutions worked with and the non-routine vaccines that were requested to be included in the national vaccination program (p=0.218).

The comparison of the participants' professional experience and their attitudes towards meningococcal vaccination is presented in Table 5. Specialist physicians (71.6%) recommended conjugate meningococcal vaccine to all patients, while 29.3% of resident physicians recommended it only to risky patients. Resident physicians (12.2%) stated that they did not recommend the vaccine (p=0.205). Among the reasons for not recommending conjugate meningococcal vaccine, the cost of the vaccine was observed to be the most common reason for 25.7% of specialist physicians and 31.7% of resident physicians (p=0.595). Specialist physicians (95.4%) and resident physicians (92.7%) gave a positive answer to the question of whether they would have their children vaccinated with meningococcal vaccine (p=0.684). It was observed that 85.7% of specialists and 80% of resident physicians administer meningococcal vaccine to their children (p=1.000). In the evaluation of non-routine vaccines recommended to be included in the national immunization schedule, it was observed that conjugate meningococcal vaccine was recommended by 48.6% of specialists and 43.9% of resident physicians. Interestingly, rotavirus vaccine was supported by 42.2% of specialists and 31.7% of resident physicians, while HPV vaccine was recommended by 9.2% of specialists and 24.4% of resident physicians (p=0.046) (Table 5).

Table 5 Evaluation of participants' attitudes towards meningococcal vaccine according to their professional experience.

	Specialist n (%)	Resident n (%)	p*
Recommend Meningococcal Vaccine			
To All Patients	78 (71.6)	24 (58.5)	0.205
To Risk Patients	18 (16.5)	12 (29.3)	
I Do Not Recommend	13 (11.9)	5 (12.2)	
Reasons for Not Recommending Meningococcal Vaccine			
Cost	28 (25.7)	13 (31.7)	0.595
Infrequency of Disease	5 (4.6)	6 (14.6)	0.071
Unwanted Effect	7 (6.4)	6 (14.6)	0.188
Low Effectiveness of Vaccine	7 (6.4)	6 (14.6)	0.188
Willingness to Have Meningococcal Vaccine for Your Child			
Yes	104 (95.4)	38 (92.7)	0.684
No	5 (4.6)	3 (7.3)	
Status of Having Meningococcal Vaccine for Your Child			
Yes	72 (85.7)	8 (80.0)	0.641
No	12 (14.3)	2 (20.0)	
Willing Meningococcal Vaccine to Be Included in the National Immunization Scheme			
Yes	107 (98.2)	41 (100.0)	1.000
No	2 (1.8)	0 (0.0)	
Vaccine Desired to Be Included in the National Immunization Scheme as a Priority			
Conjugated Meningococcal Vaccines	53 (48.6)	18 (43.9)	**0.046**
Rota Virus Vaccine	46 (42.2)	13 (31.7)	
Human Papilloma Virus Vaccine	10 (9.2)	10 (24.4)	

Discussion

In this study, the knowledge and opinions of pediatric specialists and residents about meningococcal infections and vaccines were evaluated. The findings contributed to determining the vaccine awareness and attitudes of healthcare professionals and paved the way for the development of strategic recommendations to improve the current situation regarding meningococcal vaccination.

In our study, the awareness of the participants regarding the bacterial meningitis agents was examined and the most frequently reported pathogens were determined as Neisseria meningitidis (52%) and Streptococcus pneumoniae (39.3%). With the widespread use of Haemophilus influenzae type b (Hib) and 13-valent pneumococcal conjugate vaccines in children, a general decrease in bacterial meningitis cases was observed. However, Streptococcus pneumoniae still remains one of the leading causes of meningitis in children. In addition, it was reported that the frequency of Neisseria meningitidis infections caused by Serogroup W and Serogroup B increased. The participants were aware of the bacterial meningitis agents and their frequency (15-17).

Participants' knowledge about the transmission routes and risk factors of meningococcal infections was also evaluated. Participants (79.3%) reported that humans are the only reservoir for meningococcal infections, and 97.3% reported that transmission occurs via

droplets. Among the risk factors, immunodeficiency (97.3%), splenectomy (94.7%), travel to risky areas (96%), and children under the age of two (86.7%) were frequently mentioned. However, 78% of the participants did not consider respiratory tract infection, crowded environments, smoking or exposure, and the adolescent age group as risk factors. These findings indicate a lack of awareness that respiratory tract infections, smoking exposure, and crowded environments are important risk factors for meningococcal infections (18).

When asked whether permanent immunity can develop after meningococcal infections, 80% of participants correctly answered no. This shows that the knowledge that meningococcal infections can be recurrent and that protection with vaccination should be continued is widespread among healthcare workers. This information is of great importance for the continuity of vaccination strategies.

In our study, participants stated that the serotypes causing IMD in our country were 94% W serotype, 78% A serotype, 77.3% Y and B serotypes, and 72% C serotype. In the study conducted by Ceyhan et al., the most common serogroup was W between 2009-2016, while serogroup B was detected at the highest rate in the latest data from 2017. (15). In the study conducted by Güldemir et al., N. meningitidis serogroup B was identified as the most common causative agent (19). Our physicians were aware of the serogroups commonly seen in our country.

When participants were asked about the serotypes included in conjugated meningococcal vaccines in Turkey, 98.7% correctly identified the four-component vaccine containing ACYW serotypes, while 80% correctly identified the single-component vaccine containing serotype B. In addition, 80.7% of participants correctly answered the age at which conjugated meningococcal vaccines should be administered, while 19.3% were unaware that the vaccine could be administered from two months of age onwards. In a cross-sectional study conducted in Italy among 200 pediatricians, only 28% of participants were aware of the vaccination program for children aged two and under (20). This situation shows that awareness of vaccination programs needs to be increased.

In our study, it was determined that 68% of the participants recommended conjugate meningococcal vaccines to all patients, while 20% recommended them only to risky patients. In the study conducted by Dinleyici et al. with pediatricians and pediatric infectious disease subspecialists, it was stated that 56.3% of the participants recommended conjugate meningococcal vaccination in their daily practice. When all participants were asked about their views on risk groups and routine use of meningococcal vaccines, the majority considered all children between the ages of 0-18 as a risk group. In Turkey, the vast majority of pediatricians recommend conjugate meningococcal vaccines in their daily practice. (16).

In our study, it was determined that 12% of the participants did not recommend meningococcal vaccination. The reasons for this reluctance of physicians were the cost of the vaccine, its undesirable effects, the perception that it is low effective and the rarity of the disease. In a study conducted in Latin America, it was determined that the biggest obstacles to not getting vaccinated were low education level, lack of awareness about diseases and vaccines, religious and cultural beliefs and negative socioeconomic factors. (21). Parental barriers to IMD vaccination include lack of knowledge, low perceived value of vaccines, and misperceptions about the health threats posed by vaccine-preventable diseases (8, 9, 11). In addition, the fact that IMD vaccines are not included in the national vaccination schedule may lead parents to consider these vaccines as less important (6). Such perceptions contribute to lower vaccination rates by reducing parents' trust in vaccines for their children. According to the World Health Organization (WHO), vaccine hesitancy is considered one of the top 10 threats to global health (22). In Turkey, the vaccine refusal rate in children under 2 years of age was determined as 5.9 per thousand in 2017, a 1.7-fold increase compared to 2016 (23-25). The WHO's vaccine advisory group has identified complacency, difficulties in accessing vaccines and lack of trust as the main reasons behind this hesitancy (22). Vaccination hesitancy must be addressed. Because studies have shown that the majority of parents primarily seek information and advice about vaccine-preventable diseases, vaccines, and recommended vaccination schedules from their children's healthcare providers (26, 27). The primary care physician's recommendation, the effectiveness of the vaccine, and its cost are important factors in the decision to vaccinate. When healthcare providers can effectively communicate with parents about the benefits, risks, value and necessity of vaccines, and vaccine safety, parents appear to be more confident in their decisions (28).

It was determined that 94.6% of the participants were willing to vaccinate their children and 60% had vaccinated their children. Thirty-three-point six percent of family physicians vaccinated their children with

conjugated meningococcal vaccine, and it was found that pediatricians had a higher rate of vaccinating their children than other physicians (5).

In our study, 98.7% of the participants recommended that conjugate meningococcal vaccines be included in the national immunization schedule. In the study conducted by Özdemir et al., 81.7% of the participants, in the study conducted by Duygu et al., 69.4% of the participants, and in the study conducted by Dinleyici et al., 67% of the participants stated that meningitis vaccines should be included in the national immunization schedule (5, 16, 29).

In our study, 47.4% of the participants stated that conjugate meningococcal vaccine, 39.3% rotavirus vaccine and 13.3% HPV vaccine are non-routine vaccines that should be included in the national immunization schedule as a priority. It was determined that physicians administer rotavirus and meningococcal vaccines to their children most frequently and it was observed that this situation is consistent with the recommendations of physicians (30). Unlike our study, in the study conducted by Özdemir et al., 79.3% of pediatricians recommended the inclusion of rotavirus vaccine and 68.9% of pediatricians recommended the inclusion of conjugate meningococcal vaccine in the national immunization schedule (29).

When the attitudes of physicians towards meningococcal vaccine were evaluated according to the institution they worked in, no statistically significant difference was found between physicians working in university, public hospitals and private hospitals. However, the highest rate of recommending conjugate meningococcal vaccine was observed among physicians working in private hospitals; this group was followed by physicians working in university and public hospitals, respectively. The reasons for not recommending meningococcal vaccine by physicians working in university and public hospitals were cost and undesirable side effects. The better financial situation of the patient profile in private hospitals may explain why physicians working in these hospitals recommend the vaccine more often. Similar findings were also revealed in a study conducted in Italy. The socioeconomic determinants of vaccine hesitancy and refusal were examined and it was concluded that the economic difficulties experienced by families were one of the determining factors of vaccine hesitancy (31).

When the attitudes towards conjugated meningococcal vaccines were examined according to the experience level of the participants, no significant difference was found between pediatric specialists and pediatric residents. Pediatric specialists (71.6%) and pediatric residents (58.5%) stated that they recommend the vaccine to all patients. Although the frequency of recommending the vaccine was higher in the specialist group, the reason why a statistically significant difference could not be determined may be that the number of resident physicians included in the study was lower than that of specialist physicians. However, statistically significant differences were observed in the responses given to the question of non-routine vaccines that should be added to the national immunization schedule. While pediatric specialists recommended conjugated pneumococcal vaccine and rotavirus vaccine as the priority, pediatric residents recommended HPV vaccine as the first priority.

Conclusion

Our study revealed that pediatricians and pediatric residents have sufficient knowledge about N. meningitidis infections and related vaccines and generally have a positive attitude. However, lack of awareness of some risk factors and inadequate knowledge about the vaccine administration schedule were striking findings. In addition, cost and inadequate physician recommendations were found to be the most important barriers to vaccine administration. These findings indicate that training programs for healthcare professionals should be strengthened and vaccination awareness should be increased. In addition, the inclusion of conjugated meningococcal vaccines in the national immunization schedule will increase vaccine acceptance rates. It has been demonstrated that healthcare professionals' knowledge levels and positive attitudes towards vaccination are important factors in increasing vaccine acceptance in society.

Conflict of Interest Statement
There is no conflict of interest among the authors.

Ethical Approval
Approval for the study was obtained from the Antalya Education and Research Hospital Non-Interventional Clinical Research Ethics Committee (date: 29/09/2022, decision no: 18/21). Written consent was obtained from the participants and the study was conducted in accordance with the Declaration of Helsinki.

Consent to Participate and Publish
Informed consent and written permission to publish the data were obtained from all individuals involved in the study.

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Availability of Data and Materials
Data can be requested from the authors.

Authors Contributions
HA: Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Validation; Visualization; Writing-original draft; Resources; Supervision; Writing-review & editing.

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ORIGINAL RESEARCH

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Carvacrol is a Novel Natural Therapeutic Approach Through the Inhibition of Proliferation, Autophagy and Migration in Pancreatic Ductal Adenocarcinoma Cells

Zeynep AKPINAR[1], Nilgun GURBUZ[2]

[1,2] Suleyman Demirel University, Faculty of Medicine, Department of Medical Biology, Isparta, Türkiye

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Abstract

Objective

Pancreatic ductal adenocarcinoma, which is the most common and aggressive pancreatic cancer, has the highest mortality rate of cancers because of difficulties in diagnosis and chemoresistance. As the chemotherapeutic options are very limited and insufficient for PDAC, novel effective therapeutic approaches are urgently needed for pancreatic cancer patients. Carvacrol naturally found in thyme (*Thymus vulgaris*), wild bergamot (*Citrus aurantium var. bergamia Loisel*), black cumin (*Nigella sativa*), marjoram (*Origanum scabrum, Origanum microphyllum, Origanum onites, Origanum vulgare*) and black pepper (*Lepidium flavum*) plants is shown to have antibacterial and antioxidant effects. In this study, we aimed to investigate the anticarcinogenic potential of carvacrol through proliferation and autophagy in PDAC cells.

Material and Method

To determine the anti-proliferative effects of carvacrol in Panc-1 cells, we performed the MTS assay using different carvacrol doses of 100, 200, 300, 400, 500, 600, 700 and 800 µM at 24h, 48h, 72h. The gene and protein expressions of Atg16L1 and Beclin-1, autophagy key mediators, were analyzed by RT-PCR and western blot in Panc-1 cells treated with 300 and 400 µM for 24h, 48h. Additionally, the migrative property of PDAC cells was evaluated using a wound healing assay.

Results

Based on MTS results, carvacrol significantly inhibited cell proliferation in the doses of 300 and 400 µM at 24h and 48 h in Panc-1. These same doses led to decreased autophagy and migration through, Atg16L1, and Beclin-1 expressions.

Conclusion

Our findings first revealed that carvacrol has promising value as a potential therapeutic approach for PDAC. We believe that further mechanistic investigations will be a guide for its clinical usage.

Keywords: Pancreatic cancer, carvacrol, proliferation, autophagy, migration

Correspondence: N.G/ nilgungurbuz@sdu.edu.tr
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ORCID IDs of the Authors: Z.A: 0000-0001-9547-3967; N.G: 0000-0003-4476-5593

Introduction

The pancreas, though varying in mass and volume from person to person, is divided into five anatomical sections: the head, uncinate process, neck, body, and tail, separated by fine connective tissue. It has two main functions exocrine and endocrine. In its endocrine function, it synthesizes hormones such as glucagon, insulin, and somatostatin, while in its exocrine function, it produces digestive enzymes like lipase, amylase, and trypsin. The homeostatic balance is maintained by the regeneration of intact pancreatic tissue. However, this balance is disrupted by factors such as inflammation and tissue damage, causing the acinar cells responsible for the exocrine function to undergo morphological and genetic differentiation into ductal structures through the Acinar-Ductal Metaplasia (ADM) process, leading to pancreatic intraepithelial neoplasia (PanIN) and eventually tumor formation. Pancreatic tumors include those originating from the endocrine or exocrine components of the pancreas, with pancreatic adenocarcinoma being the most common and aggressive type originating from the exocrine pancreas. Among these, pancreatic ductal adenocarcinoma (PDAC) is the most frequently seen cancer type, 90%, in the pancreas, especially when compared to acinar carcinomas and neuroendocrine tumors (1–3).

When examining the molecular mechanisms and mutations of PDAC cells, several key mutations and signaling pathways are found to play significant roles in the development and progression of this cancer type. A point mutation that activates the KRAS oncogene, a member of the RAS family and a GTPase, leads to its persistent binding to GTP, triggering the onset of cancer via three signaling pathways: the RAF/ERK pathway, phosphoinositide 3-kinase (PI3K), and RalGDS. This mutation is observed in low-grade PanINs. Mutations in the RAF family, activated by RAS through progressive phosphorylation, lead to the activation of the mitogen-activated protein kinase ERK (MAPK-ERK), contributing to the uncontrolled proliferation of cancerous cells. Thus, in the medullary type of PDAC, RAF mutations replace KRAS mutations as the primary cause. Mutations in tumor suppressor genes such as TP53, CDKN2A, and SMAD4 further the disease progression. The Transforming Growth Factor Beta (TGF-β) signaling pathway manages basic cellular processes. Binding of the tumor-suppressive SMAD4, encoded by the DPC4 gene, to TGF-β receptors on the cell surface activates SMAD proteins, which form a complex with SMAD4 to regulate gene activity in specific DNA regions, effectively triggering cell cycle and apoptosis. However, mutations in these proteins inactivate them, playing a role in cancer progression (4,5). Due to high metastatic potential, the enhanced autophagy results in PDAC cells (6,7). It is well known that autophagy serves as a survival mechanism in many cancer cells, including pancreatic cancer, in contrast to cell death (8,9). Unless autophagy is inhibited in cancer cells, apoptosis cannot be activated. Therefore, key autophagic mediators such as Beclin-1, ATG proteins, and LC3-II are overexpressed in PDAC cells (7,10). All these mutations and dysregulations in these mediators lead to uncontrolled cell division, playing a critical role in cancer pathogenesis and contributing to changes in the microenvironment of cancer cells, increasing their resistance to chemotherapy and proliferation (11).

One of the factors making PDAC so aggressive and resistant to chemotherapy is thought to be the desmoplastic tissue structure, consisting of activated fibroblasts, nerve tissue, and collagen fibers. Desmoplasia not only creates oxidative stress in cells but also reduces nutrient and drug availability. Consequently, PDAC cells reprogram their metabolism to adapt to these processes, increasing their resistance to conventional chemotherapy (12).
Current treatment for pancreatic cancer primarily involves surgical removal of the tumor. If surgery is not possible due to the tumor's conditions, treatment is attempted through three methods, which are radiation therapy combined with chemotherapy, or chemotherapy alone in the case of metastasis (13). Despite these treatment methods, the overall survival rate remains very low due to the resistance of cancer cells to chemotherapy drugs, highlighting the urgent need for developing alternative treatment methods. Research into the antiproliferative activity of natural products is crucial in this regard (14).

Carvacrol (CV; C10H14O; also known as 5-iso-propyl-2-methylphenol by the International Union of Pure and Applied Chemistry) is a liquid phenolic monoterpenoid and primarily found in the essential oil of oregano (*Origanum vulgare*). It is commercially synthesized by chemical and biotechnological techniques and is highly soluble in acetone, ether, and diethyl ether due to its lipophilic nature (14,15). Carvacrol is known for its antibacterial properties against almost all Gram-negative and Gram-positive bacteria, except for the hospital pathogen *Pseudomonas aeruginosa*. It inhibits the toxin synthesis and growth of foodborne pathogens such as *Bacillus cereus, Escherichia coli*, and Salmonella and also prevents biofilm formation in fungi like Candida, adding to its antifungal properties. Apart from these benefits, the investigations in vitro

and in vivo have revealed its anticancer properties through the apoptosis mechanism (16,17).

Despite these studies, there is no mechanistic-level research on the anti-carcinogenic efficacy of carvacrol in pancreatic cancer. Therefore, this study aims to investigate the effects of carvacrol on autophagy and cell proliferation, key mechanisms in the progression of PDAC cells, contributing to the literature and science. While literature reviews have revealed studies on carvacrol's effects on apoptosis and oxidative stress in various cancers, there is almost no information on its autophagy mechanism in pancreatic cancer, making this research pioneering in the field.

Material and Method

Cell Culture

Human PDAC cell lines having high metastatic potential (Panc-1) and non-tumorigenic human pancreatic epithelial cell line (HPDE) were cultured using Dulbecco's Modified Eagle Medium (DMEM) with different glucose concentrations specific to each cell line. Panc-1 cells were cultured in high glucose DMEM media (Invitrogen, Carlsbad, CA, USA), while HPDE in special media containing 75% of low glucose DMEM (Cegrogen Biotech, Stadtallendorf, GERMANY) supplemented with 10% FBS and 25% of M3:BaseFTM supplemented with growth factors (Incell; San Antonio, TX, USA), in the presence of 1% Penicillin/Streptomycin (Invitrogen; Carlsbad, CA, USA) under regular culture conditions, at 37 °C in a water-saturated 95% air and 5% CO2 atmosphere. When the cell density on the flask surface reached 80%, the cells were washed with 1X Dulbecco's PBS (Phosphate Buffered Saline) (Cegrogen Biotech; Stadtallendorf, GERMANY) and detached using 1X 0.25% Trypsin-EDTA (Thermo Scientific; Waltham, MA, USA). The passage number of cells was used up to 15. Cell counting was performed using Trypan Blue and a Thoma Hemocytometer before setting up experiments.

Cell Viability/Cytotoxicity Assay

The principle of the cell viability/cytotoxicity assay is based on the activity of oxidoreductase enzymes, which are indicators of cell viability and mitochondrial enzymes. These enzymes depending on NAD(P)H can convert tetrazolium to colored formazan, making the viability measurable as absorbance in a spectrophotometer by the amount of formazan produced. Panc-1 and HPDE cells were distributed into 96-well sterile microplates as 4000 cells per well and incubated overnight to allow cell attachment. The viability of Panc-1 and HPDE cells was determined using MTS Cell Proliferation Assay Kit (Promega Corporation; Madison, Wisconsin, USA) in the cells treated with carvacrol in doses of 100, 200, 300, 400, 500, 600, 700 and 800 µM for 24 h, 48 h, 72 h. At the end of the incubation period, the colored formazan products were measured at the absorbance of 490 nm using a Multiskan GO spectrophotometer (Thermo Scientific).

RT-PCR (Reverse-Transcriptase Polymerase Chain Reaction) Assay

Based on the results of the cell viability assay, Panc-1 cells were seeded into 6-well sterile microplates at 500,000 cells per well and treated with carvacrol in doses of 300 and 400 µM for 24h. At the end of the treatment, cDNA was synthesized from RNA using a cDNA synthesis kit (Applied Biosystem; Vilnius, LTU) followed by total RNA isolation with TRIzol reagent (Invitrogen; Life Technologies, Carlsbad, CA). The forward and reverse primer sequences were as follows, respectively; Beclin-1 5' GAA CCG CAA GAT AGT GGC AGA 3' and 5' CAG AGC ATG GAG CAG CAA CA 3'; ATG16L1 5' TCA GAT CTT CAT TCA GTG TTG GC 3' and 5' GCT CCT GGT TCT CTT CCG TAG T 3'; and reference gene GAPDH: 5' CAA GGT CAT CCA TGA CAA CTT TG 3' and 5' GTC CAC CAC CCT GTT GCT GTA G 3'. All RNA samples were amplified in PCR program included initial denaturation at 94℃ for 2 minutes, and then 94℃ for 30 seconds, 55℃ for 45 seconds, 72℃ for 1 minute as 35 cycles, finally additional elongation at 72℃ for 5 minutes. The PCR products were loaded onto 1.2% agarose gels in 1X TBE buffer in the presence of 10X Blue JuiceTM Gel Loading Buffer (Invitrogen, Carlsbad, CA, USA) and densitometrically analyzed with Image J software (NIH). Results were expressed as fold change compared to non-treated conditions.

Wound Healing/Migration Assay

To analyze the effect of carvacrol on cell migration, a wound healing/migration assay was conducted based on the ability of cells to migrate into a created gap. After Panc-1 cells were distributed into 6-well sterile microplates as 500,000 cells per well were incubated overnight to allow cell attachment, a line with a sterile 1 mL pipette tip was marked at the bottom of each well. The cells were then treated with 300 µM and 400 µM doses of carvacrol to be able to evaluate the possible changes in cell motility and migration. Then cells were photographed using a phase-contrast microscope (Zeiss, Jena, Germany) just before (0 h) and 24 h, 48 h later of treatment. The migrated cells to the line were counted, and photographs were taken under the microscope at 0 h and 24 h, 48 h later, with at least 5 random non-overlapping areas for each.

Statistical Analyses

All data were expressed as the means ± standard error of the mean (SEM) of three independent experiments. Statistical significance was determined using the Student t-test. P values less than 0.05 were considered statistically significant. Student's t-tests and ANOVA were calculated using GraphPad software.

Results

Carvacrol Decreases the Cell Viability in PDAC cells, not in HPDE

The effects of carvacrol on the viability of PDAC cells were determined by MTS analysis in the Panc-1 cell

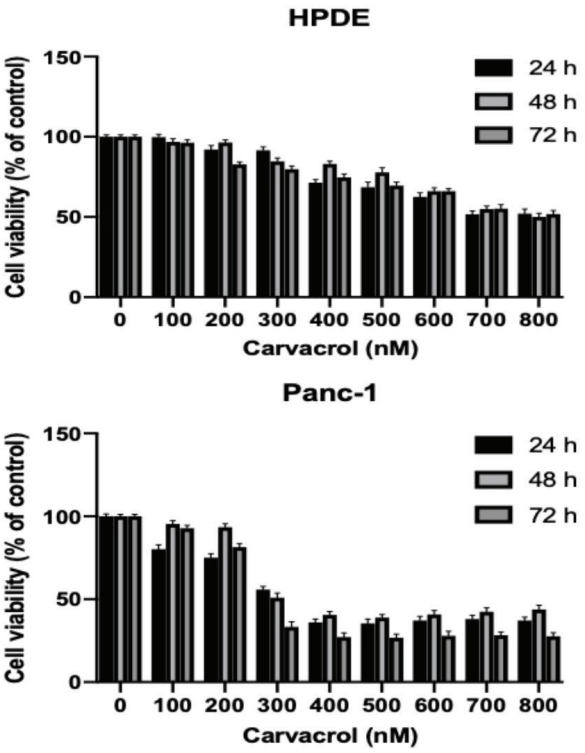

Figure 1
Carvacrol suppresses proliferation in PDAC cells, Panc-1 (A), compared to non-tumorigenic human pancreatic epithelial cells, HPDE (B). Cell proliferation was examined using the MTS assay, and the mean absorbance at 490 nm wavelength was determined at 24 h, 48 h and 72 h. Data were given as means ± SEM of three independent experiments. *p < 0.05 means significantly statistical different compared to non-treated (NT) conditions.

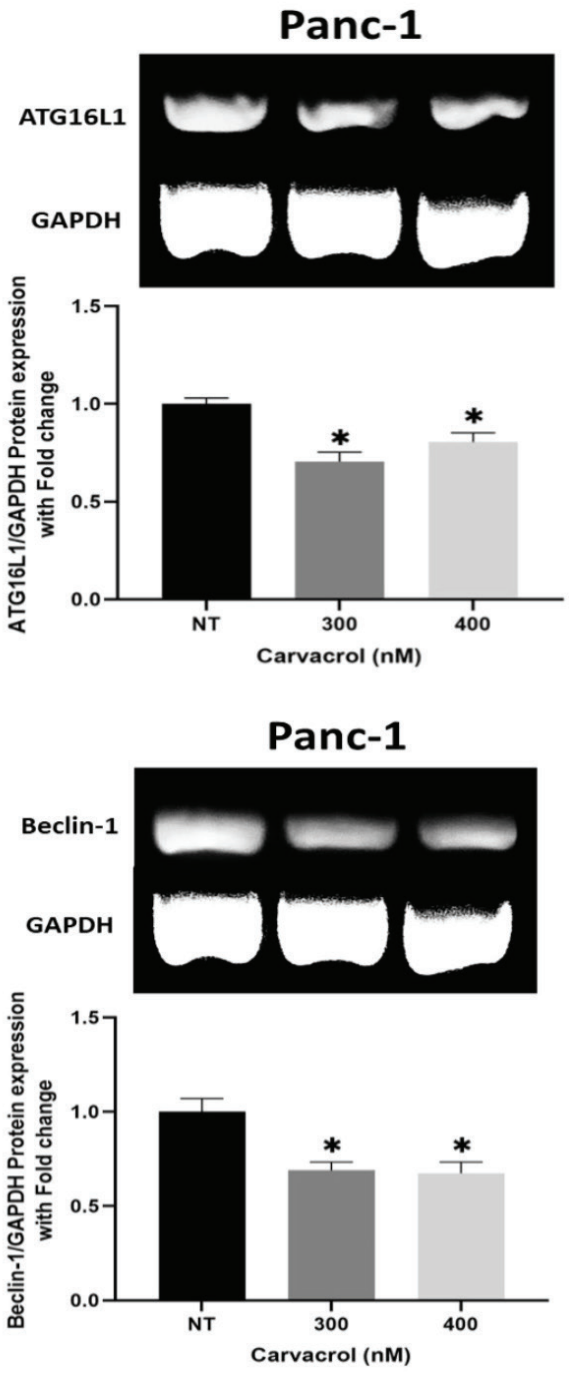

Figure 2
Carvacrol inhibits the key mediators of autophagy through ATG16L1 (A) and Beclin-1 (B) gene expressions in Panc-1 cells transfected with 300 and 400 µM carvacrol for 24h. GAPDH was used as a loading control for RT-PCR analyses. Data were normalized to the expression of loading controls and represented means ± SEM of three independent experiments. ATG16L1 and Beclin-1 gene expressions in Panc-1 were evaluated by comparing to NT conditions (*p < 0.05).

line. Of the carvacrol doses from 100 through 800 µM, the most significant carvacrol (CV) doses were found to be 300 nM and 400 µM both for 24 h and 48 h in terms of IC50 values. Panc-1 cell viability related to the treatment of 300 and 400 µM doses of carvacrol decreased to 55.8% and 36%, 50.88% and 40.5% within 24 h and 48 h, respectively, when compared to non-treated conditions, whereas 100 and 200 µM carvacrol did not cause any inhibition on cell viability, either 24 h or 48 h. In contrast to Panc-1, human non-tumorigenic pancreatic epithelial cell HPDE was not affected by carvacrol treatment. In HPDE control cells, carvacrol started to affect about 700 and 800 µM doses (Figure 1A, 1B). Therefore, 300 and 400 µM doses of carvacrol were selected for our further experiments; any adverse effects related to carvacrol occurred in HPDE.

Carvacrol Inhibits the Expression of Autophagy Mediators in Panc-1 Cells

To evaluate the anti-carcinogenic effects of carvacrol on the key oncogenic mediators, Beclin-1 and ATG-16L1, as autophagic regulators, gene expressions were analyzed by RT-PCR in the Panc-1 cell line treated with 300 and 400 µM carvacrol for 24 h. Both doses of carvacrol suppressed ATG16L1 and Beclin-1 mRNA expressions. These decreases were significantly observed, approximately 30-35% compared to non-treated conditions (Figure 2A, 2B).

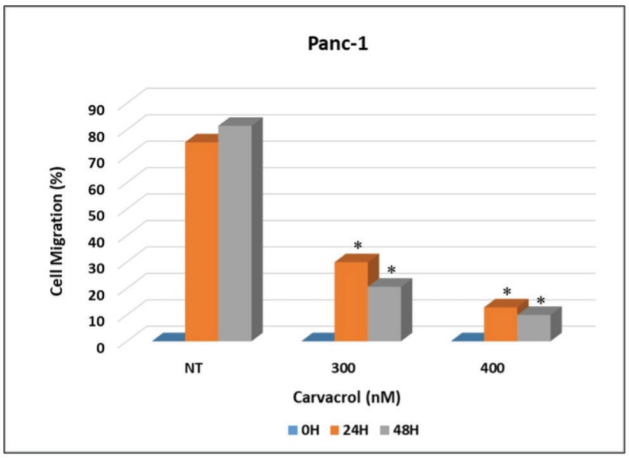

Figure 3
Carvacrol decreases migration in Panc-1 cells. Cells were counted in 5 random fields per well at 40x at 0 h, 24 h, and 48 h for migration. The migrating cells percentages of open area in the presence of 300 and 400 µM carvacrol were calculated and compared with the related controls in Panc-1 cells (*p < 0.05). Data represent means ± SEM of three independent experiments.

Carvacrol Suppresses the Migration Ability of Panc-1 Cells

Autophagy is closely associated with the aggressiveness and metastatic potential of PDAC cells. Therefore, the effect of carvacrol on cell migration was determined by the scratch wound-healing assay in Panc-1 cells. As shown in Figure 3, the distance between the edge of the wound was significantly larger than that of the control group after carvacrol treatments in a dose and time-dependent manner. Among them, a 300 µM dose of carvacrol at 24 h was observed the most effective dose because of decreased migration by 30% when compared to non-treated condition (Figure 3A, 3B).

Discussion

To the best of our knowledge, we are the first to investigate the effect of carvacrol on autophagy mediators in pancreatic cancer. In this study, we observed a 30-35% inhibition in the levels of autophagy mediators ATG-16L1 and Beclin-1 after 24 h treatments with 300 and 400 µM carvacrol in Panc-1 pancreatic ductal adenocarcinoma cells, compared to untreated condition. Additionally, we also noted nearly a 30% inhibition in the migration capability of cancer cells with a 24 h treatment of 300 µM carvacrol.

Previous studies on cancer have shown that Carvacrol-Zinc Oxide Quantum Dots (CVC-ZnO QD) suppress the expression of the anti-apoptotic protein Bcl-2 while increasing the expression of pro-apoptotic proteins Bax, caspase-9, and caspase-3 in the breast cancer cell line MDA-MB-231 (18). Additionally, high doses of carvacrol were reported to significantly inhibit cell proliferation in SW480 cells. In the hypoxic colorectal cancer cell line SW480, hypoxia was first induced by CoCl2, and then the cells were treated with carvacrol at concentrations of 400, 200, 100, 50, 25, 12.5, and 6.25 µg/mL. After 48 h treatment, the IC50 value was found to be approximately 324 µg/mL using the MTT assay. It was observed that carvacrol at concentrations of 50, 25, 12.5, and 6.25 µg/mL had a dose-dependent inhibitory effect on both cell proliferation and migration in this cell line (19).

Al-Fatlawi et al. investigated the effects of carvacrol on breast cancer cells (MCF-7), becoming the first researchers to confirm that carvacrol induces cytotoxicity and apoptosis by increasing the expression of pro-apoptotic genes. In human breast cancer cells (MCF-7), a 48 h treatment with carvacrol resulted in an IC50 value of 244.7 ± 0.71 µM. Additionally, it was reported that carvacrol induced apoptosis through the activation of the p53-dependent Bcl-2/

Bax pathway, as well as causing the expression of caspase genes (caspase-3, -9, and -6) and genomic DNA fragmentation (20).

The inhibitory effect of carvacrol on cell viability in prostate cancer cells, PC-3 and DU145, was analyzed using the CCK-8 assay. The IC50 values were found to be 498.3 µM and 430.6 µM, respectively. Carvacrol also reported was reported to reduce colony formation at doses of 250 and 500 µM in PC-3 and DU145 cell lines, respectively. Based on the related results, carvacrol has been shown to have anti-proliferative and anti-metastatic effects by blocking TRPM7 cation channel activity and acting through PI3K/Akt and MAPK/ERK signaling pathways, which led to decreased F-actin dynamics and MMP-2 protein expression (21). Compared to carvacrol, carvacrol-derived Schiff base complexes, which are carvacrol aldehyde, the Schiff base, and the copper–Schiff base complex, were found to decrease the viability of A549 cells in a dose-dependent manner as IC50 doses of 278.3 ± 4.33, 492.79 ± 4.05, and 233.49 ± 4.18 µg/mL, respectively. In addition to the inhibitory effect of cell viability, they were also found to arrest the cell cycle at the G2/M phase, reduce migration, and induce apoptosis by decreasing the expression of Bcl-2, while increasing the expression of Bax, caspase-3, and caspase-9 in A549 cell line (22).

It was observed that the proportion of cells in the S phase of the cell cycle decreased from 28.13% to 17.46% compared to untreated conditions in Tca-8113 cells, a cell line derived from clinical stage III human tongue squamous cell carcinoma, treated with 40 µM carvacrol. This effect was attributed to the inhibition of key cell cycle mediators Cyclin D1 and CDK4 and the induction of p21. Additionally, carvacrol significantly reduced colony formation at 40 µM and 80 µM concentrations compared to the control group. In the same study, the apoptotic effects of carvacrol in Tca-8113 cells were also investigated. It was found that after 24 h carvacrol treatment, the expression of pro-apoptotic proteins Bax and Cox2 decreased, and apoptotic effects were observed starting from a concentration of 20 µM, based on cell morphology. In Tca-8113, carvacrol was also found to inhibit focal adhesion kinase (FAK) activity and its signaling pathways, significantly reducing migration and invasion by decreasing the expression of ZEB1 and β-catenin proteins, as well as dose-dependently reducing MMP-2/9 protein expression (23).

In human hepatocellular carcinoma cell line HepG2, it was determined that carvacrol inhibits cell proliferation, with an IC50 value of 0.4 mmol/L. Carvacrol revealed its anti-proliferative and apoptotic effects via reducing Bcl-2 protein expression in a dose-dependent manner (24).

Apart from all these cancer types, there are very limited reports related to the potential therapeutic effects of carvacrol in pancreatic cancer. Carvacrol was first shown to have protective effects on the pancreas in acute pancreatitis by inducing endogenous antioxidant defense mechanisms and reducing malondialdehyde (MDA) levels, an indicator of oxidative stress (25). Following acute pancreatitis, Güneş et al. revealed that carvacrol exhibits anti-carcinogenic effects by reducing cell proliferation and inducing apoptosis in the Panc-1 pancreatic cancer cell line. Based on the XTT assay, the IC50 dose of carvacrol was obtained as 664.02 µM at 24 h treatment compared to the non-treated condition. This resulted from a significant increase in pro-apoptotic gene expressions such as Bax, caspase-3, caspase-8, CYCS, FADD, FAS, p53, and a decrease in Bcl-2. They also obtained the anti-metastatic potential of carvacrol because it significantly resulted in upregulating E-Cadherin, TIMP2, and TIMP3 and downregulating N-Cadherin and ZEB2 gene expressions (26).

All these reports related to the anti-carcinogenic effects of carvacrol are closely linked to our findings. Carvacrol strongly has the potential linked to anti-proliferation, anti-migration, and pro-apoptotic effects in several cancer cell lines, including PDAC, which is the most common and aggressive pancreatic cancer type. Because of the late diagnosis and frequent resistance to conventional chemotherapies of pancreatic cancer cells, there is an urgent need to provide novel natural therapeutic agents. At this point, carvacrol has promising value as a potential therapeutic approach for PDAC. We believe that further mechanistic investigations will be a guide for its clinical usage.

Conflict of Interest Statement
The authors have no conflicts of interest to declare.

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Availability of Data and Materials
Data sharing is not applicable.

Authors Contributions
ZA: Formal analysis, Writing-original draft.

NG: Conceptualization; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Supervision; Validation; Writing-review & editing.

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