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For book section;
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Gaudin S. How moon landing changed technology history [Internet]. Computerworld UK. 2009 [cited 15 June 2014]. Available from: http://www.computerworlduk.com/in-depth/it-business/2387/how-moon-landing-changed-technology-history/

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ORIGINAL RESEARCH

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Specialization Preferences of Medical Faculty Students, Consideration of Choosing Medical Pharmacology and Related Factors; An Example of Term 3

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Abstract

Objective
This study aims to determine the specialty preferences of third-year medical students and their considerations regarding choosing Medical Pharmacology as a specialty, as well as to evaluate the related factors.

Material and Method
The universe of this cross-sectional analytical study consisted of 3rd-year students of Suleyman Demirel University Faculty of Medicine. Data were collected by using a questionnaire prepared by the researchers. Analyses were conducted by using SPSS 22.0 software.

Results
The data of 143 participants were used in this study, and 85.3% of the participants indicated that they chose the medical faculty by their own will. 97.9% of the research group stated that they planned to specialize after graduation. The specialty that the participants considered most for the Medical Specialization Exam was internal medicine with 57.3%.14.7% of the group expressing a desire to specialize in medical pharmacology. The most preferred factor affecting the choice of medical pharmacology was the absence of night shifts at 49.7%.

Conclusion
In our study, it was determined that the majority of medical students planned to specialize, and many of them stated that they preferred areas of specialization within medicine. The primary rationale for preferring medical pharmacology is the absence of seizures, whereas the main reason for its disfavor is the perceived lack of professional satisfaction. Determining the factors affecting the specialty preferences of medical school students and addressing relevant issues is important for increasing the effectiveness of health service delivery. Therefore, comprehensive studies are needed on this subject.

Keywords: Medical specialization, medical education, medical pharmacology

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Introduction

One of the most important factors in evaluating healthcare service quality is the humane abilities, skills, and knowledge of physicians. Being happy with professional satisfaction, which is influenced by many factors, improves the service provided as it makes one feel that they are being rewarded both spiritually and materially for their work and that they are applying their skills in their job. Graduates of medical schools face a significant crossroads where they must make serious decisions. This crossroad involves choosing medical specialties in which they can receive medical specialization training that will impact their success and happiness. One of the determining factors in this choice is the experiences gained by physicians during their medical education. Working in an area where people feel comfortable and their abilities, personality, and mental characteristics are compatible provides more efficiency (1-5).

Physicians earn the title of "medical doctor" after six years of education in medical faculties. When they graduate from medical school, they can practice as a general practitioner and enter the specialties of surgery, internal medicine, and basic medical sciences with the Medical Specialization Exam (6-7). Medical Specialty Examination, covering Basic Medical and Clinical Medical Sciences, has been conducted annually in our country since 1987. Candidates taking the Medical Specialty Examination are placed in their preferred specialty departments according to their scores. Many factors play a role in candidates' preferences for specialty departments (4). With a good understanding and definition of these factors, candidates can be placed in specialization departments where they can practice medicine more efficiently in their fields.

According to the Health Statistics Yearbook, in 2022, the number of general practitioners was 53.697, the number of assistant physicians was 45.391, the number of specialist physicians was 95.600 and the total number of physicians was 194.688. According to the same source, there were 228 physicians per one hundred thousand people (8). The number of physicians taking the Medical Specialty Examination has been increasing with the increase in medical school quotas, and approximately one out of every ten physicians receives specialty training (7).

Pharmacology, the science of drugs, examines the interaction between drugs and the biological system and encompasses various types of research such as in vivo studies with voluntary human subjects/ experimental animals. It also investigates drug interactions with functional protein molecules by using in vitro studies including cells, tissues, bioactive molecules, or more and silico methods. (9-10). The branches of pharmacology are quite diverse and are developing and deepening. Pharmacokinetics, pharmacodynamics, and toxicology are just a few of the many branches of pharmacology (10). Pharmacology, which describes the development of treatments from natural sources, is a scientific discipline that opens new horizons in the field of science. (11).

This study aimed to determine the medical specialty preferences of third-year medical students, which can be considered as the beginning of their professional careers, the place of medical pharmacology in their preferences, and the factors affecting these preferences.

Material and Method

Type of Study, Population, and Sampling Selection

The study conducted in February 2024 is cross-sectional. The universe of the study consisted of third-year students (n=263) at Suleyman Demirel University Faculty of Medicine during the 2023-2024 academic year. No sampling was performed; the aim was to reach the entire universe. However, only 143 students could be reached due to reasons such as not participating or being on leave (53.35%).

Data Collection and Evaluation

A survey prepared by the researchers, including sociodemographic questions, specialty preferences, and factors affecting the selection or non-selection of Medical Pharmacology courses, was administered to the participants who agreed to participate in the study. The questionnaire was distributed to students via a form created by the researchers on the Internet, and data were collected accordingly. The data collection form used in the study consisted of a total of 17 questions. The variables in the study included gender, age, family type, living arrangements, parents' educational status, perception of income status, presence of healthcare professionals in the family, voluntary selection of the medical faculty, reasons for choosing the medical faculty, post-graduation specialization plans, preferred specialty in specialization, consideration of selecting medical pharmacology in specialization, and factors affecting the selection or non-selection of medical pharmacology.

Statistical Analysis

The data was evaluated using the SPSS 22.0 (Statistical Package for Social Sciences) application. Normality tests were used to determine whether

the data followed a normal distribution. Descriptive statistics were presented as numbers and percentages. The chi-square test was used to investigate two categorical variables. Categorical variables were re-categorized as family type; nuclear family and other (extended family or broken family); living with family, with friends (at home or in a dormitory), and alone (at home or in a dormitory); mother and father education level; secondary school and below (middle school or primary school), high school and above (high school or university); income status perception; income less than expenses, income equal to or more than expenses; presence of Specialty plans include Medical Specialty Examination and overseas (or other). To identify the factors influencing the choice of Medical Pharmacology as a specialty, variables found to be significant in univariate studies were added to the model, and logistic regression analysis was done. The Hosmer-Lemeshow test was employed to assess model fit. All tests were approved with a statistical significance criterion of $p<0.05$.

Results

Data from 143 participants were utilized in this study. The mean age of the research group was 21.37±1.23

Table 1 Distribution of the research group according to sociodemographic characteristics

Variables	n (%)
Gender	
Male	64 (44,8)
Female	79 (55,2)
Family Type	
Nuclear family	130 (90,9)
Extended family	11 (7,7)
Dispersed family	2 (1,4)
Who do you live with?	
With family	35 (24,5)
With friends at home	15 (10,5)
With friends at dormitory	24 (16,8)
Alone at home	63 (44,1)
Alone at dormitory	6 (4,2)
Mother's education level	
Elementary school graduate	33 (23,1)
Middle school graduate	14 (9,8)
High school graduate	35 (24,5)
University graduate	61 (42,7)
Father's education level	
Elementary school graduate	16 (11,2)
Middle school graduate	6 (4,2)
High school graduate	25 (17,5)
University graduate	96 (67,1)
Perceived income status	
Income exceeds expenses	50 (35,0)
Income equals expenses	73 (51,0)
Income falls short of expenses	20 (14,0)

years (ranging from 20 to 29). 55.2% of the participants are women and 90.9% belong to a nuclear family structure. Regarding living arrangements, 44.1% of the research group lived at home alone while 24.5% lived with their families. It was observed that the majority of the parents had a university education, and 42.7% of the mothers and 67.1% of the fathers were university graduates. Among the participants, 69.2% did not have any healthcare professionals in their families, 18.9% had non-physician healthcare professionals, and 11.9% had physicians in their families.

85.3% of the participants stated that they chose to study medical school willingly. 28.0% of the participants

Table 2 Distribution of the research group according to the presence of healthcare workers in the family, willingly choosing medical school, and preferred specialty characteristics

Variables	n (%)
Presence of healthcare worker in the family	
Yes, physician	17 (11,9)
Yes, non-physician	27 (18,9)
No	99 (69,2)
Did you choose medical school intentionally?	
Yes	122 (85,3)
No	21 (14,7)
What is your purpose in choosing medical school?	
Perceived as an ideal profession	25 (17,5)
Desire to help people	33 (23,1)
Prestige	14 (9,8)
Belief that the salary will be high	13 (9,1)
Family's desire	7 (4,9)
Thought of professional satisfaction	40 (28,0)
High score in university entrance exam	11 (7,7)
Are you planning to specialize?	
Yes	140 (97,9)
No	3 (2,1)
What is your specialization plan?	
National Medical Specialty Examination	109 (76,2)
Specialization abroad	33 (23,1)
Other	1 (0,7)
Which group does the specialty you consider for specialization belong to?	
Internal medical sciences specialties	82 (57,3)
Surgical medical sciences specialties	49 (34,3)
Basic medical sciences specialties	12 (8,4)
Are you considering selecting Medical Pharmacology as a specialty?	
Yes	21 (14,7)
No	122 (85,3)

Table 3 Distribution of the research group based on the criteria influencing whether or not Medical Pharmacology was chosen as a specialization

	n (%)*
Pharmacology is a branch I enjoy and it aligns with my interests	41 (28,7)
Academic career plans	18 (12,6)
Absence of on-call duty in the department	71 (49,7)
Working on a shift basis	40 (28,0)
Absence of malpractice risk	65 (45,5)
Absence of physical or verbal violence risk	69 (48,3)
Patients not being in critical condition and not requiring urgent intervention	58 (40,6)
Lower professional satisfaction	96 (67,1)
Concerns about salary	38 (26,6)
Unclear boundaries in professional definition in specialization	62 (43,4)

*Multiple options could be selected

stated that the most common reason for choosing a medical school was the expectation of professional satisfaction, followed by the desire to help people with 23.1%. Seeing medicine as an ideal profession (17.5%) and the desire for respect (9.8%) were other significant reasons cited.

The research group comprised 97.9% of participants who stated that they intended to specialize after graduation. Among them, specialization through the Medical Specialty Examination was the most preferred option with 76.2%, followed by pursuing specialization abroad with 23.2%.

Among the participants, 57.3% expressed their consideration of choosing a branch within the field of internal medical sciences specialties for specialization through the Medical Specialty Examination. Although it was found that females tended to prefer internal medicine specialties more frequently compared to males, however, this difference was not statistically significant (p=0.09). Moreover, 14.7% of the group expressed their interest in specializing in medical pharmacology. The inclination to choose medical pharmacology as a specialty was slightly higher among females compared to males, but again, this difference was not statistically significant (p=0.25). Descriptive characteristics of the participants are presented in Table 1 and Table 2.

The most preferred factor affecting participants' consideration of choosing medical pharmacology as a specialty was the absence of night shifts with 49.7%. The absence of the risk of physical and verbal violence was the second most common factor with 48.3%, followed by the absence of the risk of malpractice, which is the most common factor, with 45.5%. The distribution of factors affecting the selection of medical pharmacology by the group is presented in Table 3. The most preferred factor affecting the group's decision not to choose medical pharmacology was the lack of professional satisfaction with 67.1%. This was followed by those who stated that the boundaries of the professional definition in specialization were not clear with 43.4%. The distribution of factors influencing the group's decision not to choose medical pharmacology is presented in Table 3.

Table 4 and Table 5 show the distribution of factors influencing the decision to specialize in medical pharmacology. Those with a health worker in their family, those considering pursuing an internal branch as a specialty, and those who stated that medical pharmacology is a branch they enjoy were significantly more likely to choose medical pharmacology as a specialty (p= 0.022; p= 0.037; p= 0.001, respectively). Those who believe professional satisfaction in medical pharmacology is low were considerably less likely to choose medical pharmacology as a specialty (p=0.003). Gender, family type, living with, mother's education level, father's education level, perception of income status, willingly choosing a medical school, specialization planning, specialty plan, academic career plan, medical pharmacology as a non-shift

Table 4 Distribution of the research group according to the variables affecting the decision to choose medical pharmacology as a specialty

	Considering Choosing Medical Pharmacology			
	Yes		No	
Variable	**Number**	**%**	**Number**	**%**
Gender Male	7	10,9	57	89,1
Female	14	17,7	65	82,3
p= 0,184				
Family Type				
Nuclear family	18	13,8	112	86,2
Other	3	23,1	10	76,9
p=0,293				
Who do you live with?				
With family	3	8,6	32	91,4
With friends	5	12,8	34	87,2
Alone	13	18,8	56	81,2
p= 0,349				
Mother's education level				
Middle School and Below	7	14,9	40	85,1
High School and Above	14	14,6	82	85,4
p= 0,572				
Father's education level				
Middle School and Below	3	13,6	19	86,4
High School and Above	18	14,9	103	85,1
p= 0,590				
Perceived income status				
Income falls short of expenses	5	25,0	15	75,0
Income equals expenses or exceeds	16	13,0	107	87,0
p=0,144				
Presence of healthcare worker in the family				
Yes	11	25,0	33	75,0
No	10	10,1	89	89,9
p= 0,022				
Did you choose medical school intentionally?				
Yes	18	14,8	104	85,2
No	3	14,3	18	85,7
p= 0,629				
Are you planning to specialize?				
Yes	21	15,0	119	85,0
No	0	0,0	3	100,0
p= 0,619				
What is your specialization plan?				
Medical Specialty Examination	16	14,7	93	85,3
Specialization abroad	5	14,7	29	85,3
p= 0, 595				
Which group does the specialty you consider for specialization belong to?				
Internal	14	17,1	68	82,9
Surgical	3	6,1	46	93,9
Basic	4	33,3	8	66,7
p= 0,037				

Table 5 The research group is successful in deciding on the field of treatment, distribution of pharmacology, according to expert viewpoints and advancements

Considering Choosing Medical Pharmacology				
	Yes		No	
Variable	**Number**	**%**	**Number**	**%**
Pharmacology is a branch I enjoy and it aligns with my interests				
Yes	13	31,7	28	68,3
No	8	7,8	94	92,2
p=0,001				
Academic career plans				
Yes	2	11,1	16	88,9
No	19	15,2	106	84,8
p=0,486				
Absence of on-call duty in the department				
Yes	11	15,5	60	84,5
No	10	13,9	62	86,1
p=0,486				
Working on a shift basis				
Yes	7	17,5	33	82,5
No	14	13,6	89	86,4
p=0,362				
Absence of malpractice risk				
Yes	12	18,5	53	81,5
No	9	11,5	69	88,5
p=0,177				
Absence of physical or verbal violence risk				
Yes	9	13,0	60	87,0
No	12	16,2	62	83,8
p=0,383				
Patients not being in critical condition and not requiring urgent intervention				
Yes	12	20,7	46	79,3
No	9	10,6	76	89,4
p=0,077				
Lower professional satisfaction				
Yes	8	8,3	88	91,7
No	13	27,7	34	72,3
p=0,003				
Concerns about salary				
Yes	6	15,8	32	84,2
No	15	14,3	90	85,7
p=0,505				
Unclear boundaries in professional definition in specialization				
Yes	11	17,7	51	82,3
No	10	12,3	71	87,7
p=0,252				

Table 6 Regression analysis results of variables found to be linked in univariate analyses while choosing medicinal pharmacology

Considering Choosing Medical Pharmacology			
Variable	**[B]**	**%95 Confidence Interval**	**p**
Presence of healthcare worker in the family (No=0, Yes=1)	0,98	0,934-7,686	0,067
Considering Choosing an Internal Branch in Specialization (No=0, Yes=1)	0,55	0,572-5,252	0,331
Pharmacology is a branch I enjoy and it aligns with my interests (No=0, Yes=1)	1,91	2,295-20,016	**0,001**
Lower professional satisfaction (Yes=0, No=1)	1,55	1,603-14,014	**0,005**

-2 Log Likelihood: 92,728; Cox&Snell R^2: 0,170; Nagelkerke R^2: 0,300

department; Working in shifts, no risk of malpractice, no risk of physical or verbal violence, patients being treated not being in an emergency and not requiring emergency intervention, salary concerns, and lack of clear professional boundaries in the specialty of medical pharmacology did not significantly influence choosing medical pharmacology as a specialty (respectively p= 0.184; p= 0.293; p= 0.349; p= 0.572; p= 0.590; p= 0.144; p= 0.629; p= 0.619; p= 0.595; p= 0.486; p= 0.486; p= 0.362; p= 0.177; p= 0.383; p= 0.077; p= 0.505; p= 0.252).

Having a health worker in the family increases the likelihood of selecting medical pharmacology as a specialty by 0.98 times, considering an internal branch by 0.55 times, liking medical pharmacology by 1.91 times, and finding professional satisfaction in medical pharmacology not low by 1.55 times (p=0.067; p=0.331; p=0.001 and p=0.005, respectively). The regression results are presented in Table 6.

Discussion

In this study, research was conducted on third-year medical students who had not yet started clinical training on their specialty preferences and preferences for choosing a branch of pharmacology.

The most frequently cited reasons for choosing medical school were professional satisfaction, the desire to help people, and the perception of medicine as an ideal profession. This finding is consistent with the literature. In a study conducted in Poland, the reasons for choosing medical school were found to be as follows: the most common reason was an interest in medicine, followed by the desire to help people and the prestige associated with being a physician (12). Similarly, studies conducted in Turkey also found that the respectability of being a doctor, the desire to help people, and career aspirations were the most common reasons for choosing medical school (13-15). Medicine, one of the oldest professions in history, is not only an admired profession that keeps ideals at the top but also one of the most prestigious professions of today. Physicians are professionals who, in addition to their knowledge and experience, undertake many missions such as understanding and empathizing with patients' thoughts and feelings (16-17) With the mission of protecting the health and welfare of societies, physicians continue to maintain this respected tradition from past to present.

In this study, the research group planned a high rate of specialization. In the literature, many studies conducted in Germany, France, and Turkey showed a high percentage of those who wished to specialize after graduating from medical school (13,18-24). This study also reveals that a very high percentage of third-year students do not intend to pursue a career as a general practitioner in the future. General practice in our country refers to doctors who have completed their medical education (22). Medical educators should analyze the fact that the majority of students do not prefer such an important healthcare service in their training. The relatively high desire to specialize in Turkey can be attributed to problems such as the low professional prestige of general practitioners, inadequate economic conditions, and limited specialization opportunities (22, 25-26).

When we look at the preferred branches of specialization in medicine, the most common ones are

internal medicine specialties, followed by surgical medical sciences specialties, and lastly, basic medical sciences specialties. There are studies parallel to the results of this study (15, 27-28). Factors such as the number of shifts, duration of residency, working hours, and stress induced by working conditions may affect students' choices (5,29). The fact that basic medicine and pharmacology specialties were less preferred in this study can be attributed to the fact that the research group was in the third year and had not yet transitioned to clinical medicine education.

In this study, the most important factors for choosing Medical Pharmacology as a specialty were the absence of on-call duties, lower risk of violence, and no risk of malpractice. Additionally, students who expressed that Medical Pharmacology is their preferred field had a statistically significant higher likelihood of choosing this specialty. The prominence of violence and malpractice risks in the top three concerns, particularly affecting third-year students who have three years until graduation, highlights the severity of the issue. Low professional satisfaction emerged as the most significant reason for not choosing Medical Pharmacology, with those reporting low satisfaction having a significantly higher inclination to avoid the field. Furthermore, a quarter of the students surveyed expressed concerns about salary. The fact that the country’s intelligent and educated youth have salary concerns, especially after medical education and specialization in Medical Pharmacology, is a significant issue. No study has been found in the literature that directly focuses on pharmacology selection. However, when factors such as night duties, overtime hours, and the risk of violence in the medical profession are considered, even third-year students are influenced in their specialty choices. In a study, students ranked professional satisfaction as the primary reason for their specialty preferences (22). In another study, when students were questioned about their reasons for specialty preferences, the top three reasons were 'providing happiness', 'professional satisfaction', and 'quality of residency training' (7). In another study, when their opinions about the necessity of being an expert were questioned, the highest rate was found in professional satisfaction. In the same study, they also stated 'their own interests' among their reasons for choosing specialization. (13).

According to this study, there was no significant difference found between gender and specialty preferences. In many studies in the literature, no significant difference has been found between gender and specialty preference (6, 30-32). However, there are also studies with opposite findings. In studies conducted by Tekin et al. in 2013, Açıkgöz B et al. in 2019, and Yılmaz N et al. in 2021, it was concluded that men tended to prefer surgical specialties more (22,29,33). Similarly, Khader et al.'s study in 2011 also reached the same conclusion (34). Finding different results regarding gender suggests that gender alone may not be a determining factor in choosing a medical specialty.

In conclusion, it has emerged that third-year medical students plan to specialize at a high rate but do not consider pursuing a career as general practitioners in the future. The lack of interest in such an important area of healthcare should be taken into account by educators. Additionally, factors such as on-call duties, risk of violence, malpractice risk, professional satisfaction, and salary concerns have influenced preferences for the Medical Pharmacology specialty. It is quite concerning that these worries exist among students who have not yet started clinical practice. Identifying and addressing the motivations, concerns, and attitudes of future doctors, along with implementing appropriate measures, will be crucial for effective and efficient healthcare.

Conflict of Interest Statement
There is no financial conflict of interest with any organization, institution, or person related to our article and there is no conflict of interest between the authors.

Ethical Approval
Before the study, ethical approval was obtained from the Ethics Committee of Suleyman Demirel Faculty of Medicine (Decision No: 15, dated 03.01.2024), and permission was received from the Dean's Office of Suleyman Demirel University Faculty of Medicine on January 12, 2024, for the conduct of the study. Participants were given necessary information about the purpose of the research and the scientific evaluation of the data. The study was conducted in accordance with the principles set forth in the Declaration of Helsinki.

Consent to Participate and Publish
Written informed consent to participate and publish was obtained from all individual participants included in the study.

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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

SO: Conceptualization of the study; Formal analysis; Investigation; Methodology; Visualization; Writing original draft; Writing review & editing.

ED: Conceptualization of the study; Data curation; Formal analysis; Investigation; Methodology; Writing original draft.

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ORIGINAL RESEARCH

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Violence Against Healthcare Professionals: A Retrospective Review of a University Hospital in Turkey

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Abstract

Objective
Workplace violence (WPV) against healthcare professionals (HCPs) is increasing globally. This study retrospectively examines White Code Records (WCR) of WPV cases reported at a university hospital in Turkey.

Material and Method
The study analyzed 106 WCR cases recorded between January 2018 and September 2024. Data included the content and timing of violent incidents, along with demographic details of HCPs and perpetrators. Descriptive statistics, Monte-Carlo simulation, and Fisher's exact test were used to understand the differences by years and logistic regression was used to analyze the conditions affecting the type of violence.

Results
The analysis revealed that the highest incidence of violence occurred in 2022 (25.5%), coinciding with the post-COVID-19 period. Verbal violence was predominant, comprising 83% of cases. WPV incidents were most frequently reported in outpatient polyclinics and imaging departments (42.5%). More than half of the cases (55.7%) occurred in the second half of the year, with 68.9% taking place during daytime hours. Behavioral problems of perpetrators were identified as the leading cause (39.6%) of violent events. Healthcare professionals involved in the incidents had an average age of 31 ± 6.55 years, with 61.3% being female. A majority (67.9%) of HCPs had postgraduate education, with physicians constituting a significant proportion. The average age of perpetrators was 40.1 ± 12.2 years, and 68.9% were male. Notably, 67% of the perpetrators were relatives of patients. Statistically significant year-by-year variations were observed in the type of violence reported. Variables such as daytime incidents, HCP education levels, and perpetrator gender significantly influenced the type of violence ($p < 0.05$).

Conclusion
The findings reveal key trends in WPV in healthcare, with incidents primarily caused by behavioral issues of male relatives of patients. Most affected HCPs are young, female, and physicians. This suggests that gender norms influence violence in healthcare.

Keywords: Workplace violence, verbal violence, healthcare workers, physicians, nurses

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Introduction

The prevalence of workplace violence (WPV) against healthcare professionals (HCPs) remains alarmingly high, posing a significant threat to public health (1,2). WPV is the use of force against an individual or group of people in the workplace, resulting in physical and psychological injury or even death (3). These may be verbal, psychological, or even physical. HCPs may become exposed to WPV because of the stressful and chaotic healthcare environment. It is imperative to ensure that HCPs have a safe and secure working environment (4). Research shows that a significant portion of WPV in the healthcare sector goes unreported (5,6), suggesting that the incidents observed represent only a fraction of the overall problem, akin to the tip of an iceberg.

While overall WPV may be declining in the US, there is a concerning trend of increasing WPV in the healthcare sector (7). One in five health professionals worldwide is subjected to physical violence each year, and at least two are subjected to verbal violence (8,9). WPV can have several negative impacts on the physical and mental health, job satisfaction, and performance of HCPs (10). WPV can have a serious consequence on the physical and mental health of HCPs and the quality of care provided to patients (11).

Over the years, there has been a consistent and steady growth in research on WPV against HCPs worldwide, spanning from 1992 to 2019. This growth is evident in both the increasing number of research documents and the corresponding citations (12). Furthermore, the COVID-19 outbreak, which was declared by the World Health Organization (WHO) in February 2020, increased the workload of HCPs and exacerbated violence against them due to an influx of anxious and concerned people (13-15).

The prevalence of WPV among HCPs in emergency services, polyclinics/waiting rooms, geriatric units, and psychiatric departments is notably high (16,17). Several factors contribute to violence against HCPs in various settings. Sun et al. (2022) reported that gender differences played a role in WPV among Chinese healthcare providers. The study results show that females were at a higher risk of experiencing WPV due to their gender roles. On the other hand, many studies showed that nurses were more likely to be victims of violence than other HCPs (5,18,19). Studies showed that nurses were at risk of verbal and physical violence and that incidents were not mostly reported (20).

Employers need to take proactive measures to prevent WPV and to provide support to victims. Understanding the factors associated with WPV is essential to prevent and mitigate its consequences (21). One solution is to improve communication between patients and HCPs (22). In addition, people with underlying mental and substance use disorders may have a higher incidence of violent behavior. Therefore, frontline clinicians need to be knowledgeable and competent in the management of patients with aggressive behavior to alleviate the condition and prevent or reduce violence (23). Systematic reviews of studies conducted since 1992 suggest that training techniques for dealing with mostly belligerent patients are now the standard practice (24).

Implementing measures to prevent WPV is important for healthcare facilities. To minimize WPV against HCPs, more safety programs and training need to be implemented as well as efficient reporting systems and a policy of zero tolerance (25). In addition to efforts to prevent or reduce physical violence against HCPs, non-physical violence should be given importance and focused on patient perpetrators (26). An increasing number of countries are implementing stricter penalties for perpetrators of WPV against HCPs (27).

Workplace violence contributes significantly to absenteeism among HCPs because of injuries sustained in violent incidents or fear of personal safety. In addition, some workers may leave their jobs out of fear for their safety, leading to higher turnover rates in workplaces where WPV is common (28). The quality of patient care is negatively affected by absenteeism and high turnover, as they result in understaffing, increased workloads for those who are still on the job and reduced quality of care.

Globally, there is a concerning observation of high levels of violence against HCPs in Turkey. The incidence of violence directed at HCPs in Turkey is steadily increasing each year, leading to adverse psychological and physical effects on those exposed to such violence (29). Due to the increase in cases of violence, it is a necessity to take measures both in health institutions and at the national level. As part of these measures, the Ministry of Health first introduced the "White Code Application" in all hospitals in the country in 2011 (30,31). The White Code Records (WCR) is a system that is activated by HCPs. Providing legal assistance to HCPs while ensuring their safety is the main purpose of this system. In the White Code process, violence against HCPs is evaluated within the scope of a public case. At the same time, the Ministry of Health aims to guide the fight against violence by collecting detailed data on violence (32).

As of today, WPV remains a persistent and unresolved issue, primarily due to factors such as using social media to target HCPs and the absence of sufficient deterrence against such crimes. In addition to legal measures, the importance of scientific approaches in addressing this problem has been increasingly recognized. By conducting an in-depth analysis of violence cases and evaluating the situation qualitatively based on the results obtained, this study aims to shed light on the development of preventive regulations. The study focuses on a retrospective evaluation of quantitative data derived from WCR in a university hospital.

Materıal and Method

Design and Participants

In this retrospective study, we conducted an analysis of WCR documenting incidents of WPV perpetrated by patients and their relatives against HCPs in a university hospital. All White Code notifications reported during the seven-year study period were included in this study. A total of 106 cases of violence were identified between January 2018 and September 2024. No sampling was performed, as the study aimed to include all reported cases of violence. The sample size was calculated using the G*POWER 3.1 statistical program. Based on a medium effect size (0.1), a significance level of 0.05, and a power of 80%, the required sample size for regression analysis was determined to be 100.

Data Collection

A data collection form prepared by the researchers was used in this study. Through this data collection form, the files received from the White Code Unit were analyzed. The data collection form included demographic information about HCPs and perpetrators of violence, and the content, causes, and details of the violence (year, month, time, department). The causes of violence were identified by integrating the categories available in the Turkish Ministry of Health's White Code data system, following the relevant literature (33,34). Inappropriate demands encompassed requests for procedures with incomplete documentation demands for prescription medication or reports, and requests for examinations or procedures outside of scheduled appointment times. Under the service complaints category, dissatisfaction with treatment, demands for a different physician, and requests for faster treatment or procedures were included. The behavioral issues of the perpetrators involved threats, bullying, insults, swearing, and attempts at physical violence. Additionally, actions such as visiting outside of accompanying hours, unauthorized filming or photographing with hidden cameras, and entering restricted areas were categorized as rule violations.

Data Analysis

IBM SPSS Statistics version 24.0 was used for data analyses. Descriptive statistics, Fisher’s exact test with Monte-Carlo simulation, and logistic regression were employed to compare the data. For Fisher's exact test, Cramér's V coefficient was calculated to assess the strength of the association between variables (35). Statistical analyses were conducted with a 95% confidence interval (CI), and significance was determined at $p<0.05$.

Results

When the 106 notifications from the WCR were analyzed according to years, there were five (4.7%) cases of violence in 2018, 17 (16%) in 2019, three (2.8%) in 2020, 13 (12.3%) in 2021, 27 (25.5%) in 2022, 22 (20.8%) in 2023, and 19 (17.9%) in 2024. According to the type of violence from the WCR, 83% (n=88) were verbal violence and 17% (n=18) were physical violence. Psychological violence was excluded from this study, as it was not defined in the records. During the study period, four individuals experienced violence for the second time as victims. According to the September 2024 data, there were 1154 HCPs in the hospital, and the rate of violence experienced by HCPs in the last year was 2.34%. The highest number of WCRs occurred in the second half of the year and accounted for 55.7% (n=59) of the incidents. Additionally, a significant majority of incidents, 68.9% (n=73), took place during regular working hours from 08.00 to 16.00. The study identified polyclinic and imaging units as the most common areas of violence, accounting for 42.5% (n=45) of the reported cases. Behavioral problems of the perpetrators (39.6%) were the most common cause of WPV incidents (Table 1).
The sociodemographic characteristics of the HCPs and perpetrators are shown in Table 2. According to the data, the mean age of HCPs affected by WPV was 31±6.55 years. Among the victims, 65 (61.3%) were female, 72 (67.9%) had postgraduate education, and 72 (67.9%) were physicians. Regarding the perpetrators of violence, the mean age was 40.1±12.2, 73 (68.9%) were male, and 71 (67%) were patients’ relatives.

Table 3 shows whether there is a significant difference between physical or verbal violence in different years. There is a statistically significant difference in the incidence of physical violence between 2021 and 2022, as well as between 2022 and 2024 ($p<0.05$). Specifically, 5 cases were reported in 2021, none

Table 1 Descriptive Statistics of Workplace Violence

Characteristics	Number (n)	Percentage (%)
Year		
2018	5	4.7
2019	17	16
2020	3	2.8
2021	13	12.3
2022	27	25.5
2023	22	20.8
2024	19	17.9
Type of Violence		
Physical	18	17
Verbal	88	83
Half of The Year		
1st (Jan-Jun)	47	44.3
2nd (Jul-Dec)	59	55.7
Shift Period		
08 am -16 pm	73	68.9
16 pm -08 am	33	31.1
Area of healthcare		
Emergency	40	37.7
Clinics	21	19.8
Polyclinic and Imaging	45	42.5
WPV Causes		
Inappropriate demand	21	19.8
Complaint about service	22	20.8
Perpetrators' behavioral problems	42	39.6
Disobeying the rules	21	19.8

in 2022, and 6 cases in 2024. Similarly, there is a significant difference in verbal violence between 2021 and 2022 and between 2022 and 2024 ($p<0.05$). The significant difference corresponds to a high effect size (0.402).

The results of the logistic regression regarding certain variables that may influence the type of violence directed toward HCPs are presented in Table 4. In the analysis, the reference groups were defined as: experiencing verbal violence for the type of violence variable, the year 2024 for the year variable, daytime for the shift period variable, second half of the year for the quarter of the year variable, being over the age of 30 for HCPs age variable, being male for HCPs gender variable, being a nurse for the job position variable, having postgraduate education for the education level variable, being over the age of 30 for the perpetrator's age variable, being male for the perpetrator's gender variable, and being a patient's relative. All assessments were made about these reference groups. According to logistic regression analysis, it was found that experiencing violence during the day shift, the educational level of the HCPs, and the gender

Table 2 Descriptive Statistics of Healthcare Professionals and Perpetrators

Characteristics	Number (n)	Percentage (%)
HCPs' Age		
31±6.55 (Minimum= 20, Maximum= 55)		
<30	70	66
≥31	36	34
HCPs' Gender		
Female	65	61.3
Male	41	38.7
HCPs' Educational Status		
Undergraduate level	34	32.1
Postgraduate level	72	67.9
HCPs' Worker Role		
Physician	72	67.9
Nurse	18	17
Other	16	15.1
Perpetrators' Age		
40.1±12.2 (Minimum= 20, Maximum = 69)		
<30	27	25.5
≥31	79	74.5
Perpetrators' Gender		
Female	33	31.1
Male	73	68.9
Perpetrators' Role		
Patient	35	33
Patient' relative	71	67

Table 3 Exploring Differences in Physical and Verbal Violence between Different Years

Violence Type		Years							Total	X^2	p
		2018	2019	2020	2021	2022	2023	2024			
Physical Violence	Frequency (f)	$0_{a, b, c}$	$5_{a, b, c}$	$0_{a, b, c}$	5_{c}	0_{b}	$2_{a, b, c}$	$6_{a, c}$	18	16.904	**0.004**
	Percentage (%)	0	27.8	0	27.8	0	11.1	33.3	100		
	Year (%)	0	29.4	0	38.5	0.0	9.1	31.6	17		
Verbal Violence	Frequency (f)	$5_{a, b, c}$	$12_{a, b, c}$	$3_{a, b, c}$	8_{c}	27_{b}	$20_{a, b, c}$	$13_{a, c}$	88		
	Percentage (%)	5.7	13.6	3.4	9.1	30.7	22.7	14.8	100		
	Year (%)	100	70.6	100	61.5	100	90.9	68.4	83		

a, b, c: Significant difference indicator between cells, there is a significant difference between cells that do not share the same character.

Table 4 The Relationship Between the Type of Violence and Various Variables

Characteristics	B	S.E.	p	Exp(B)	95% CI for EXP(B)	
					Lower	Upper
Year	-.300	.199	.132	.741	.501	1.095
Shift period	1.362	.682	.046	3.903	1.026	14.852
Half of the year	.471	.662	.477	1.602	.438	5.866
HCPs' age group	.548	.674	.417	1.729	.461	6.486
HCPs' gender	.481	.630	.446	1.617	.470	5.561
HCPs' worker role	.515	.451	.254	1.673	.691	4.053
HCPs' educational status	2.237	1.046	.032	9.367	1.205	72.807
Perpetrators' age group	.933	.676	.168	2.542	.675	9.571
Perpetrators' gender	-2.652	1.115	.017	.070	.008	.627
Perpetrators' role	.517	.633	.414	1.677	.485	5.803

of the perpetrator had a significant effect on the type of violence ($p<0.05$). If violence is experienced during the daytime shift, the probability of being subjected to verbal violence increases approximately four times compared to physical violence (Odds Ratio: 3.903). If HCPs had a postgraduate education level, the likelihood of experiencing verbal violence compared to physical violence increased by nine times (Odds Ratio: 9.367). If the perpetrator was male, the likelihood of the HCPs experiencing physical violence, compared to verbal violence, increased by 14 times (Odds Ratio: 14.285).

Discussion

Workplace violence against HCPs has been widely recognized as a significant public health issue (12). Despite this recognition, violence against HCPs remains both more prevalent and underreported in surveys conducted in Turkey and globally (3,32,36). Furthermore, some studies from Turkish hospitals have reported higher prevalence rates of violence (30,32,37–40).

In a comprehensive study conducted across 30 countries, the rate of physical violence against HCPs was found to be 19.3%. In contrast, our study observed a slightly lower but comparable rate of 17%. It is known that some demographic variables such as education level are among the important factors affecting individuals' tendency to violence (41). According to data from the Turkish Statistical Institute, the prevalence of physical violence increases as the level of education decreases. Notably, Çanakkale province, where this study was conducted, has the second-highest literacy rate in Turkey (42,43). Additionally, the West Marmara region, which includes Çanakkale, is among the regions with the lowest rates of physical violence (42). These factors may explain why the observed violence rates in this study are lower compared to those reported in other studies.

The patriarchal belief in the superiority of male over female, reinforced by cultural norms, significantly increases the likelihood of male resorting to acts of violence (44). Consistent with this, previous studies have demonstrated that males are more prone to engaging in violent behaviors due to gender norms (45,46). The findings of this study indicate that when the perpetrator is male, the likelihood of the HCPs experiencing physical violence, rather than verbal violence increases by 14 times (Table 4). Although no significant association was found between the gender of HCPs and the type of violence they experienced in this study, some studies have suggested that male HCPs are at greater risk of physical assault (47).

Workplace violence among HCPs was primarily directed toward females (61.3%). Although females were more frequently victimized by violence, as observed in our findings, gender did not have a statistically significant impact on WPV (7). However, the risk of WPV is thought to be higher in nursing, a profession predominantly occupied by females (48).

Similarly, one study reported that female physicians have a lower sense of security compared to male physicians (49). While the influence of Turkey's patriarchal societal structure is considered a potential factor contributing WPV, it is essential to note that the findings from the European Working Conditions Survey (EWCTS) 2021, encompassing 36 European countries, also highlight that female are more at risk of experiencing WPV (50).

Looking at violence by year, there was a decrease from 2020 with the COVID-19 pandemic beginning, but the acceleration, which increased from 2021, reached a record level in 2022 (n=27). There is also evidence from some studies that there was an increase in WPV against HCPs after the COVID-19 pandemic (4,15,28). For example, a meta-analysis found that nurses were particularly vulnerable during the pandemic, facing an overall prevalence of violence of 47% (13). In this study, physical violence cases increased from 5 in 2021 to zero in 2022 and 6 in 2024 ($p<0.05$). Similarly, verbal violence cases increased from 8 in 2021 to 27 in 2022 and decreased to 13 in 2024 ($p<0.05$). It may be thought that this situation may be related to COVID-19 and that verbal violence was resorted to by avoiding physical contact while the effects of the pandemic continued.

When patients and their relatives are dissatisfied with treatment, they often resort to threats and sometimes even violence (51). In this study, behavioral problems of perpetrators were identified as the most common source of violence. Perpetrators of violence against HCPs often exhibit behavioral problems, as highlighted in various studies. Research indicates that these perpetrators frequently have psychiatric disorders, anger management issues, and impulsivity. For example, a study found that 50% of individuals who committed violence against HCPs were diagnosed with a psychiatric disorder. Additionally, these perpetrators showed significantly higher levels of anger and impulsiveness compared to the control group (52). In this study, the average age of the perpetrators of violence was significantly higher than that of the HCPs. The evidence indicates that older perpetrators were responsible for perpetrating violence against the HCPs.

Most incidents of WPV against HCPs occurred during the second half of the year (55.7%) and daytime hours (68.9%). This pattern may be attributed to the increased presence of patients and their relatives during these periods. Other studies have reported similar findings supporting this observation (53). Furthermore, violence was most frequently reported in emergency departments, where HCPs are known to face a higher risk of violence compared to the general population (30,54). In this study, nearly half of the reported incidents occurred in outpatient polyclinics and imaging areas (42.5%), followed by the emergency department, which accounted for 37.7%.

Some publications that address violence against HCPs as an occupational problem, as well as publications, such as our study, that collectively examine violence in the healthcare sector. In this study, physicians were identified as the group most frequently exposed to violence, accounting for 67.9% of the reported incidents. Remarkably, this finding aligns with other studies conducted in Turkey, which also reported physicians as the group most vulnerable to WPV (30,32,37–40). Additionally, a recent review of 78 studies reported that nurses experienced more psychological violence than physicians (9,55).

Limitations

This study has some limitations. The violence cases in this study consist only of reported cases. In addition, types of violence other than physical and verbal violence were not reported. For example, psychological violence. Therefore, different types of violence can be examined in future studies. Finally, the WCR of a university hospital was analyzed in our study. Therefore, it is not appropriate to generalize the results to all of Turkey.

Conclusion

The findings reveal key trends in WPV in healthcare, found that violence was most common against physicians, that physical violence was less likely to occur than verbal violence, and that incidents of violence increased as the pandemic progressed. WPV was most prevalent in outpatient polyclinics and imaging areas, particularly during the second half of the year and during the daytime when the hospital experienced its highest activity and patient flow. Although female was more likely to experience violence, there was no correlation between gender, educational level, and physical violence. However, all perpetrators of physical violence were male, and this was statistically significant. The cases of violence were mostly caused by behavioural problems of male relatives of patients. Most of the HCPs exposed to violence were young female physicians, suggesting that gender norms—particularly patriarchal attitudes—significantly impact violence in health services.

These findings may help healthcare facilities consider a few down-to-earth measures to reduce violence.

One step is strengthening psychosocial support and security efforts, especially for female HCPs who face higher risks. It can also be helpful to organize training sessions on communication, conflict resolution, and personal safety so that staff feel more confident when dealing with difficult situations. Clear-cut rules against violence and straightforward reporting and follow-up procedures can encourage everyone to stay accountable. Additionally, hospitals might benefit from collaborating with community groups and local authorities to highlight the consequences of aggressive behavior—not just for HCPs but also for patient care in general. Finally, teaching patients and their relatives about respectful communication and the harms of violence could help lower tensions and change expectations. By weaving these approaches together, healthcare organizations stand a better chance of creating a safer, more supportive environment for everyone involved.

Conflict of Interest Statement
There is no conflict of interest.

Ethical Approval
The Ethics Committee of Çanakkale Onsekiz Mart University Institute of Postgraduate Education granted approval for this clinical research on April 28, 2022, with decision number 09/52. HCPs who experienced violence were informed that their data might be used for scientific research, and explicit consent was obtained from them. In compliance with GDPR guidelines, all personal information was stripped from the data and anonymized to ensure confidentiality. 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 are available on request due to privacy or other restrictions.

Authors Contributions
AK: Concept, Literature Review, Analysis and Interpretation, Article Writing

MAO: Concept, Literature Review, Analysis and Interpretation, Supervision

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ORIGINAL RESEARCH

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Outcomes and Complications of Parotidectomy in Benign and Malignant Salivary Gland Tumors: A Single-Center Retrospective Study

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Abstract

Objective

To retrospectively evaluate the demographic data, imaging, biopsy, and surgical outcomes of 98 patients who underwent parotidectomy.

Material and Method

Ninety-eight patients followed up and operated on for parotid masses between January 2020 and December 2023 in the ENT department of Antalya Training and Research Hospital were included in the study. The patient's gender, age, presenting complaints, comorbidities, fine-needle biopsy results, imaging results, surgical procedures performed, pathology results, and complications were evaluated.

Results

Among the patients who underwent surgery, 39 were female (39.8%) and 59 were male (60.2%). 96.8% of the patients reported experiencing swelling in front of the ear. Pathological results revealed that 81.7% of the patients were operated on for benign reasons, while 18.3% were operated on for malignant reasons. On average, after 2 years of follow-up, 97% of our patients continue their follow-ups in good health without disease.

Conclusion

Most salivary gland tumors are found in the parotid gland, with around 80% of these being benign. In this study, consistent with the literature, 81.7% of the operated cases were benign pathologies.

Keywords: Salivary gland, parotidectomy, pleomorphic adenoma, warthin tumor

Introduction

Salivary gland tumors are uncommon, representing around 0.6% of all body tumors and 26% of head and neck tumors. A significant portion of these gland tumors, about 75-80%, arise in the parotid gland, and approximately 80% are benign. Studies indicate that pleomorphic adenoma is the most common benign parotid tumor, whereas mucoepidermoid carcinoma is the most common malignant parotid tumor (1).

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Benign tumors typically present as mobile, painless, and slow-growing masses, whereas malignant masses can present with more severe symptoms, such as pain and facial paralysis. It is important to complement the patient's clinical presentation with objective data. A detailed head and neck examination should be followed by, ultrasound (USG), computed tomography (CT), or magnetic resonance imaging (MRI) to help determine the nature of the tumor mass. Fine-needle aspiration biopsy (FNAB) can also help with diagnosis. The literature indicates that FNAB has a sensitivity of 80% and a specificity of up to 95% for parotid masses (2).

The primary treatment for parotid gland masses is surgery, with the procedure varying based on the mass' location (superficial or deep lobe) and whether it is benign or malignant.

In this study, we retrospectively evaluated the treatment of patients who had parotid masses who underwent parotidectomy in our clinic between 2020 and 2023.

Material and Method

98 patients who had undergone a parotidectomy at the Antalya Training and Research Hospital's Ear, Nose, and Throat (ENT) clinic for various complaints between January 2020 and December 2023 were included in this study. Detailed preoperative ENT examinations, USG, CT, and MRI evaluations, were reviewed from the patient files. Depending on the location of the mass (either superficial or deep lobe), superficial or total parotidectomy was performed based on the suspected pathology. Intraoperative nerve monitoring was applied, and patients were hospitalized and examined until the drains were removed and the elevated flaps settled. Patients' demographic data findings and postoperative facial examination findings were recorded and evaluated statistically.

The statistical analysis of the obtained data was performed using the SPSS for Windows 11.5 (Chicago INC.) software package. In the evaluations: the Chi-Square test was used to compare categorical variables between groups, the Kappa test was used to assess the concordance of pathology results with FNAB, the independent samples t-test was applied for comparisons of continuous variables between two groups, and One-Way Analysis of Variance (ANOVA) was utilized for comparisons among more than two groups.

The threshold for statistical significance was set at 0.05.

Results

Of the patients who underwent parotidectomy in our clinic, 39 were female (39.8%) and 59 were male (60.2%). Details regarding gender and pathologies are shown in Table 1. The average age of the patients was 52.2 years (range 17-77 years). Almost all operated patients (96.8%) presented with a complaint of swelling in front of the ear. Only one (1%) patient presented incidentally, one (1%) was referred for follow-up MRI, and one (1%) presented with facial paralysis. No additional diseases were found in 59 (60.2%) patients.

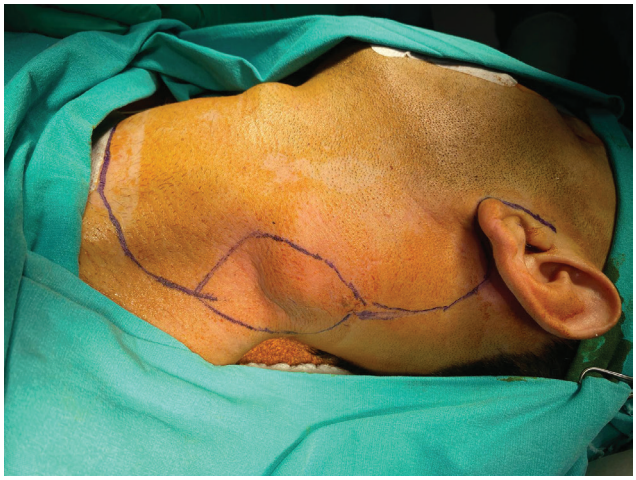

Figure 1
High-grade mucoepidermoid carcinoma case, preoperative

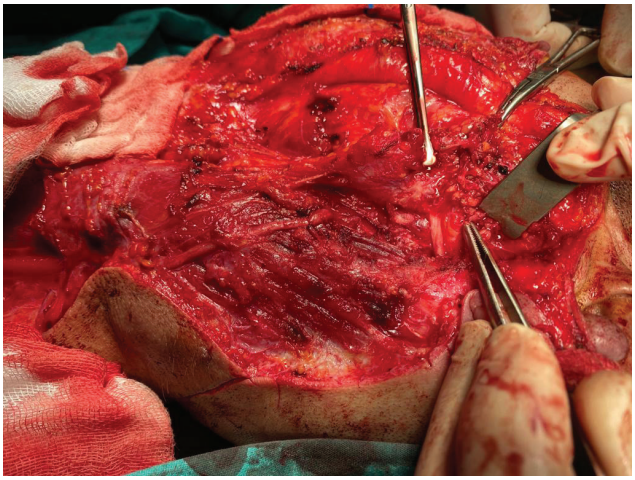

Figure 2
High-grade mucoepidermoid carcinoma, Facial nerve involvement (black arrow)

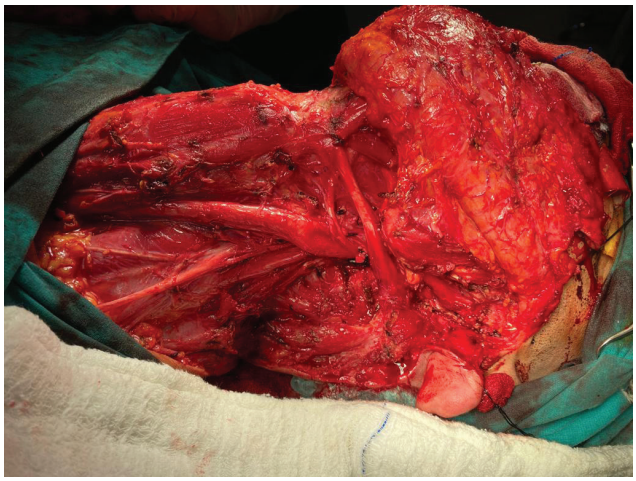

Figure 3
High-grade mucoepidermoid carcinoma case, radical parotidectomy + left radical neck dissection

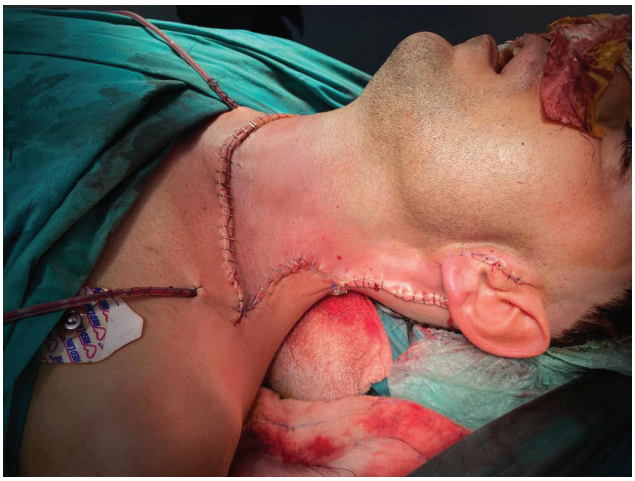

Figure 4
High-grade mucoepidermoid carcinoma case, reconstruction with sliding skin flap

Table 1 Diagnosis and gender distribution of the cases

			Pathology				Total
			Warthin	Pleomorfic	Other bening	Malign	
Gender	f	number	10	18	5	6	39
		% within pathology	26,3%	60,0%	41,7%	33,3%	40,0%
	m	number	28	12	7	12	59
		% within pathology	73,7%	40,0%	58,3%	66,7%	60,0%
Total		number	38	30	12	18	98
		% within pathology	100,0%	100,0%	100,0%	100,0%	100,0%

Table 2 Distribution of cases diagnosed as malignant

Diagnosis	Number Of Cases	%
Mucoepidermoid carcinoma	6	6,2
Adenoid cystic carcinoma	1	1,02
Asinik cell carcinoma	3	3,1
Squamoz cell carcinoma	2	2,04
Myoepithelial carcinoma	1	1,02
Secretuar carcinoma	1	1,02
Oncocytic carcinoma	1	1,02
Dermatofibrosarcoma	1	1,02

Of the patients who underwent surgery, 84 (85.7%) had superficial parotidectomy, and 14 (14.3%) had total parotidectomy. Final pathology revealed Warthin tumors in 38 (38.8%) patients, pleomorphic adenoma in 30 (30.6%) patients, and canalicular adenoma, basal cell adenoma, oncocytoma, oncocytic papillary cystadenoma, lipoma, or chronic sialadenitis in 12(12.2%) patients. Malignant pathologies were detected in 18 (18.3%) of the 98 patients.

Among the malignant tumors, squamous cell carcinoma (SCC), metastatic SCC, high-grade and low-grade mucoepidermoid carcinoma, acinic cell carcinoma, adenoid cystic carcinoma, and oncocytic carcinoma were identified. These are also showed in details in Table 2. Of the patients with at least a 12-month follow-up, one patient with high-grade mucoepidermoid carcinoma died. One patient with a fixed skin tumor and parotid SCC had a recurrence. During the follow-up period of our study, no recurrences were encountered among the other patients who had surgery.

Discussion

According to the World Health Organization (WHO), salivary gland tumors account for approximately 3-6% of all head and neck tumors. The annual incidence worldwide ranges between 0.5-2 cases per 100,000 people (3). These tumors can be benign or malignant, with malignant tumors being either primary or metastatic. Most salivary gland tumors (80%) occur in the parotid gland, and about 80% of these are known to be benign (1). While studies on parotid tumors do not show a distinct gender predominance, some subtypes, such as Warthin tumors, can be prevalent in males (4,5).

Parotid tumors can occur at any age but are mostly diagnosed between ages 40 and 60 years. The average age of the patients in our study was 52.2 years, with a patient-gender ratio of 39.8% females to 60.2% males.

95.8% of our patient cohort complained of swelling in front of the ear. Considering this, it should be noted that a patient presenting with a swelling in the parotid region is likely to be directed toward surgical evaluation.

According to WHO data, approximately 22.3% of the global population was smoking in 2020 (6). Although cigarette use has declined worldwide in recent years, this trend does not apply to Turkey. In our study, 37.8% of the patients were active smokers, while 3.1% were ex-smokers. Regarding pathologies, 69.4% of the patients who were operated on for Warthin tumors were active smokers, while 5.6% reported smoking use in the past. Only 23.3% of patients who were operated on for pleomorphic adenoma were smokers. Similarly, only 16.7% of patients with other benign tumor diagnoses were smokers. Surprisingly, only 11.8% of patients diagnosed with malignant parotid tumors had a history of smoking.

In this study, and consistent with the literature, 81.7% of the patients underwent surgery for benign pathologies. Benign tumors are typically characterized by well-defined borders, and encapsulation, and are not aggressive in nature. According to the current literature, the most frequently observed benign parotid tumors are pleomorphic adenomas, followed by Warthin tumors (7).

In our study, the commonest observed benign pathologies were Warthin tumors (38.8%), followed by pleomorphic adenomas (30.6%).

Pleomorphic adenoma is a benign tumor with significant morphological variability and includes both epithelial and myoepithelial elements. Although the rates for malignant transformation in the literature vary (1.6%- 9.4%), this tumor group is known for its potential for malignant change (8,9). In our cohort, no cases of malignant transformation were identified.

Pathologically, Warthin tumors are reported to originate from heterotopic salivary duct inclusions found in intraparotid or periparotid lymph nodes (10). Bilateral Warthin tumor cases have been reported to range between 5- 17% (11). In our study, bilateral Warthin tumors were detected in 2 (5.8%) patients.

We think it would be proper to note the changing prevalence of warthin tumors since its original description in 1929 (12). During the last years, several European studies showed the increasing prevalence of warthin tumors; where the most common explanation was the increase of tobacco consumption (13). However, a study from Austria showed that WT prevalence continued to increase even with the decreasing tobacco use (14). Therefore, it was stated that not only smoking but also other etiological factors, such as an increased body mass index (BMI) or metabolic syndrome should be taken into consideration.

In our study, we found that 34% of patients diagnosed with WT had diabetes, hypertension, or both. Additionally, 38% of these patients did not even smoke. Therefore, although we could not access

BMI data, considering the etiology of these chronic diseases, we can suggest that obesity might also be an etiological factor in the development of WT. It is also possible that the observed differences in the ratios of pleomorphic adenoma and Warthin tumors in our study could be related to the limited number of cases.

12.6% of the cases in our cohort, consisted of other benign tumoral masses. Among these, the most common were chronic sialadenitis (3.1%), lipoma (2%), basal cell adenoma (3.1%), and oncocytoma (2%).

Basal cell adenoma is a rare tumor of the salivary gland and reported to occur at a rate of 1-3.7%. Basal cell adenoma is a trabecular lesion with round isomorphic cells, a prominent basal membrane, interwoven and present in adult patients. It is also characterized by the presence of loose and hyaline stroma (15). This lesion, mostly located in the parotid gland, can be treated with partial parotidectomy. However, if the membranous subtype of basal cell adenoma is present, total parotidectomy is preferred. Although very rare, recurrence and malignant transformation have been reported in membranous basal cell adenoma (16). In this study, three (3.1%) of 98 cases were basal cell adenomas. All patients, diagnosed when older than 50 and 70 years, underwent superficial parotidectomy, with no malignant transformation or recurrence observed.

Oncocytic adenomas are rare salivary gland tumors composed of oncocytes, accounting for 1-2% of these tumors (17). These cases are typically diagnosed in patients older than 60 years, with a history of radiotherapy in approximately 20% of them. In our study, 2% of the patients were reported to have oncocytic adenoma. Superficial parotidectomy was performed on patients diagnosed at ages older than 50 and 70 years. Apart from diabetes mellitus and hypertension, no significant comorbidities were observed in the patients.

Parotid malignant tumors, while not constituting a high proportion among head and neck malignancies, hold significant importance among salivary gland tumors. In our study, 18.3% of the surgical cases were reported as malignant. Consistent with the literature, the most frequently observed malignancy in this study was mucoepidermoid carcinoma.
Mucoepidermoid carcinoma is the most common malignant salivary gland tumor in pediatric and adult patients, with a prevalence of 10-15%. Although observed in both major and minor salivary glands, mucoepidermoid carcinoma is most frequently detected in the parotid gland (18). The photographs of a patient operated on for parotid mucoepidermoid carcinoma are shown in Figures 1, 2, 3, and 4.

Mucoepidermoid carcinomas present in two patterns: high-grade and low-grade. The low-grade subtype has a very good prognosis with a 5-year survival rate of up to 98%, while the high-grade subtype has a much lower survival rate of around 67% (19). In our study, mucoepidermoid carcinomas were observed in six (6.2%) of 98 patients and 33.3% of malignant tumors. By the end of the 3-year follow-up, one of our two patients diagnosed with high-grade mucoepidermoid carcinoma had died.

Although adenoid cystic carcinoma is the second most common malignancy of the parotid gland, it constitutes the most common malignancy in salivary glands overall and is reported to have an affinity for the minor salivary glands. Considering perineural invasion, which is a specific characteristic of this tumor, caution should be exercised in terms of late metastasis. The lung is the most common site of distant metastasis: unfortunately, long-term survival is found to be as low as 20-40% (20).

Acinic cell carcinoma is the third most common malignancy of the parotid gland and is the second most common salivary gland malignancy in pediatric patients. Acinic cell carcinoma is a slow-growing tumor with relatively good early-stage survival rates; however, the 20-year disease-free survival rate drops to 42% (21). In our study, acinic cell carcinoma accounted for 16.7% while adenoid cystic carcinoma constituted 5.5% of the malignant pathology group. All cases continued to be followed up and were tumor-free at 2 years.

Primary SCC of the salivary glands is rare; however, considering metastatic cases, it constitutes 11% of major salivary gland malignancies. These tumors are highly aggressive, with a 5-year survival rate of approximately 50% (22). Considering the patient group in our study, two (11.1%) of 18 malignant cases were SCCs. One of these cases was primary SCC, while the other presented as skin tumor metastasis. At their 3-year follow-up, both patients receive additional treatment due to recurrence.

Salivary gland tumors have a wide range of diagnoses, given the diverse group of cells they originate from. This diversity can sometimes complicate the decisions of both the pathologist and the surgeon. However, in cases with suspected malignancy, it is

more appropriate to perform a fine needle aspiration and decide whether to perform a total parotidectomy intraoperatively using frozen tissue section analysis. For benign tumors not located in the deep lobe and for many low-grade malignant tumors, partial parotidectomy is a sufficient surgical treatment.

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

Ethical Approval
This study was conducted in accordance with decision No. 3/12, dated March 21, 2024, which was granted by the Clinical Research Ethics Committee of Antalya Training and Research Hospital. The study adhered to the Helsinki Declaration.

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Availability of Data and Materials
Data are available on request due to patients' privacy.

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

YY: Conceptualization; Formal analysis; Investigation; Methodology; Validation.

MY: Investigation; Validation; Writing-original draft.

NE: Formal analysis; Investigation; Visualization

ÖEG: Resources; Supervision; Writing-review & editing.

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ORIGINAL RESEARCH

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The Effect of Nebivolol on Acute Brain Damage in a Rat Model of Lps-Induced Inflammation

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Abstract

Objective
This research seeks to determine whether nebivolol (NEB) can prevent brain damage caused by lipopolysaccharide (LPS).

Material and Method
The thirty-two female Wistar Albino rats were divided into four groups, each containing eight rats: LPS (5mg/kg single dose i.p.), Control, LPS+NEB, and NEB (1 ml of 10 kg mg/kg given by oral gavage every day for three days). In brain tissues, the following parameters were measured: tumor necrosis factor-alpha (TNF-α), vascular endothelial growth factor A (VEGFA), caspase-3 (Cas-3), total antioxidant status (TAS), total oxidant status (TOS), and oxidative stress index (OSI).

Results
In the LPS group, there was an increase in the levels of TOS, VEGFA, Cas-3, and TNF-α. NEB therapy markedly reduced TOS, VEGFA, TNF-α, and Cas-3 levels. In both the control and NEB groups, histopathological analysis of the brain, hippocampus, and cerebellum demonstrated normal tissue architecture. In the LPS group, there were mild to moderate hemorrhages and severe hyperemia in the meningeal and parenchymal arteries of the brain and cerebellum. The LPS + NEB group's histopathology results were significantly ameliorated by NEB treatment.

Conclusion
NEB treatments anti-inflammatory, anti-apoptotic, and antioxidant characteristics helped mitigate the brain damage caused by LPS. NEB may help to reduce the severity of LPS-induced damage.

Keywords: LPS, Brain, Nebivolol, oxidative stress, inflammation, apoptosis

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Introduction

Neuroinflammation is an important factor contributing to cognitive impairment, various neurodegenerative diseases, and brain injury (1,2). Hypoxia-ischaemia (HI), insulted as a result, exaggerates the brain injury. Loss of oligodendroglial precursors, myelination abnormalities, astrogliosis (especially in the periventricular regions), and bilateral ventricular dilatation are the hallmarks of the inflammatory response. Furthermore, inflammation is the most powerful predictor of brain lesions (3,4). Inflammatory cells and their mediators can directly reach the central nervous system (afferent fibers of the abdominal vagus nerve, non-tight connection areas of the blood-brain barrier, etc.), activate cellular death mechanisms, and destroy cellular functions (5).

A significant part of the gram-negative bacterial wall, lipopolysaccharide (LPS) is an endotoxin that can trigger the creation of immune cells, pro-inflammatory cytokines, and cytotoxic agents like TNF-α, nitric oxide (NO), and interleukin-1 (IL-1), leading to numerous severe inflammatory disorders and dementia (6-9). Therefore, it is used in experimental inflammation models to mimic the systemic inflammatory response (10). Translocation of mitogen-activated protein kinases (MAPK), an upstream regulator of nuclear transcription factor-κB (NF-κB), is crucial for developing this neurodegeneration. The production of factors like VEGF and NO, as well as inflammatory cytokines like TNF-α, IL-6, and IL-1β, is stimulated by translocated nuclear factor-κB (NF-κB) (8).

Vascular Endothelial Growth Factor (VEGF) and its primary isoform VEGFA are pivotal signaling proteins in both physiological and pathological angiogenesis. VEGFA, commonly called VEGF, is primarily responsible for promoting blood vessel formation by interacting with specific receptors on vascular endothelial cells. This signaling pathway is crucial for vascular development during embryogenesis and tissue regeneration and repair processes in adults. VEGFA has important roles in angiogenesis and neuroprotection processes in the brain (11). It is also known that VEGFA enhances the permeability between cells and increases inflammation (12). Therefore, an imbalance in the VEGFA / VEGF receptor pathway has been predicted as an indicator of prognosis in inflammation (13,14).

Acute inflammation may be accompanied by an overabundance of a situation that is beneficial to the organism in small or medium amounts, which causes oxidative stress, often referred to as reactive oxygen species (ROS), to be produced at the cellular level. The shift of the balance between the pro-oxidant / antioxidant system can incline to the direction of oxidants, due to excessive ROS production and can damage the tissues. (15, 16). In the evaluation of the oxidant/antioxidant system, TOS and OSI are used as oxidant parameters, TAS are used as antioxidant parameters, and these parameters are oxidative stress indicators (16-18).

NEB is a beta-1 adrenergic receptor antagonist that is used to treat hypertension (19). NEB has antioxidant, vasodilator, and anti-inflammatory properties. NEB inhibits NF-κB, which results in a decrease in the levels of various pro-inflammatory cytokines. It also has antiapoptotic effects (20-22).

In the light of this study aims to investigate the ameliorative role of NEB in LPS-induced brain injury, considering its effect in reducing oxidative damage, inflammation, and apoptosis.

Material and Method

Animals and Ethical Acceptance

32 female Wistar Albino rats weighing 250-350 grams lived in a room with controlled temperature (21°C to 22°C) and humidity (60 ± 5%), as well as a 12:12 h light/dark cycle. All rats were given regular commercial chow (Korkuteli Yem, Antalya, Turkey).

Experimental Design

The four groups of rats—Control, LPS, NEB, and LPS+NEB groups—each had eight rats. (Figure 1)

1-Control Group (n = 8); Once a day for three days, a single dosage of one milliliter (ml) of saline was given orally via gavage, and thirty minutes later, a single dose of one milliliter of PS was given intraperitoneally (i.p.).

2-LPS Group (n = 8); 1 ml of saline was administered by oral gavage on 1-3 days and dissolved in saline and i.p. 5 mg/kg LPS (048K4126, Sigma Aldrich, USA) was administered 30 minutes after the last oral gavage saline administration (23).

3-NEB Group (n = 8); A single dose of 1 ml i.p. saline was given 30 minutes after the last oral gavage NEB treatment, and 1 ml of 10 mg/kg NEB was given by oral gavage every one to three days (22).

4-LPS + NEB (n = 8); 30 minutes after the last oral gavage NEB treatment, i.p. 5 mg/kg LPS (048K4126, Sigma Aldrich, USA) was given. 1 ml of 10 mg/kg NEB

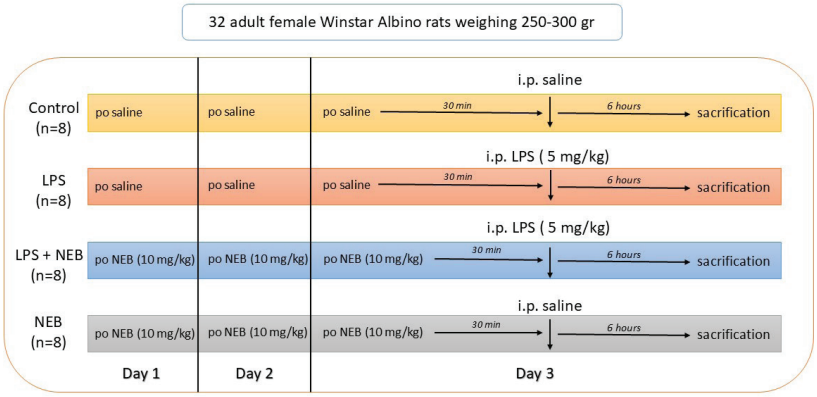

Figure 1
Experimental Design.
Po, Per Oral; i.p., İntraperitoenal; LPS, lipopolysaccharide; NEB, nebivolol.

was provided by oral gavage every one to three days and dissolved in saline.

All rats were given injections of 10 mg/kg Xylazine (Alfazin, Alfasan IBV) and 90 mg/kg ketamine intraperitoneally (i.p.) (Alfamin, Alfasan International B.V., Netherlands) to induce anesthesia six hours after the LPS was administered. After the trial, After the brain was removed, a sagittal cut at the level of the midbrain was made to separate the two hemispheres. One hemisphere was placed in formaldehyde for histopathological and immunohistochemical analyses, while the other hemisphere was further divided. One part was stored at -20°C for biochemical analyses, and the remaining part was allocated for genetic analyses and stored at -80°C.

Biochemical Analysis
Determination of the Parameters of Oxidative Stress
Rat brain tissues, weighing about 150 mg apiece, were homogenized using a phosphate-buffered saline (PBS) solution. After homogenization, samples were centrifuged at 10,000 rpm for 10 min. Using an automated analyzer fitted with Erel's colorimetric method, the values of the Oxidative Stress Index (OSI), Total Oxidant Status (TOS), and Total Antioxidant Status (TAS) in the samples were determined (Beckman Coulter, USA). After computing [(TOS, µmol/l) / (TAS, mmol Trolox eq/l)×100], the OSI value was determined (24,25).

Reverse Transcription-Quantitative Polymerase Chain Reaction (RT-qPCR)
Total RNA was extracted from rat tissue samples using a GeneJET RNA purification kit (Thermo Fisher Scientific) according to the manufacturer's protocol. The cDNA synthesis was performed from approximately 500 - 1 µg of RNA sample using the iScriptTM cDNA Synthesis kit (Bio-Rad Laboratories, Hercules, CA). Subsequently, a qRT-PCR assay was carried out using an iTaq Universal SYBR® Green Supermix (Bio-Rad Laboratories, Hercules, CA) with conditions consisting of 30 seconds at 95°C followed by 40 cycles of 5 seconds at 95°C and 10 seconds at 60°C. A fluorescence signal was detected on a CFX connect instrument (Bio-Rad Laboratories, Hercules, CA). The sequences of the primer were designed to amplify VEGFA (Forward 5'-GGA AGA GAG AGA GAG AGA GAG AC-3', Reverse 5'-GAC TGG TCC GAT GAA AGA TCC-3'). Per cDNA samples were analyzed in triplicates for each PCR. The expression of GAPDH (Forward 5'-CAA GGT CAT CCC AGA GCT GAA-3', Reverse 5'-CAT GTA GGC CAT GAG GTC CAC-3') was used for normalization. The relative gene expression was determined using the 2–ΔΔCt method. The results were presented as a fold change in the graph.

Analysis of Immunohistochemistry and Histopathology
Histopathological Analysis
Brain and cerebellum samples were obtained and stored in a 10% neutral formalin solution. Samples

were routinely processed and paraffin-embedded after fixing. After the paraffin blocks cooled, 5 µm thick sections were stained with hematoxylin-Eosin (HE). Analysis of the samples was done using a light microscope.

Immunohistochemical Analysis:
Furthermore, two series of sections from each block drawn on poly-L-lysine coated slides were immunohistochemically stained for the expression of TNF-α (Anti-TNFα Antibody (52B83):sc-52746, 1/100 dilution) and caspase-3 (Anti-caspase-3 Antibody (E-8): sc-7272) Santa Cruz (Texas, USA) using the streptavidin-biotin technique as directed by the manufacturer. After the sections were treated with the primary antibodies for 60 minutes, biotinylated secondary antibodies and streptavidin-alkaline phosphatase conjugate were used for immunohistochemistry. DISCLOSE The secondary antibody was a Mouse and Rabbit Specific HRP/DAB Detection IHC kit (ab80436) (Abcam, Cambridge, UK). As the chromogen, diaminobenzidine (DAB) was employed. Primary antibodies were substituted with antigen dilution solution for the negative controls.

For each antibody, slices were examined independently for immunohistochemical examination. Semiquantitative analysis was utilized to determine the degree of immunohistochemical reactivity of the cells with markers, using a grading score ranging from (0) to (3) as follows: 0 denote no expression, 1 focal and weak staining, 2 diffuse and weak staining, and 3 diffuse and marked staining respectively. In each part, ten distinct locations were inspected under 40X objective magnification for evaluation (Olympus Co., Tokyo, Japan). After being saved, the outcomes were statistically examined.

Statistical Analysis
The presentation of the variables was as mean ± standard deviation. The groups' biochemical, histopathological, and genetic results were compared using ANOVA (post hoc LSD and Duncan) tests. Statistical computations were performed utilizing the SPSS 18.0 program pack (SPSS Inc., Chicago, IL, USA). A significance threshold of P<0.05 was used.

Results

Oxidative Stress Markers in Brain Tissue
When comparing the LPS group to the control group, the levels of TOS were found to be significantly greater, and when comparing the LPS+NEB group to the LPS group, they were shown to be reduced (p = 0,016 and p = 0,029, respectively). Compared to the LPS group, the NEB group's TOS levels were considerably lower (p = 0.001). OSI levels were significantly greater (p = 0,018) in the LPS group compared to the control group, whereas they were lower in the NEB group (p = 0,005). TAS levels were significantly greater in the NEB group than in the LPS group (p = 0,011) (Table 1).

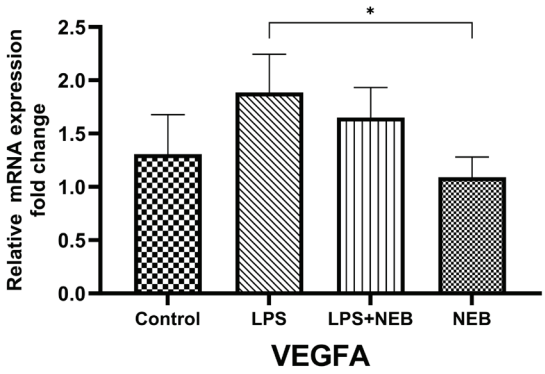

Figure 2
Analysis of VEGFA mRNA expression level by qRT-PCR.
The total RNA was extracted, and the VEGF gene expression was assessed by qRT-PCR assay. Gene expression was standardized based on GAPDH expression, and relative gene expression was calculated using the 2–ΔΔCt method. One-way ANOVA was used to compare the groups, and a post hoc LSD test was used to evaluate the results. The graph's fold change was used to display the results. The control value was 1. The values are shown as means ± SD. Vascular endothelial growth factor, VEGF; * p<0.05; LPS, lipopolysaccharide; NEB, nebivolol.

Table 1 Oxidative stress markers for brain tissues.

	Control	LPS	LPS+NEB	NEB	P value
TAS	0,67 ± 0,05	0,57 ± 0,14	0,65 ± 0,44	0,71 ± 0,10	Control vs. LPS = NS LPS vs. LPS+NEB = NS LPS vs. NEB < 0.05
TOS	8,15 ± 1,05	9,83 ± 1,32	8,32 ± 1,09	7,33 ± 1,68	Control vs. LPS < 0.05 LPS vs. LPS+NEB < 0,05 LPS vs. NEB = 0,001
OSI	1,22 ± 0,16	1,91 ± 1,03	1,28 ± 0,16	1,06 ± 0,30	Control vs. LPS <0,05 LPS vs. LPS+NEB <0,05 LPS vs. NEB < 0,01

Data are means ± SD. Comparison between groups and results of oxidative stress markers were assessed by a one-way ANOVA test that followed by post hoc Tukey's multiple comparison test. LPS, lipopolysaccharide; NEB, nebivolol; TAS, total antioxidant status; TOS, total oxidant status; OSI, oxidative stress index.

Relative VEGFA mRNA Expression Levels in Brain Tissues

The LPS group's relative VEGFA mRNA level rose significantly in comparison to the NEB group (p=0,013). In the LPS group, relative VEGFA mRNA levels were lower than in the LPS+ NEB group and greater than in the control group, respectively. Nevertheless, these differences did not reach statistical significance (p> 0.05). (Figure 2)

Histopathological and Immunohistochemical Results

In both the control and NEB groups, histopathological analysis of the brain, hippocampus, and cerebellum demonstrated normal tissue architecture. In the LPS group, there were mild to moderate hemorrhages and severe hyperemia in the meningeal and parenchymal arteries of the brain and cerebellum. The brains and cerebellum of the LPS group showed a large number

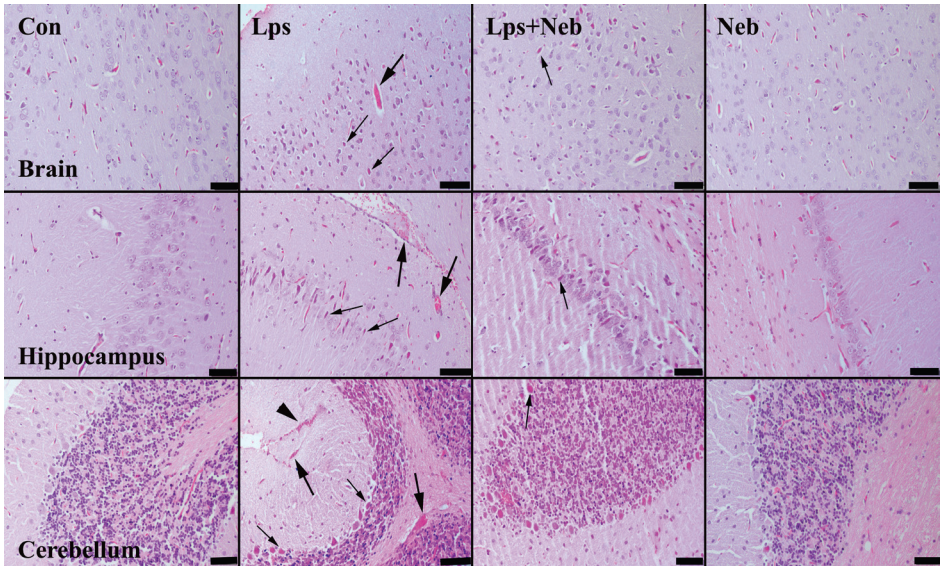

Figure 3

Histopathological appearance of the brain cortex, hippocampus, and cerebellum between the groups. Several degenerative pink coloured neurons in the brain cortex, hippocampus, and cerebellum (thin arrows), severe hyperemia (thick arrows), hemorrhages in the cerebellum (arrowhead), lipopolysaccharide (LPS), NEB (nebivolol), and H&E (scale bars= 50µm)

of deteriorated neurons, whereas normal neurons were observed in the control and NEB groups. Purkinje cells were the most afflicted cells in the cerebellum. The histopathology results in the LPS + NEB group were dramatically improved compared to the LPS group (Figure 3).

Cas-3 and TNF-α immunostained slides were examined using immunohistochemistry, and the LPS group showed significant expression of both markers. Figures 4-5 show that the LPS + NEB group's expressions were reduced compared to the LPS group. The results of the statistical analysis of immunohistochemistry scoring are displayed in Figure 6.

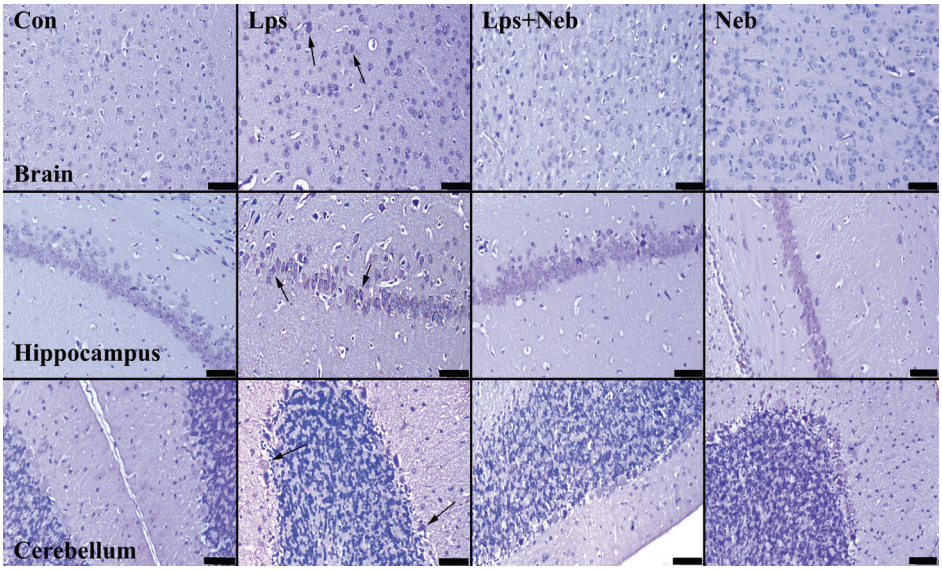

Figure 4

Cas-3 immunohistochemistry findings among the groups.

Increased expressions in neurons (arrows) in the brain cortex, hippocampus, and cerebellum in the LPS group, LPS, lipopolysaccharide; NEB, nebivolol, Streptavidin biotin peroxidase method, scale bars= 50µm

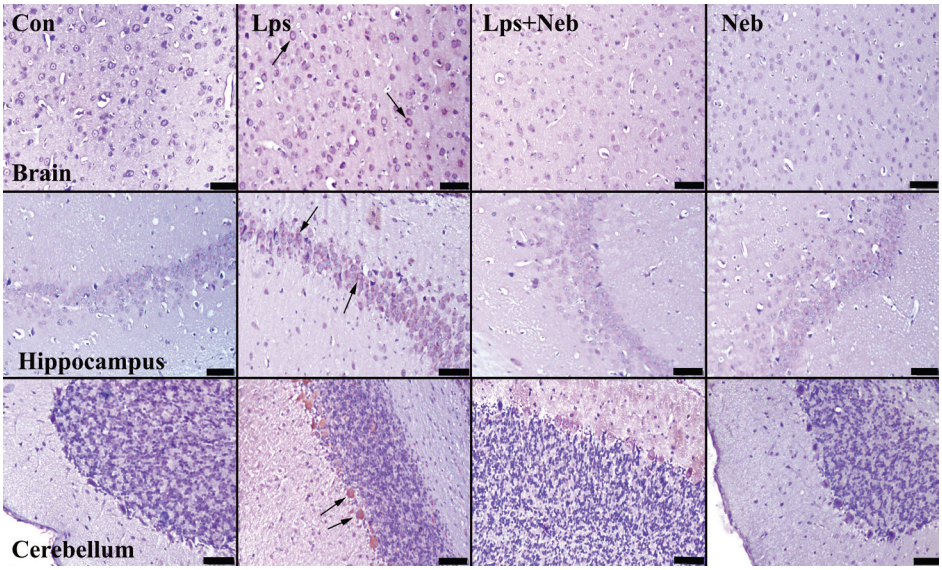

Figure 5

TNF-α immunohistochemistry findings among the groups.

Markedly increased in expressions in neurons (arrows) in brain cortex, hippocampus, and cerebellum in LPS group, LPS, lipopolysaccharide; NEB, nebivolol, Streptavidin biotin peroxidase method, scale bars= 50µm.

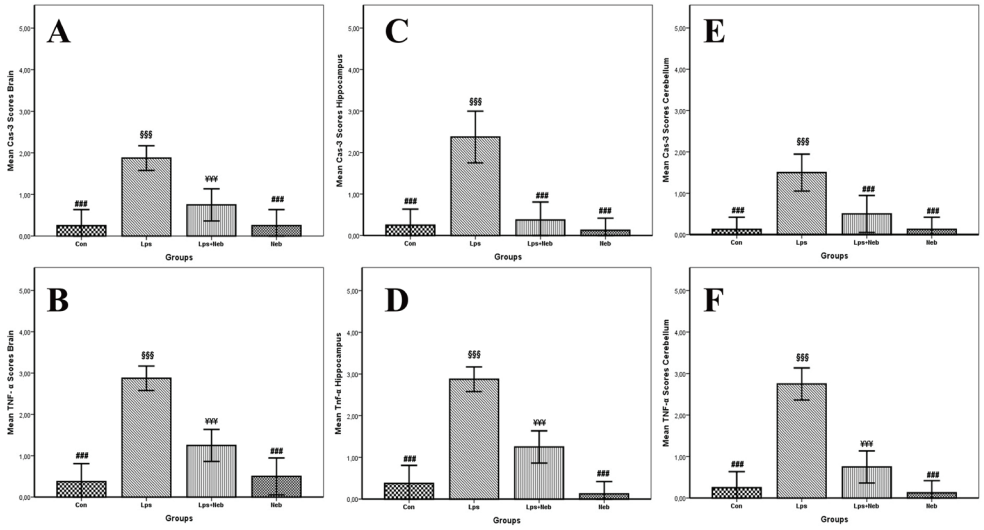

Figure 6

Illustration of the mean values of the immunohistochemical scores between the groups. (A) Cas-3 scores of the brain cortex, (B) TNF-α scores of the brain cortex, (C) Cas-3 scores of the hippocampus, (D) TNF-α scores of the hippocampus, (E) Cas-3 scores of the cerebellum, (F) TNF-α scores of the cerebellum.

Discussion

Endotoxin levels in blood plasma increase in the presence of an infection in the body. In the case of very high endotoxin levels inflammatory diseases may occur, unless not sufficiently cleared from the blood (26). An increased inflammatory response results from the endotoxin LPS, which increases the synthesis of pro-inflammatory cytokines like TNF-α and IL-1 (6–10). In the current investigation, we found that NEB protects brain tissue against oxidative stress, inflammation, and apoptosis caused by LPS.

NEB, a third-generation beta-1 adrenergic receptor antagonist, has higher β-receptor affinity compared to other beta-blockers and has a beta-3 receptor partial agonist function. In addition to beta-1 receptor antagonism in cardiac muscles, beta-3 agonism activates endothelial and neuronal nitric oxide synthase and causes NO-mediated vasodilation (19,20). Rats exposed to cisplatin-induced nephrotoxicity showed nephroprotective effects from NEB, and it was noted that these effects might be mediated by the compound's anti-inflammatory, antiapoptotic, and antioxidant properties. The application of Nx-nitro-L-arginine methyl ester, a non-specific NOS inhibitor, altered the protective effect of NEB in the same trial) (21). Also, NEB has been shown to have a nephroprotective effect by improving renal histopathology and decreasing serum renal function parameters (22). Colak et al reported that, after ischemia reperfusion damage was developed in the ovaries of rats, increased levels of malondialdehyde and TNF-α, oxidative stress and inflammation markers, were reduced by NEB (27).

Kumar et al. evaluated oxidative stress and antioxidant status in sepsis and reported that the balance between oxidants/antioxidants has a key role in sepsis (28). Furthermore, the uncontrolled and exaggerated peripheral inflammatory response that occurs in sepsis may cause neuroinflammation. Along with neuroinflammation, disruption in the blood-brain barrier is accompanied by glial activation, and as a result, proinflammatory cytokines increase (5, 29). Microglia activation is the primary indicator of neuroinflammation, resulting in the release of cytokines like IL-1, TNF-α, and NO (9). LPS binding to TLR-4 receptors in microglia cells activates the NF-κB and mitogen activated protein kinase pathways, leading to the production of proinflammatory cytokines (TNF-α, IL-1β, and IL-6). NO and prostaglandin E2 mediators, which further aggravate this inflammatory process, are released as a result of the upregulation of nitric oxide synthase and cyclooxygenase-2 enzymes (30). According to Hun et al., LPS caused the synthesis of prostaglandin E2, TNF-a, IL-1β, and IL-6 (31).

The impairment of balance between the oxidant and antioxidant system causes increased ROS levels that lead to oxidative damage and oxidative stress via

damaging cell structures such as protein, lipid, and DNA (16). ROS is beneficial for the organism at low concentrations (such as defense against infectious agents, and cellular signaling functions) (15). In Chen et al.'s study, LPS decreased the activity of antioxidant enzymes that cause cardiac damage caused by LPS, such as glutathione peroxidase, catalase, and superoxide dismutase (32). In another study, LPS has been shown to increase ROS production in microglia cells (33). In our investigation, six hours following the application of LPS, TOS levels, and OSI levels statistically rose, but TOS levels were dramatically reduced by NEB administration. Moreover, albeit this was not statistically significant, NEB treatment raised TAS levels. Insufficiency of the administered dose could be the explanation. As such, additional research at varying doses could help clarify NEB's antioxidant action.

As known VEGF, a vasoactive glycoprotein, has important roles in vascularization, angiogenesis, and neuroprotection. Besides increasing microvascular permeability, it induces cell migration and increases endothelial mitogenesis. VEGFA is released by proteases in inflammation and its levels increase in sepsis, which is an exaggerated inflammatory response (11-14, 34, 35). Braile et al. showed activation of VEGFA release from neutrophils via LPS administration (13). In another study, Li et al. reported significantly higher levels of VEGFA in the lungs of the LPS group rats (36). Correlative to the literature in our study, LPS application increased VEGFA levels, and NEB treatment improved increasing VEGFA levels, but this was not statistically significant. In cases where such inflammation occurs, it is known that apoptosis develops with the activation of various intracellular pathways.

Apoptosis is essential to the processes of cell division and death. Cas-3 is essential to the process of apoptosis (37-39). According to Mohamed et al., NEB can protect the heart against doxorubicin-induced cardiotoxicity via modulating TNF-α, inducible nitric oxide synthase, and Cas-3 (40). In this study, LPS administration increased the Cas-3 levels, and a statistically significant decrease was observed with NEB treatment in the LPS + NEB group. According to these findings, we can interpret that the antiapoptotic effects of NEB contribute to the improvement of inflammation induced by LPS.

The limitations of our study include: 1- The use of only female animals, 2- The inability to investigate detailed cellular signaling mechanisms, 3- The lack of assessment of protein-level expression of examined genes due to financial constraints, and 4- The absence of evaluation of NEB's effects over longer periods and at varying doses. In our future studies, we aim to include animals of both sexes to determine whether the neuroprotective effects of NEB are sex dependent. Additionally, it is essential to investigate various signaling mechanisms and document their protein-level expressions to contribute to the scientific literature.

Conclusion

NEB may be considered a potential therapeutic agent in brain damage within neuroinflammation via protective effects due to anti-inflammatory, antioxidant, and anti-apoptotic features. However different dose studies may be useful in elucidating the effects of NEB.

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Conflict of Interest Statement
Dr. Oguzoglu Ali Serdar and the co-authors have no conflicts of interest to declare in association with this study.

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 Suleyman Demirel University, Isparta (Ethic No:11.09.2020/06-02).

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Availability of Data and Materials
A data availability statement should be included.

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

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

DD: Methodology; Validation; Visualization;

SA: Formal analysis; Validation; Visualization

YE: Genetic analysis

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

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ORIGINAL RESEARCH

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Evaluation of Süleyman Demirel University Faculty of Medicine Simulated Patient Applications within the Scope of "The ASPiH Standards 2023"

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Abstract

Objective
Simulated patient (SP) applications are the general name for educational activities in the field of health that are carried out through simulated patients playing the role of trained patients. This study aimed to evaluate the Simulated Patient Laboratory of Süleyman Demirel University within the scope of the Association for Simulated Practice in Healthcare (ASPiH) 2023 Accreditation Standards".

Material and Method
This study used a qualitative research design. “Süleyman Demirel University Faculty of Medicine Simulated Patient Laboratory” was determined as the study area. Based on the decision of the board of the Department of Medical Education and Informatics, medical faculty members who took an active role in every stage of the accreditation process in the evaluation of the subject of the study were selected as in-house evaluators, and two trainers who participated in simulated patient applications and organized training in a different institution were selected as peer evaluators and invited to participate in the study. At the end of the laboratory evaluation, a report was prepared and presented to laboratory supervisors and medical faculty members.

Results
Within the scope of the study, the laboratory was evaluated over 17 standards for one day. The evaluation revealed that 15 (88%) items were fully met and two (12%) items were partially met.

Conclusion
The accreditation of our laboratory by an international organization is valuable for the quality processes of our faculty of medicine. As a result of this study, we believe that our laboratory can undergo international accreditation by conducting improvement studies in areas necessary for improvement.

Keywords: Accreditation, simulated patient applications, simulated patient laboratory

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Introduction

Simulated patient (SP) applications are the general name for educational activities in the field of healthcare carried out through simulated patients playing the role of trained patients. (1). These applications involve the use of simulated patients who are specially trained to mimic diseases in a consistent and reproducible manner (2). Simulated patient applications are considered an important teaching tool in medical education and are frequently recommended to be included in modern medical education programs (1,3,4). These applications provide students with the opportunity to practice real and authentic patient-patient interactions and contribute to the development of their clinical skills (2). It also helps develop students' communication skills, patient care skills, and clinical thinking abilities (5–7). Simulated patient practice increases students' skills in providing safe and ethical patient care and makes future healthcare professionals more competent (8).

Simulated patients are specially trained to consistently mimic different disease states and symptoms. This structure offers students the chance to practice in situations and environments similar to the real world and provides students with the opportunity to learn by repeating, and the practices are carried out in environments that will not violate patient safety (1). Students both reinforce their positive behaviors and realize aspects that need improvement through the feedback they receive after the application and the analysis sessions they attend (6,9). In the literature, students stated that simulated patient practices were useful in improving their communication skills and preparing for real patient encounters (10). While real patient encounters are seen as important for learning clinical skills in the literature, simulated patient interactions are valuable in terms of receiving feedback and improving communication skills (10). These practices are considered an important androgogical tool in medical education and are widely used as part of modern medical education programs.

The accreditation process for pre-graduate medical education programs in our country is carried out by the "Association for Evaluation and Accreditation of Medical Education Programs (TEPDAD)," which is authorized by the Council of Higher Education (YÖK) and recognized by the World Federation of Medical Education (WFME). According to the current accreditation standards of the association, “Development Standard 7.1.2. Faculty of Medicine; it must offer training and evaluation opportunities with simulated/standardized patients.” Based on this article, the need to develop training and evaluation opportunities for simulated/standardized patients has emerged in medical faculties wishing to be accredited. To meet this need and carry out planned training activities with simulated patient applications, it is recommended to establish simulated patient laboratories. (11). These laboratories simulated outpatient clinic rooms and offered audio and video recording opportunities to enable feedback and self-evaluation simultaneously. Twelve recommendations were listed by Ker to establish simulated patient laboratories (12). These recommendations should consider the training level and experience of the simulated patients. The management and organization of simulated patients should be ensured. Simulated patient feedback and self-evaluation skills should be improved. Regular time should be devoted to training the simulated patients. An environment in which simulated patients are used for training must be suitable. The scenarios to be used in training simulated patients should be carefully designed. The training process for the simulated patients should be continuously evaluated. Resources and materials for training the simulated patients should be provided. The success and challenges in training simulated patients should be documented. Guidance and mentoring programs should be established to educate simulated patients. Simulated patient laboratories must be effectively managed.

For simulated patient applications to have national and international validity, these laboratories and applications must comply with accreditation standards. (13). The Association for Simulated Practice in Healthcare (ASPiH) was founded in 2008 and set accreditation standards for current simulated patient practices and laboratories in 2023. (13). These standards were established to promote best practices and improve the quality of simulated patient education. Thus, it aims to increase the practical skills and competencies of graduating health professionals in patient care by ensuring the effectiveness and quality of simulated patient applications in medical education.

In 2019, a simulated patient laboratory consisting of three interview rooms was established at Süleyman Demirel University Faculty of Medicine (14). After the pandemic, four simulated patients were employed as part-time temporary workers, and the simulated patients were certified with a 15-day training program developed by the Department of Medical Education and Informatics. By 2023, practices were included in the education program by the National Core Education Program-2020, and active student meetings are still

ongoing (14). For the sustainable development of the laboratory, there is a need to evaluate it within the scope of international standards.

This study aimed to evaluate the Süleyman Demirel University Simulated Patient Laboratory within the scope of ASPiH 2023 Accreditation Standards".

Material and Method

This study used a qualitative research design. “Süleyman Demirel University Faculty of Medicine Simulated Patient Laboratory” was determined as the study area. Ethics committee approval was received from the Süleyman Demirel University Health Sciences Ethics Committee, dated 29.05.2024 and numbered 76/9, and written approval was received from the Süleyman Demirel University Faculty of Medicine Dean's Office. Based on the decision of the Board of the Department of Medical Education and Informatics, medical faculty members who played an active role in the evaluation of the subject of the study at every stage of the accreditation process were selected as in-house evaluators, and two educators who participated in simulated patient applications and organized training in a different institution were selected as peer evaluators and invited to participate in the study. Before the study, evaluators were provided with the opportunity to examine standards in detail. Within the scope of accreditation standards, the " Süleyman Demirel University Faculty of Medicine Simulated Patient Laboratory" was evaluated for one day. The process was completed with a book, which is a self-evaluation report written for laboratories and applications, on-site evaluation, and interviews. In the consensus of the evaluators, a decision was made regarding the meeting status of each standard and suggestions were made for the development process. At the end of the laboratory evaluation, a report was prepared and presented to laboratory managers and medical faculty administrations.

Results

Within the scope of the study, the laboratory was evaluated according to 17 standards for one day. As a result of the evaluation, it was determined that 15 (88%) items were fully met and 2 (12%) items were partially met.

Although the psychological safety standard is partially met within the basic values standards, it is recommended to make an official correspondence/ protocol with the Department of Psychiatry for a more formal management of the process. It has been concluded that, although the equality, diversity, and inclusion (ECC) standard is naturally achieved, there is no effort in this regard, and this inclusion is met informally. In this regard, it has been suggested that the feedback of experts in the field of Equality, Diversity, and Cultural Competency (ECD) may be useful and that formal support should be obtained on this issue. Although the employment process of simulated patients has been completed according to the sustainability standard, it is recommended to make a maintenance contract for laboratory equipment. For the standard of excellence, it is recommended to prepare and implement a laboratory "improvement form" while ensuring that the institution's total quality management policy is secured in the context of the approaches of the Faculty of Medicine Management and Department of Medical Education and Informatics and national accreditation standards. It was determined that all training and application standards were met. Although many items are met in the resource management standards, it is recommended to prepare a directive, inspection form, and calendar to move the process to the formal basis for the standards related to inspection. Explanations of the items that were met and suggestions for the items that were partially met are shown in detail in the table below (Table 1).

Medical faculty members in medical education and informatics lead the design of simulated patient applications, with medical educators having received doctoral-level training. Simulated patient training was conducted using a program developed by the medical educators. The simulated patients were certified after 15 days of training. Medical educators collaborate with term coordinators and education management boards to create content that is in line with the National Core Education Program-2020. Simulated patients worked with medical educators to prepare scenarios and undergo preliminary preparation for their roles (Table 2).

All practices were aligned with the feedback, learning outcomes, and core training programs. Undergraduate education scenarios comply with the National Core Education Program-2020, and the "Breaking Bad News" training in Term 6 was added based on student feedback. Specialist training was conducted as a CPD activity, following assistant feedback. Simulated patient applications were designed in accordance with the learning outcomes. Evaluation and research were incorporated during the planning stage, and measurements were performed. Students evaluated the simulated patients and the laboratory, and this feedback was used in continuous improvement mechanisms (Table 3).

Table 1 Evaluation of core values of Süleyman Demirel University simulated patient laboratory with the ASPiH 2023 accreditation standards

Core Values	SDÜTF applications and approaches	Evaluation	Evaluation status
1. All individuals involved in the design, delivery, evaluation, and translation of simulated practice should adhere to the ASPiH core values:			
i. Safety	In our laboratory, the psychological safety of simulated patients and students is prioritized. Within the scope of training on giving bad news, a training program was designed in consultation with the Department of Psychiatry. Medical educators are present as observers in all interviews. They can intervene themselves when necessary and even receive support from the Department of Psychiatry.	xx	Partially covers It is recommended to correspond with the Department of Psychiatry for all applications, determine a protocol, and expand this protocol to include not only students but also simulated patients.
ii. Equity, diversity, and inclusion	The principles and rules established for all people involved in the design and delivery of simulation-based activities in our laboratory, students, faculty, staff, patients, service users, carers, families, and communities, are carried out with an approach that includes the principles of equality, diversity, and inclusion. As per the recruitment criteria, it is aimed to reach all students based on talent and knowledge criteria. In this respect, although there is natural compliance with the EÇK principles, there is no structured approach to this issue. All individuals participating in the simulation behave in a way that adheres to the four principles of biomedical ethics (autonomy, beneficence, nonmaleficence, and justice).	xx	Partially covered Feedback can be obtained from Equity, Diversity, and Inclusion (EDI) experts. Training on EDI can be planned. Structuring can be provided in a way that takes into account EDI principles, and a separate policy can be determined for disadvantaged groups.
iii. Sustainability	Support for technical equipment is available in our laboratory. Our simulated patients work as part-time temporary workers.	xx	Partially covered A regular maintenance contract can be made.
iv. Excellence.	Our laboratory is constantly monitored and evaluated by our department. All processes in the application are being developed. Continuous improvements are made with constant feedback. In addition, scientific studies and scientific production are constantly carried out in different patterns.	xx	Partially covered A laboratory improvement form can be prepared. A framework directive, control mechanism, and work schedule can be created to ensure continuity.

xxx is covered, xx is partially covered, x is not covered

Table 2 Evaluation of core values of Süleyman Demirel University simulated patient laboratory with the ASPiH 2023 accreditation standards

Faculty			
2. All individuals involved in the design, delivery, evaluation, and translation of simulated practice should be trained and committed to continuous professional development.	The entire design of our simulated patient applications was carried out by faculty members of the Department of Medical Education and Informatics. Medical educators have received doctoral level training in their field. Simulated patient training was carried out with the training program developed by medical educators.	xxx	It is covered
3. Simulation technicians should have received training for the simulation activity they support. Required to undertake.	Our simulated patients were certified after receiving 15 days of training.	xxx	It is covered
4. Simulation educators and trainers must possess competence in simulation as well as appropriate content knowledge.	Our medical educators produce UÇEP-2020 compatible content in cooperation with term coordinators and education management boards.	xxx	It is covered
5. Simulated participant.	Our simulated patients prepare scenarios with medical educators. Preliminary preparation and study are carried out for their roles.	xxx	It is covered

xxx is covered, xx is partially covered, x is not covered

Table 3 Evaluation of preparation and planning of Süleyman Demirel University simulated patient laboratory with the aspih 2023 accreditation standards

Activity			
Preparation and planning			
6. The intended learning outcomes must be relevant and aligned with learning needs.	All our practices are related to feedback, learning outcomes, and/or core training programs. The scenarios of our undergraduate education are compatible with UÇEP-2020. Term 6 “Breaking bad news” training was added in line with student feedback. The “Breaking bad news” training in our specialist training is implemented as a CPD activity in line with assistant feedback.	xxx	It is covered
7. The simulation modality, fidelity, and activity design should be determined by the intended learning outcomes.	Simulated patient applications are carried out in line with the learning outcomes.	xxx	It is covered
8. Evaluation and research should be considered during the planning stage.	In all our applications, measurements are made at the planning stage. All students evaluate simulated patients and the laboratory. The feedback obtained is evaluated as an active data source in continuous improvement mechanisms.	xxx	It is covered

xxx is covered, xx is partially covered, x is not covered

Table 4 Evaluation of facilitation of Süleyman Demirel University simulated patient laboratory with the aspih 2023 accreditation standards

Facilitation			
9. The individual or team facilitating the activity should have training and experience in facilitation, including establishing psychological safety and debriefing.	Medical educators are present in all training in our laboratory. When necessary, support is received from the Department of Psychiatry.	xxx	It is covered
10. The activity must be initiated by a briefing or pre-briefing which helps create a safe environment where learning can take place.	All training in our laboratory starts with a briefing.	xxx	It is covered
11. The purpose of the activity should be to ensure the achievement of the intended learning outcomes. Team and system performance.	The main purpose of all our applications is determined as learning outcomes.	xxx	It is covered
12. The simulated experience must include a facilitated reflection or debriefing in which the participants should explore and develop strategies to improve individual,	In all our trainings, feedback is given and a debriefing session is held.	xxx	It is covered
13. The use of simulation for summative assessments should prioritize validity, reliability, and psychological safety.	Feedback is given to students in all our applications. Scoring is done with the skill evaluation rubric. The entire process is observed by medical educators and psychological safety is prioritized.	xxx	It is covered

xxx is covered, xx is partially covered, x is not covered

Medical educators facilitate all laboratory training and support from the psychiatry department is available when needed. Each session began with brief briefings to ensure a safe learning environment. The primary goal of all the applications is to achieve learning outcomes. Every training session included a debriefing session where participants received feedback and explored strategies for improvement. In simulation-based summative assessments, feedback is given, scoring is performed using a skill evaluation rubric, and the process is overseen by medical educators, with psychological safety as a priority (Table 4).

Feedback was collected from all participants during the laboratory training sessions. Ethical approval was obtained from all the research conducted in the laboratory. The laboratory has a clear vision and mission, aligns with organizational and stakeholder needs, and works in collaboration with the Interprofessional Applied Education Laboratory. It is open to internal and external stakeholders and supports individual research and postgraduate studies. Laboratory operations are informally overseen by the medical faculty administration, although formal directives, forms, and inspection calendars could enhance oversight. TEPDAD accreditation standards, specifically “Development Standard 7.1.2”, ensure prioritization, quality assurance, and safety. Key performance indicators of the laboratory were established (Table 5).

Discussion

Simulated patient methodology is among the cornerstones of medical education and offers a structured and effective way to train future healthcare professionals (15,16). Developing skills such as communication skills and clinical reasoning with simulated patients before contact with a real patient is valuable in terms of patient safety (12,17). Students can hone their clinical skills and improve patient care outcomes through simulated patient practice (8,18,19). By adhering to established best practices and standards, educators can ensure the quality and consistency of simulated patient interactions, ultimately benefiting both students and patients in the healthcare system (8). Currently, simulated patient applications are included in many health professional training programs in our country (20–23). Simulated patients can take part in these trainings as training tools, trainers, and evaluators/raters (5–8,23,24).

Table 5 Evaluation of evaluation and research and resource management of Süleyman Demirel University simulated patient laboratory with the aspih 2023 accreditation standards

Evaluation and research			
14. The activity should be evaluated by participants and faculty to inform future activities and, where applicable, system improvement.	Feedback is received from all participants who receive training in our laboratory.	xxx	It is covered
15. Simulation-related research should be of high quality and carried out ethically.	Ethics committee approval is obtained for all studies carried out in our laboratory.	xxx	It is covered
Resource management			
16. There should be a clear vision, mission, and strategy to sustain and grow simulation practice in alignment with wider organizational and stakeholders' needs.	The vision and mission of our laboratory have been defined. Our laboratory works in harmony with the Interprofessional Applied Education Laboratory. It is open to internal and external stakeholder use. Individual research and postgraduate thesis studies are also carried out.	xxx	It is covered
17. Designated leads with organizational influence, appropriate expertise, and accountability should oversee the design and delivery of simulation activities and use of resources.	Our laboratory and practices are informally supervised by the faculty administration.	xx	Partially covered Directives, a form, and an inspection calendar can be created for the inspection of laboratories and applications.
18. Robust policies should be in place to ensure prioritization, financial support, quality assurance, and safety.	Within TEPDAD accreditation standards; "GS.7.1.2. "Providing training and evaluation opportunities with simulated/standardized patients". This article is valuable for prioritization, quality assurance, and security in the faculty. The key performance indicators of our laboratory have been defined.	xxx	It is covered

xxx is covered, xx is partially covered, x is not covered

Today, simulated patient laboratories have become an important part of medical education and health care (8,12,25). Establishing these laboratories and employing simulated patients is a labor-intensive process (17,26–28). Simulation of an outpatient clinic or inpatient room is recommended, especially for laboratories (1,29). After laboratory installation, the recruitment, training, and management/evaluation of simulated patients and evaluation of the impact begin (30). It is recommended to develop a structured training program, especially for the training of simulated patients (12,13,30).

According to the findings of this study, a more formal management process is needed in certain areas of the laboratory. This can enable laboratory activities to be managed more effectively and efficiently using resources (13). In particular, it has been suggested that regulations should be made on issues such as the employment of simulated patients and the maintenance of laboratory equipment. Implementing these recommendations can make the daily operation of the laboratory smoother and improve the overall quality of the educational programs. The study showed that all training and practice standards were

met (12). However, the evaluation recommends that the auditing process for resource management standards become more formal. These developments can make laboratory budget management more effective and help prevent unnecessary expenses. Such arrangements can increase the long-term sustainability and operational efficiency of laboratories.

Although the fact that this is an internal evaluation is among the limitations of this study, the evaluation of laboratories and applications according to international standards is valuable in terms of the vision of the faculty. In addition, laboratories and applications were developed using feedback obtained from the evaluation of these standards. In this context, a laboratory's internal evaluation process is considered a valuable resource for the official accreditation process. With comprehensive studies designed in the future, more scientific information will be obtained about simulated patient laboratories and simulated patient applications in our country.

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Conflict of Interest Statement
The authors declare that there is no conflict of interest.

Ethical Approval
Written permission was obtained from the developer of the scale, and ethics committee approval was obtained from the Süleyman Demirel University Health Sciences Ethics Committee (dated 29.05.2024 and numbered 76/9). In the ethics committee approval, a commitment was made that there was no relationship between the data collection process of the participating students and the educational processes. Ethical approval indicates that the study adhered to ethical standards regarding human participants, including considerations of privacy, consent, fairness, and beneficence. This approval confirmed that the research was conducted in accordance with ethical principles and that the rights of the participants were protected.

This study was conducted in accordance with the principles outlined in the Declaration of Helsinki.

Consent to Participate and Publish
Written consent was obtained from all participants who expressed their opinions within the scope of the research.

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Availability of Data and Materials
In this study, the authors assumed that if the requests are deemed appropriate, the data stored in the data warehouse can be easily accessed by others.

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

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

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ORIGINAL RESEARCH

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The Role of Computed Tomography in Detection of Lung Metastases in Patients with Colorectal Carcinoma

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Abstract

Objective

At the time of diagnosis, 20% of colorectal carcinomas (CRC) are metastatic, and 1-9% of these metastatic patients have lung metastasis. Current guidelines recommend thoracic computed tomography (TCT) for preoperative staging, but this is costly and involves significant radiation exposure. Additionally, in most cases, the treatment plan does not change. Therefore, a comparison between chest X-ray (CXR) and computed tomography (CT) in patients with CRC was conducted.

Material and Method

In this study, 630 patients admitted to our hospital between May 2019 and May 2023 for CRC were retrospectively screened. According to follow-up records, the presence of lung metastasis was confirmed based on biopsy, CT, and/or PET/CT results. Thirteen patients with lung metastasis were classified as Group 1, and 31 patients without lung metastasis, identified using propensity score matching, were classified as Group 2, totaling 44 patients for analysis.

Results

Preoperative screening revealed that metastatic lesions were detected by CXR in 4 out of 13 patients in Group 1, with an average lesion diameter of 1.5 cm (min: 0.5, max: 5.0 cm). The average diameter of lesions detected by TCT in the remaining 9 patients, which were not visible on CXR, was 7 mm. The sensitivity and specificity of TCT and CXR were found to be 30.77% and 100%, respectively (Table 2). The positive predictive value and negative predictive value of CXR were 100% (39.76%-100%) and 77.50% (70.57%-83.19%), respectively.

Conclusion

Although current guidelines recommend TCT for screening, our study found that TCT did not lead to a change in the treatment plan for patients. The use of TCT in the staging of CRC patients may need to be reconsidered.

Keywords: Colorectal Cancer, Chest X-ray, Thoracic Computed Tomography

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Introduction

Colorectal carcinomas (CRC) are the third most common type of cancer worldwide, with 20% of patients being metastatic at the time of diagnosis (1). Among metastatic cases, only 1-9% involve lung metastases (2–4). In the diagnosis and follow-up of lung metastases, posteroanterior chest X-ray (PAACG), computed tomography (CT) (whether high-resolution or not), and positron emission tomography/computed tomography (PET/CT) are used, with CT having high sensitivity in detecting metastases (5). However, due to the relatively low incidence of lung metastasis in CRC patients, the use of thoracic computed tomography (TCT) is not highly recommended (2,6).

Another issue with TCT is that it detects nonspecific nodules in 20-30% of cases, which are rarely malignant (5,7). Consequently, the detection of nodules on TCT rarely leads to a change in the treatment paradigm (7). Therefore, the routine use of TCT for screening purposes in all CRC cases presents challenges such as time consumption, additional costs, and radiation exposure. Some studies have found TCT screening to be ineffective based on these factors (8,9). For similar reasons, PET/CT is recommended only in special circumstances, such as when CT, magnetic resonance imaging (MRI), and contrast agents are contraindicated, or to assess suspicious areas identified on CT/MRI (6).

At our center, routine TCT is requested for CRC patients by guideline recommendations. This study aims to evaluate the necessity and utility of imaging methods for detecting lung metastases in patients diagnosed and treated at our center.

Material and Method

In this study, patients admitted to our hospital between May 2019 and May 2023 for CRC were retrospectively screened. Ethical approval numbered E1-23-3547 was obtained from the Ethics Committee of the Hospital for this study. Demographic data, laboratory, radiological parameters, and pathology reports of the 630 patients admitted during this period were examined, and their stages were determined. The patients' treatment processes were then reviewed, and their follow-up records were examined.

The lung metastasis status of CRC patients was obtained from oncological follow-up records. The presence of lung metastasis was confirmed based on biopsy, CT, and/or PET/CT results. Thirteen patients with lung metastasis were classified as Group 1, and 31 patients without lung metastasis, identified using propensity score matching, were classified as Group 2, totaling 44 patients for analysis. The chest X-rays and computed tomographies of the patients were re-evaluated by a radiology specialist working specifically in this field. The location, size, and PET/CT involvement of the nodules in the radiological examinations were re-evaluated.

The clinical features, laboratory values, radiological examinations, and treatments of patients diagnosed with lung metastasis were examined in their subsequent follow-ups.

Statistics Analysis

Descriptive statistics were used to summarize the demographic and clinical characteristics of the patients. Continuous variables were expressed as mean ± standard deviation (SD) or median (IQR) depending on the distribution. Categorical variables were expressed as frequency and percentage. Data analysis was performed using SPSS (Statistical Package for Social Sciences) version 26.0. The normality of continuous variables was evaluated using the Shapiro-Wilk test. Descriptive statistics were presented to provide a comprehensive overview of the data. Since the study was observational and did not include group comparisons or hypothesis testing, no inferential statistical tests were performed.

Results

The average age of the 44 patients included in the study was 64 years (min: 42, max: 82), with 17 (38.6%) female and 27 (61.3%) male patients. The average age of patients in Group 1 was 60 years (min: 45, max: 73), and in Group 2, it was 65 years (min: 42, max: 82). When looking at the tumor localization of the patients, the tumor locations in Group 1 and Group 2 were as follows: cecum and ascending colon, transverse colon, descending colon, sigmoid colon, rectum; with 5 (38.5%) and 12 (38.3%), 0 (0%) and 1 (3.2%), 0 (0%) and 2 (6.4%), 1 (6.9%) and 4 (12.9%), 7 (53.4%) and 12 (38.3%) respectively. According to the preoperative CT TNM classification, the number of patients with T1 tumors in Group 1 and Group 2 were 0 (0%) and 3 (9.7%), T2 tumors were 0 (0%) and 2 (6.5%), T3 tumors were 7 (63.6%) and 20 (64.5%), and T4 tumors were 4 (36.4%) and 6 (19.4%) respectively. In the pathology specimens, Group 1 and 2 had 3 (27.3%) and 16 (51.6%) N0 patients, 6 (54.5%) and 9 (29.0%) N1 patients, 0 (0%) and 3 (9.7%) N2a patients, and 2 (18.2%) and 3 (9.7%) N2b patients respectively (Table 1). All patients with lung metastasis detected on preoperative scans also had liver metastasis.

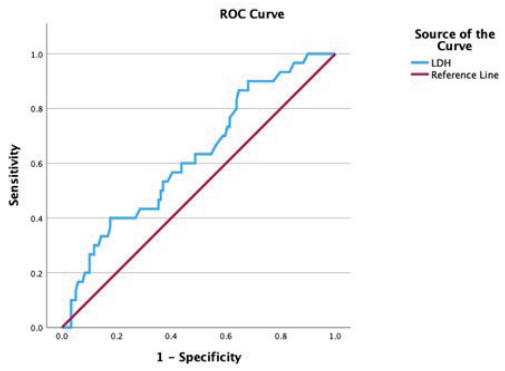

Figure 1
ROC analysis of LDH for Bowel Resection

Preoperative screenings revealed that metastatic lesions were detected by CXR in 4 out of 13 patients in Group 1, with an average lesion diameter of 1.5 cm (min: 0.5, max: 5.0 cm). The average diameter of lesions detected by TCT in the remaining 9 patients, which were not visible on CXR, was 7 mm. In two of the metastatic patients, the diagnosis was confirmed by biopsy, and the rest were confirmed to be metastatic by PET/CT. None of the patients with lung metastasis underwent lung resection. Nine of the patients underwent palliative surgery, and three symptomatic patients underwent palliative/resective surgery. The concordance analysis between TCT and CXR in detecting lung metastasis showed a kappa value of 0.760, indicating high concordance. The sensitivity and specificity of TCT and CXR were found to be 30.77% and 100%, respectively (Table 2). The positive predictive value and negative predictive value of CXR were 100% (39.76%-100%) and 77.50% (70.57%-83.19%), respectively (Figure 1).

Table 1 Demografic Characteristics of Patients

N		Lung Metastasis +					Lung Metastasis -				
		N	Mean	Min	Max	Column%	N	Mean	Min	Max	Column%
Age		13	60	45	73		31	65	42	82	
Gender	Female	7				53,8	10				32,3
	Male	6				46,2	21				63,7
Tumour Localisation	Rectum	2				15,4	10				32,3
	Rectosigmoid	5				38,5	2				6,5
	Hepatic Fleksura	0				0	7				22,6
	Sigmoid Colon	1				7,7	4				12,9
	Splenic Fleksura	4				30,8	1				3,2
	Caecum	1				7,7	4				12,9
	Ascenden Colon	0				0	1				3,2
	Descenden Colon	0				0	1				3,2
	Transvers Colon	0				0	1				3,2
T Stage	T1	0				0	3				9,7
	T2	0				0	2				6,5
	T3	7				63,6	20				64,5
	T4	4				36,4	6				19,4
N Stage	N0	3				27,3	16				51,6
	N1	6				54,5	9				29
	N2	0				0	3				9,7
	N3	2				18,2	3				9,7

Table 2 Cohens Kappa Statistics

Negative		X-Ray Metastasis		Total	Kappa	Sensitivite %	Spesifite %
		Negative	Positive				
TCT Metastasis	Negative	31	0	31	k:0.760	69.2	100
	Positive	4	9	13			
Total		35	9	44			

Discussion

Even though current guidelines and literature acknowledge the rarity of lung metastasis and the lack of impact of thoracic computed tomography (TCT) on treatment modalities, TCT is still recommended for preoperative staging (6,10,11). However, in this study, it was observed that TCT did not have any effect on surgical treatment strategies at the time of initial diagnosis.

The primary goal of staging in colorectal cancer (CRC) and other cancers is to select the most appropriate treatment method for the patient (7). TCT has been reported to detect metastases with high sensitivity (73%) and specificity (74%) (12–14). This is attributed to TCT's ability to reveal even 2-3 mm nodules in appropriate slices of the lung. The problems that arise here are that TCT also shows nonmetastatic and insignificant lesions in the lungs and that these small lesions are not always easily identifiable, necessitating additional procedures (5,14). In patients with indeterminate lung lesions, three different approaches are followed: (1) incisional or excisional biopsy of the lesion, (2) performing PET/CT, and (3) follow-up with re-evaluation when the lesion exceeds 1 cm (3,15,16). Most CRC patients with lung metastasis already have liver metastasis, initially receive adjuvant chemotherapy, and only 21.1-32.5% undergo metastasectomy (6,17,18). Additionally, the treatment plan for patients with lung metastasis rarely changes, and definitive treatment for lung metastasis is typically received by the patient only after a certain period (3,7,19). In the study conducted by Grosman et al. (7), out of 200 patients, lung metastasectomy was performed in only 2 patients ten months after the initial diagnosis. Similarly, in a study by Lazzoron et al. (3) involving 223 patients, metastasectomy was performed in only 5 patients, on average, one year later. In the present study, TCT was performed on all 630 patients, but only 13 had lung metastasis, and these patients continued with adjuvant chemotherapy, with no lung metastasectomy performed.

In colorectal cancers, the rate of lung metastasis without liver metastasis is reported to be 5-6%, while in rectal cancers, this rate is reported to be 10-18% (7,19,20). In the study conducted by Kim et al. (21), it was reported that patients with lymph node metastasis might have lung metastasis without liver metastasis. Contrary to the literature, in this study, all patients with lung metastasis had liver metastasis, and no significant difference was found between rectum-rectosigmoid cancers and other colon segments regarding lung metastases. In another study conducted by Brent et al. (5), 439 patients were prospectively examined, and among them, 45 had indeterminate pulmonary nodules, with only 5 of those having lung metastasis. All patients with lung metastasis were reported as N1 or N2. In another study conducted by Kim Hye et al. (19), it was reported that the rate of pulmonary metastasis was higher in T3-T4 tumors and in cases with positive lymph nodes. In the study by Lazzoron et al. (3), the risk of lung metastasis was reported to be 11 times higher in patients with T4 tumors compared to those with T1 tumors. Although Hogan et al. (2) found no statistical difference between T stage and lung metastasis in their study, the rate of lung metastasis was higher in T3-T4 tumors, and the rate of lung metastasis was statistically higher in cases with lymph node metastasis. In the present study, all thirteen patients with lung metastasis had T3-T4 tumors, but five had no suspicious lymph nodes in their preoperative imaging.

Although posteroanterior chest X-ray (PAACG) is considered a primitive examination, it can detect lesions as small as 7-8 mm and identify them with a

sensitivity of 33-73% (12,13,19). These lesions have a false negative rate of about 20%. However, this rate can be reduced further with repeated imaging (22,23). Despite this disadvantage of PAACG, in a study by McIntosh involving 403 CRC patients, only 3 out of 7 patients with lung metastasis were not detected by PAACG (24). In another study by Povoski involving 100 patients, only 4 out of 11 patients with lung metastasis were not detected by PAACG (12). In another study by Kronawitter, 202 patients with negative PAACG were examined, and 10 of them were found to have lung metastasis on TCT (25). Similarly, in this study, only 4 out of 13 patients with lung metastasis were not detected by PAACG, and the sensitivity rate was found to be 69.2%.

Although TCT does not change the treatment plan at the time of initial diagnosis, lung metastasis usually emerges approximately fifteen months after the initial diagnosis (26). During this period and afterward, it is recommended that patients be followed up with computed tomography. The use of TCT in diagnosis and follow-up increases the radiation exposure that the patient receives, and when contrast-enhanced scans are performed, there is a risk of contrast nephropathy and contrast allergy. Requesting TCT in patients with T3-T4 tumors, those with lymph node metastasis (radiologically or pathologically), and after positive findings on PAACG will not only reduce these types of complications but also unnecessary costs.

The most significant limitation of this study is that it is a retrospective study, and as a result, some information may be missing, and the sample size is limited. Another limitation is the small number of patients diagnosed with lung metastasis. Therefore, the number of patients without lung metastasis to be compared was kept small for the propensity score match analysis. Determining the necessity and cost-performance relationship of TCT with prospective randomized studies would be more valuable.

Conclusion

Although current guidelines recommend thoracic computed tomography (TCT) for screening purposes, the detection of indeterminate nodules and the fact that lung metastasis rarely alters the treatment plan, coupled with the observation that most nodules detected on TCT are also visible on posteroanterior chest X-ray (PAACG), make the use of TCT as a first-line investigation still problematic. It may be considered to request TCT as an additional investigation in patients who have nodules detected on PAACG, those with T3-T4 tumors, and those with liver metastasis, to minimize radiation exposure.

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

Ethical Approval
Ankara Bilkent City Hospital Ethic Committee 10.05.2023/ E1-23-3547.The study was conducted by the principles outlined in the Declaration of Helsinki.

Consent to Participate and Publish
Because of studies retrospective nature, there is no consent.

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

Authors Contributions
HFM: Conceptualization (lead), data curation (lead), investigation (lead), methodology (lead), project administration (lead), resources (lead), supervision (lead), writing-original draft (lead) and writing-review and editing (lead);

FS: Conceptualization (equal), resources (supporting), software (equal) and visualization (supporting), writing-original draft,

ECİ: Conceptualization (equal), resources (supporting), software (equal) and visualization (supporting), writing-original draft,

BCD.: Conceptualization (equal), resources (supporting), software (equal) and visualization (supporting), writing-original draft,

MT: Conceptualization, resources (supporting), software (equal) and visualization (supporting), writing-original draft,

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ORIGINAL RESEARCH

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Respiratory School (Smoke and COPD)

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Abstract

Objective

Within the scope of TUBITAK Nature and Science Schools Projects, it aimed to provide training for teachers working in schools connected to National Education in Isparta Province to raise awareness about smoking and harms.

Material and Method

Two hundred and thirty-one teachers (112 (48.48%) men and 119 (51.52%) women) participated in the training. A pre-test and post-test control group-free experimental study design was applied to measure training effectiveness during the Respiratory School project. At the end of the training, participants were asked to write their positive and negative feedback about the project using the so-called critical events technique. The questionnaire included information on satisfaction with education and trainers, demographic characteristics and thoughts about smoking, and the effect of education. A total of 196 participants completed the questionnaire.

Results

Most participants were women (52.9%) aged 36 to 45 (46.2%). Most of the participants had teaching experience between 16 and 25 years. There was a

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significant improvement in correct answers between the pre-test and the post-test on all training days ($p < 0.001$). The Cronbach's alpha was 0.904, and the average response to training questions was 4.68. The reliability of our findings is underscored by a Cronbach alpha value of 0.969, with a mean expression score of 4.77, highlighting the reliability of the participants' responses. Approximately 26% of the participants (n=44) reported smoking. However, the incidence of smoking among family members of the participants was markedly higher, 47.9% (n=79). Similarly, the proportion of participants who had a spouse smoking was noteworthy (31.4%; n=48). had attempted to quit smoking at some point, but only three participants (5.8%) sought help in their quitting efforts. Of the participants, 75% (n=33) acknowledged that they had quit smoking but later resumed.

Conclusion
The teachers who participated in the training found it helpful and increased targeted awareness.

Keywords: Respiratory school, Smoke, COPD, CET

Introduction

The main task of the respiratory system is to provide the oxygen needed by the organism from the atmosphere and remove the released carbon dioxide from the organism. The respiratory system comprises the respiratory organ (lungs) and the respiratory tract (1).

All tobacco products, especially cigarettes, such as cigars, pipes, cigarettes, and hookahs for tobacco use, show similar effects and damages. New products thought to be less harmful or harmless than tobacco in the mid-20th century have been developed. These include cigarette types (light, mild, ultralight, etc.), smoke-like products that produce smoke, and smokeless tobacco products. However, they have been shown to have similar effects and harms (2-5). Cigarettes include tobacco, paper, filter parts, additives, pesticides, fertilizers, fumigants, and processing agents used during fabrication. As a result, more than 4000 harmful substances are found in cigarette smoke. In 1985, the International Agency for Research on Cancer adopted cigarette smoke as the first group of carcinogens for human beings. It is crucial to understand that the substances in the smoke are not just harmful, but are pharmacologically active, mutagenic, or toxic (6, 7).

Maraş grass indisputably increases the risk of oral and pharyngeal cancers, just like other smokeless tobacco products. Numerous studies confirm the link between smokeless tobacco use and cancer. According to a study published in 2008, the risk of oral, pharyngeal, pancreatic, esophagus, and lung cancer increases (8-10).

The most significant harm caused by smoking to human health is to the lungs, and is often a cause of chronic obstructive lung disease. Chronic Obstructive Pulmonary Disease (COPD), according to the Global Strategy for the Diagnosis, Management, and Prevention of Chronic Obstructive Pulmonary Disease 2025 report, is a heterogeneous lung condition characterized by chronic respiratory systems (dyspnea, cough, sputum production and/or exacerbations) due to abnormalities of the airways (bronchitis, bronchiolitis) and/or alveoli (emphysema) that cause persistent, often progressive, airflow obstruction (11). COPD is one of the leading causes of death in most countries. For example, it was the third leading cause of death in the United States. It is estimated that around three million deaths annually due to COPD worldwide, and the increased prevalence of smoking in low- and middle-income countries (LMIC), combined with aging populations in high-income countries, will lead to more than 5.4 million annual deaths from COPD and related conditions by 2060 (12).). In a study investigating mortality from COPD in our country, the mortality rate in 427 COPD patients followed for four years was 17.3% (13). As a result, COPD is a public health problem that has a high mortality, morbidity, and economic burden for our country and all over the world.

A large volume of data has been accumulated on tobacco and health issues around the world. Numerous studies have been published that support the strong association of tobacco use with various adverse human health effects, most prominently with cancer, cardiovascular disease, and COPD. Although susceptibility to smoking-related flow restriction is rather individual due to the interaction between environmental factors and the host in patients with COPD, cessation of smoking is the primary treatment to prevent the development and progression of COPD (14). Therefore, smokers should be encouraged to quit. We believe that informing our teachers, who are important role models in society regarding smoking

and COPD, will contribute to the students. As the most critical risk factor in the development of COPD in the respiratory school project, the results of smoking were reported with theoretical and practical applications.

Material And Method

The project is not only the transfer of knowledge and skills, but also social sensitivity, health, quality of life, and role models. The project involves transferring knowledge, skills, and social sensitivity to improve health, and quality of life, and provide role models.

There is an agreement outlining a project involving 250 teachers, which includes both smoking and non-smoking educators. The target audience will be selected by the Research and Development Unit of the Isparta National Education Directorate, a partner institution with the state secondary schools in the region. The Research and Development Unit has determined half of the teachers will participate in the training. The Research and Development Unit hung the project poster in a visible place in the Directorate of National Education and Schools, and it turned the poster until the end of the project. The selected target groups for this initiative are secondary and high school teachers. The project aims to empower teachers to become role models in society by increasing their awareness of smoking and related diseases. It seeks to ground this awareness in scientific knowledge, thereby enhancing the project's impact and effectiveness. After the project, it is expected that teachers, who are the target audience, will have a positive change in their perspective on smoking, gain empathic thinking skills, and increase their knowledge and understanding of the long-term effects of smoking. This change aims to measure and evaluate how the gains and expectations will be realized;

1. To measure the effectiveness of training given during the 'Respiratory School' project process, an experimental study design without a pre-test and a post-test control group was applied. A total of 30 questions were prepared during the day. The pretest-posttest questions were formed from a total of 8 different training contents. Participants were asked questions before and after the start of the training. For five days, tests were performed on 206 participants.

2. At the end of the training, the participants of the Respiratory School project were asked to write their positive and negative feedback about the project using a method called the critical incidents technique. The project activity was evaluated with this form.

3. A questionnaire was applied to the participants to test the satisfaction of the training and effectiveness of the training during the 3rd Respiratory School project. The questionnaire consisted of two main sections. The first part consisted of expressions of satisfaction about education and trainers. After the completion of the training programs, a survey was conducted at the end of the day. A total of 196 participants completed the questionnaire. Less than 20% of participants were present in the sessions held on Monday and Thursday, and more than 20% on other days.

4. Poster and poster works were made.

5. In addition, the bag activity law and the smoking bans applied in our country and the world were taught.

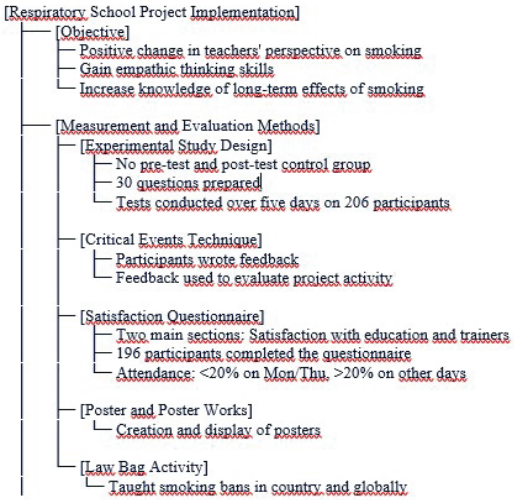

Figure 1
Method as a flow chart

As a result, practical and theoretical information about smoking and chronic diseases was conveyed to teachers within the scope of the project. Therefore, changes and gains in teachers' sensitivity and awareness, empathic thinking, social skills, and characteristics of entrepreneurship were measured and evaluated with the methods mentioned above.

At the end of the project, the training materials were shared in printed and digital media for the teachers who participated in the project to make the gains permanent for the participants and develop. Materials to be provided in relation to the project: general information, case studies, articles, events, videos, etc. The topics will be shared, and brochures, books, and training material will also be provided.

Statistical analysis of the study was performed with the SPSS 20.0 program. Descriptive measures were presented as frequency (percentage) for categorical and mean±SD (median, min-max) for numerical variables. The normality assumption was checked by Kolmogorov-Smirnov test, and the scores were distributed normally. Paired sample Student t-test was used for comparison of two dependent groups for pre and post-test scores, and one-way analysis of variance (ANOVA) was used for comparison of multiple groups. Pairwise comparisons of significant results were performed using the Tukey HSD post-hoc test. In all analyses, $p<0.05$ was considered statistically significant result.

Results

Most of the participants were women (52.9%) aged 36 to 45 (46.2%). The study found that 16.4% of participants were between 25 and 35 years old, whereas 37.4% were between 46 and 65. The teaching experience differed between 11-25 years, where 68.2% had 16-25 years, and 14.7% had 11-15 years of experience. Most participants (94.7%) were married. Approximately one-quarter of the participants (26%) reported being smokers; however, the rate of smoking within their families was higher at 47.9%. Additionally, 31.4% had a spouse. Among those who had used or were currently using cigarettes, more than half (57.4%) expressed a desire to quit smoking. However, only 3 participants (5.8%) sought help to quit. Among the participants who had previously stopped smoking, 75% indicated that they had resumed smoking. The reasons for restarting smoking included psychological factors (45.5%), environmental influences (33.3%), and physical factors (21.2%).

A questionnaire was applied to the participants to test their satisfaction with the training and effectiveness of the training during the “Respiratory School” project. A total of 196 participants completed the questionnaire. Less than 20% of the participants were present at the sessions held on Monday and Thursday, and more than 20% on other days.

Questions were asked about their status of smoking. A total of 40 participants answered these questions. The items 'I don't intend to quit in the next 6 months' and 'I intend to smoke in the future, but I intend to reduce it' had equal (27.5%) ratios. The item 'I am planning to quit within 30 days' was selected by 10 participants (25%). The item 'I can stop in 6 months, but not in 1 month' was selected by 6 participants (15%), while only 2 participants (5%) preferred 'I have no intention to reduce.' For the items on the effectiveness of the training in the project, 86.8% of the participants chose 'I increased my level of knowledge,' 82.3% chose 'I think I should keep my students away from smoking as much as possible,' 15.8% of the implied 'I started to give up smoking.' One participant answered, 'I have no expectations.' 9.2% of all participants had alcohol use, and only one participant stopped using it.

The mean and median smoking age was 20.44 ± 3.98 (20) years. The monthly expenditure on cigarette consumption was 241,27 ± 186,88 (200) TRY. On the other hand, the answer was 2.5 ± 1.38 times for the question of how many times smoking was stopped before.

The average values of the correct pretest and posttest answers were 14.61 and 22.78, respectively. The difference between the correct pretest and posttest answers was significant on all training days ($p <0.001$). Similarly, the difference between the pretest and posttest scores was significant. The final test scores were higher. The mean score of the final test was 75.95, and the mean of the pretest was 48.72. The difference between the pretest scores on Tuesday and Thursday was significant ($p = 0.020$). However, Wednesday and Friday were the days when the difference between the final test scores was significant ($p <0.001$). Information showing the descriptive measures of the correct answers and scores from the pretest and after the test according to the training days is presented in Tables 1 and 2. The pretest and posttest scores according to days are presented in Figure 2 and Figure 9.

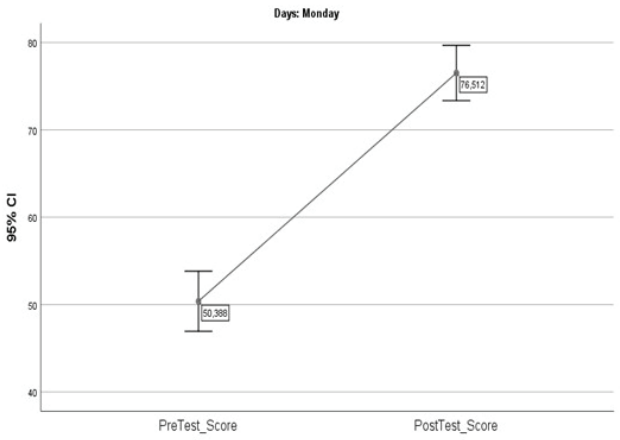

Figure 2
Pre- and post-test scores of Monday training

The training satisfaction section included 10 items. Participants reported an average satisfaction score of 4.68. The reliability of these items was high, with a Cronbach's alpha of 0.904. In addition, there were

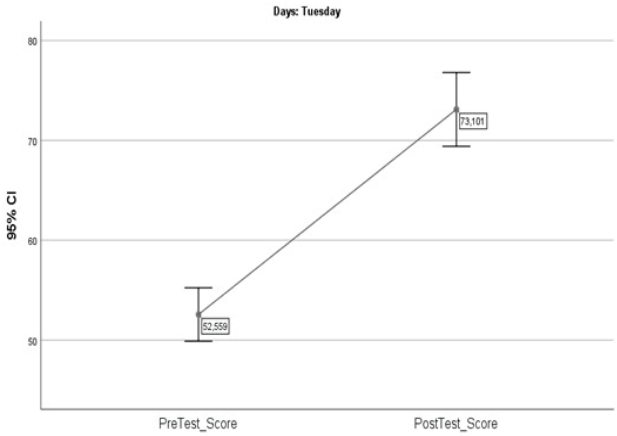

Figure 3
Pre- and post-test scores of Tuesday training

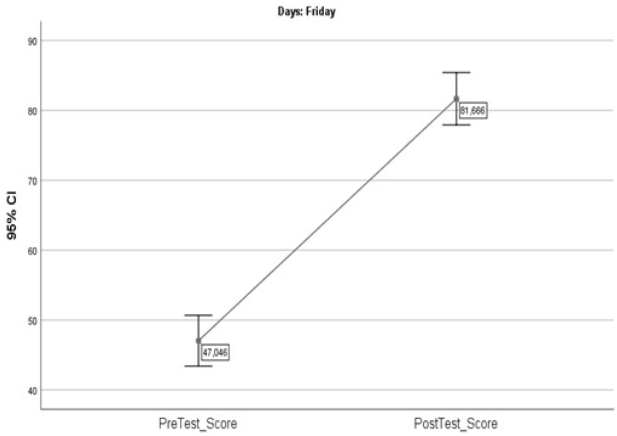

Figure 6
Pre- and post-test scores of Friday training

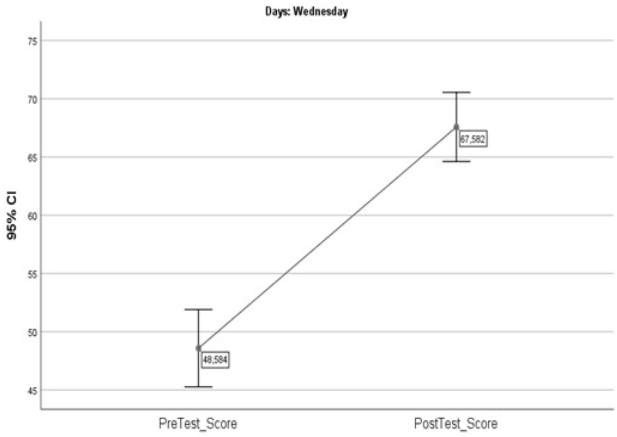

Figure 4
Pre- and post-test scores of Wednesday training

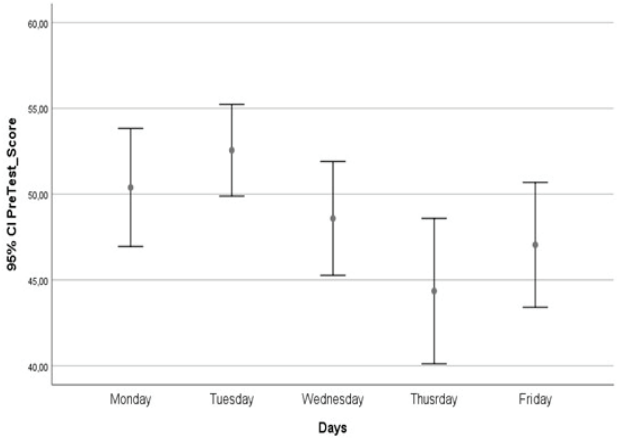

Figure 7
Pre-test scores according to days

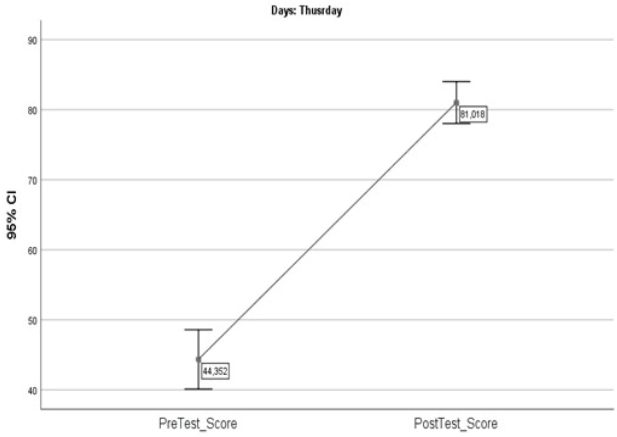

Figure 5
Pre- and post-test scores of Thursday training

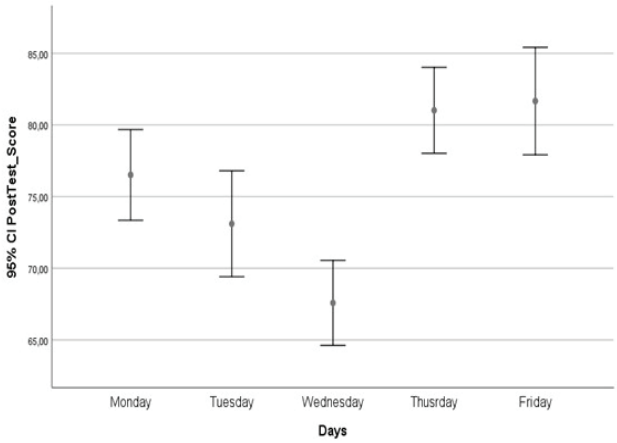

Figure 8
Post-test scores according to days

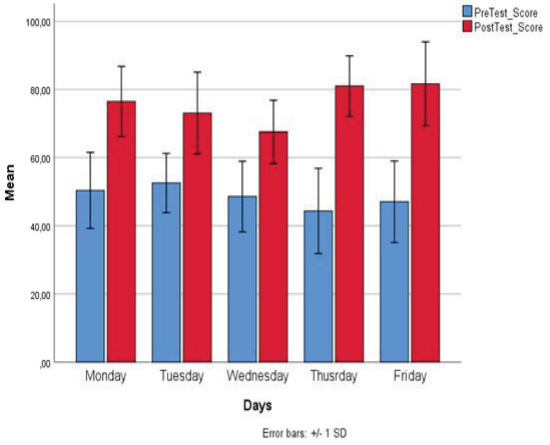

Figure 9
Pre- and post-test scores according to days

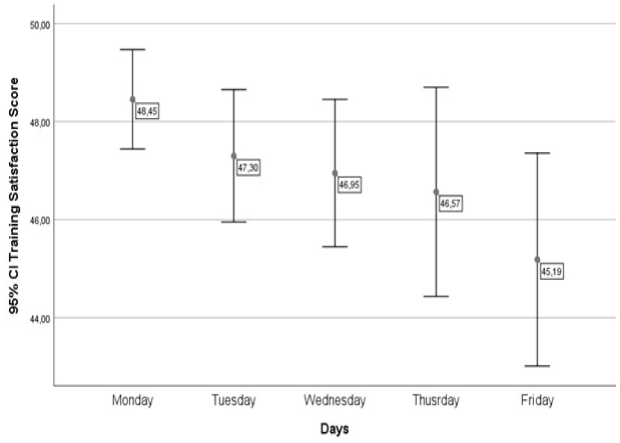

Figure 10
Training satisfaction scores according to days

Table 1 Pre- and Post-test correct answers according to training days

Training day		Pre-test correct answers	Post-test correct answers	$P_{re\text{-}post\text{-}test}$
Monday	Participant number	43	43	<0.001
	Mean±SD	15.11±3.35	22.95±3.08	
	Median, min, max	16. 8. 21	23. 15. 28	
Tuesday	Participant number	43*	43	<0.001
	Mean±SD	15.76±2.60	21.93±3.60	
	Median, min, max	16. 10. 21	22. 6. 27	
Wednesday	Participant number	40	40**	<0.001
	Mean±SD	14.57±3.11	20.27±2.78	
	Median, min, max	15. 8. 20	21. 10. 25	
Thursday	Participant number	36*	36	<0.001
	Mean±SD	13.30±3.75	24.30±2.65	
	Median, min, max	13. 7. 20	25. 17. 28	
Friday	Participant number	44	44**	<0.001
	Mean±SD	14.11±3.59	24.50±3.70	
	Median, min, max	14. 5. 21	25. 11. 29	
Total	Participant number	206	206	
	Mean±SD	14.61±3.36	22.78±3.54	
	Median, min, max	15. 5. 21	23. 6. 29	
	P_{days}	0.020	<0.001	

SD: Standard deviation,
*: the significance of Pre-test correct answers between days
**: the significance of Post-test correct answers between days

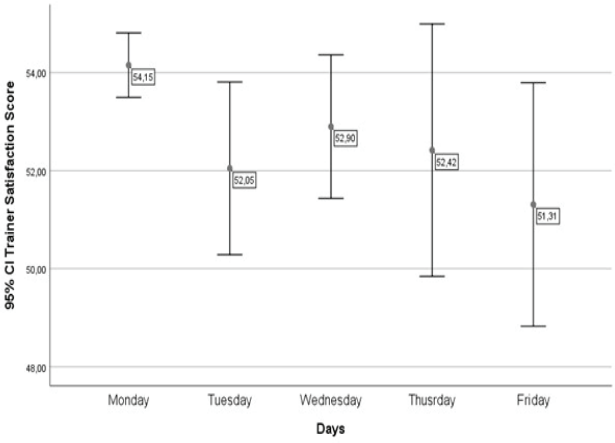

Figure 11
Trainer satisfaction scores according to days

11 items related to the trainers, which also received a relatively high average satisfaction score of 4.77, with Cronbach's alpha of 0.969. No significant differences were observed in satisfaction scores between different groups on various days. Generally, the average scores were quite similar. On the first day of the week, the educational satisfaction score was the highest, but it tended to decline as the week progressed. The trend for the trainers' satisfaction was similar, although participants reported higher satisfaction on Wednesday compared to Tuesday. Table 3 presents the satisfaction scores, and smoking characteristics based on the training days. Figures 10 and 11 illustrate these trends more clearly. The participants expressed positive opinions 92% about the project. These are:

Table 2 Pre- and Post-test scores according to training days

Training day		**Pre-test scores**	**Post-test scores**	**$P_{re\text{-}post\text{-}test}$**
Monday	Participant number	43	43	<0.001
	Mean±SD	50.38±11.17	76.51±10.28	
	Median, min, max	16. 8. 21	23. 15. 28	
Tuesday	Participant number	43*	43	<0.001
	Mean±SD	52.55±8.69	73.10±12.00	
	Median, min, max	16. 10. 21	22. 6. 27	
Wednesday	Participant number	40	40**	<0.001
	Mean±SD	48.58±10.37	67.58±9.27	
	Median, min, max	15. 8. 20	21. 10. 25	
Thursday	Participant number	36*	36	<0.001
	Mean±SD	44.35±12.51	81.01±8.86	
	Median, min, max	13. 7. 20	25. 17. 28	
Friday	Participant number	44	44**	<0.001
	Mean±SD	47.04±11.96	81.66±12.33	
	Median, min, max	14. 5. 21	25. 11. 29	
Total	Participant number	206	206	
	Mean±SD	48.72±11.22	75.95±11.82	
	Median, min, max	15. 5. 21	23. 6. 29	
	P_{days}	0.020	<0.001	

SD: Standard deviation,
*: the significance of Pre-test correct answers between days
**: the significance of Post-test correct answers between days

Table 3 Training satisfaction scores according to the days

		Training satisfaction scores	Trainers satisfaction scores	Smoking beginning age (year)	Monthly expenditure (TRY)	Smoking gives up trials (times)
Days		**Mean±SD**				
Monday sessions		48,45±2,86	54,15±1,85	18,66±4,27	307,14±142,67	2,28±1,38
Tuesday sessions		47,30±4,38	52,04±5,72	20,75±2060	228,57±191,17	2,33±1,15
Wednesday sessions		46,95±4,70	52,89±4,51	21,00±3,53	154,28±115,20	3,30±1,15
Thursday sessions		45,56±6,39	52,41±7,60	18,54±3,04	222,0±147,72	1,66±1,21
Friday sessions		45,19±7,05	51,30±7,96	22,36±5,27	335,83±275,76	2,25±1,89
Total		46,82±5,41	52,48±6,06	20,44±3,98	241,27±186,88	2,50±1,38
	p	0,255	0,206			

- has increased awareness of cigarette and addiction,

- decided to make activities related to smoking,

- in which the training and the trainers were sufficient and sufficient visual,

- in which auditory and experimental materials were used in education,

- corrected wrongs as known correctly,

- has understood the damages of cigarettes to all systems,

- was well planned and education was sufficient,

- in which the drama activities make the activity more effective,

- was composed of teamwork and well academic level,

- in which the education materials contributed to the given training,

- can be applied to different participant groups.

Moreover, negative opinions as 8% were found, such as:

- insufficient transfer of education to students,

- lack of concrete examples,

- lack of active participation,

- games and practices,

- re-explaining some issues,

- long-term and long words in the Latin language,

- insufficient physical conditions in the training room,

- inadequate visual materials.

Discussion

The most important result of the Respiratory School project was to raise awareness among teachers about the development of COPD and changes in quality of life in long-term smoking in the context of the relationship between the respiratory system and smoking. By reaching the aim of theoretical and practical training given during education, the negative effects of smoking were transferred to teachers who were role models in society, and they have copied this role.

In a study conducted by Perincek G among teachers; The age at first smoking was 17.5±3.5 years and the most common reason for starting smoking was curiosity (57%). Nicotine use among participants who smoked addiction was very low (43.5%). In our study, 26% of teachers smoked. In this context, the high level of smoking among prospective teachers may give the impression that the number of smoking teachers will be high in the future. Smoking was also found to be high among students who smoke in their families. In our study, the rate of smoking was high in the families of teachers who smoked (15).

In a study of primary school teachers investigating smoking prevalence and factors affecting smoking prevalence, 36.7% (n=793) of all participants were found to be smokers. The mean age at first smoking was 18.2±4.5 years and the mean duration of smoking was 18.5±8.5 years. The prevalence of smoking cessation was 28.8% and the prevalence of smoking was higher in men than in women (16). In the study conducted by Gencer et al., to determine smoking habits and behavioral behaviors related to this habit among teachers, a questionnaire about smoking habits was applied to 172 teachers working in a primary school. 50 (29.1%) of the teachers who participated in the survey smoked (17). In another study, 860 teachers who work in 21 primary schools in the city center of Kayseri were selected using a stratified random sampling method from 210 public schools and agreed to participate in the study to determine their behaviors and opinions. 31.5% of the teachers smoked, 54.2% of the teachers smoked more than 11 cigarettes per day, and 93.7% of the teachers smoked in the school areas. Of the teachers included in the study, 69.8% of those who informed their students about the health damages of cigarettes, and those who knew about the damages of cigarettes, 69.7% of those who knew about the law numbered 4207, 69.2% of those who thought it was the responsibility of the teacher to reduce the smoking rate. It was found that 75.2% of the participants in the seminar on the harms of smoking do not smoke. The study determined that non-smoking teachers had more positive attitudes towards smoking by students (18). Similarly, in our research, non-smokers focused on education, and increased awareness levels were higher.

The attitudes of teachers about the ban on smoking both indoors and in gardens of educational institutions were examined. The attitudes of teachers about the ban on smoking in educational institutions indoors and in gardens were examined. The change in smoking behavior of 545 (34.6%) teachers who answered two questionnaires was evaluated by all teachers of 33 high schools in the central district of Denizli before the law (May 2008) and one year after (September 2009). At the end of one year, the teachers' quitting rate was 20.8% in the education group and 12.2% in the control group. Variables thought to be effective in smoking cessation: teachers' gender, age, smoking cessation education, dependence levels, smoking status of their spouses, and whether they support the law on smoking were analyzed (19). Coşkun et al. determined the smoking levels of teachers in Bursa and their opinions about the law prohibiting smoking in indoor areas, which started to be implemented in our country. Twenty-two questionnaire forms were distributed to all schools in Bursa. A total of 8291 teachers were included in the study. The smoking ratio of the males (n=3519, mean age 40.6±0.1) was 33.6%, and for the females (n=4772, mean age 34.9±0.1) was 25.4%. In response to the question ‘What do you think about the prohibition of smoking in public indoor areas according to the Law on the Prevention and Control of Harms of Tobacco Products, which entered into force on 19 May 2008?’, 75.8% of teachers answered it as a necessary law, while 14.3% stated they found the law unnecessary (20). When we compare the findings of these studies with the data from the Respiratory School project, they are in the same direction. In the Respiratory School project, branch and elementary school teachers were trained indiscriminately. Furthermore, the fact that the smoking rate among teachers is considerably high reveals how accurate it is in terms of the selection of the target audience in the project. In another study evaluating smoking habits among teachers in schools, the mean age of the participants was 38.9 ± 8.9 years. When smoking habits were evaluated, it was found that 291 (44.1%) were smokers, 252 (38.2%) were nonsmokers, and 117 (17.7%) had stopped smoking. 43.2% of women and 44.8% of men were smoking. The data in the School of Respiration project are in the same direction, which makes it inevitable that the target audience should be teachers (21).

Within the scope of a project called 'Preventing Substance Addiction in Schools', guidance training, class/branch teachers, and informing about substance use and addiction. 508 guidance teachers, 2599 class / branch teachers, and 284 parents who participated in the training carried out within the scope of the Drug Addiction Prevention Project in schools were administered the first post test. The effectiveness of the training was measured by examining the difference between the total line numbers of the first test and the total line numbers of the last test. To check whether completion of the first test affected the effectiveness of the training, only the final test was applied to 15% of the participants in each training. The total number of questions correctly answered by the guidance

counselor, the class / branch teacher, and parents in the first and last tests increased. The highest increase in the number of correctly answered questions was observed between the first and last tests by class / branch teachers, parents, and guidance teachers, respectively. The difference between the groups was found to be statistically significant when the mean increase in the number of lines obtained in the first test was compared with each other. As the project output, it has been shown that the applied training increases the knowledge level of guidance teachers, class/ branch teachers, and parents (22). Similar results were obtained in the Respiratory School project on cigarette addiction, and the difference between the first and last test scores of the teachers was significant. In particular, the effectiveness of the training was demonstrated by the first and last results of the test and other awareness surveys.

The training program implemented within the scope of the 'Respiratory School' project has achieved its goal. At the end of the training, it was found that participants gained more knowledge and behavior. Although approximately one-quarter of the participants were smoking, most of the participants, except for a small number of participants, were determined to quit smoking in the short term. The social aspects of such projects should be applied to all segments of society, considering the power of society, and contribute to our fight against smoking, which steals our silent and insidious health.

Since the post-test was conducted immediately after the training, the recall factor may have affected the findings of the study. This was taken into consideration as a limitation of the study.

Suggestions
Carrying out the training to the vocational high schools and the families of the students there. To be held in a different and wider time than the seminar period. Raising awareness as a subject in the local and national press. Repeating this training to teachers in certain time periods. Dissemination in Turkey. Education of patients with COPD is planned for the continuation of the project. Repeating this training in schools with lung models. Choosing secondary and high schools as target groups.

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

Ethical approval
This article does not contain any studies with human or animal subjects.

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Data available on request from the authors.

Authors Contributions
MS: Project administration; Conceptualization; Data curation; Formal analysis; Investigation; Methodology; Validation; Visualization; Original writing draft.

OO: Data curation; Formal analysis; Investigation; Methodology; Validation.

OC: Methodology; Validation; Visualization.

OO: Investigation; Methodology; Project administration; Resources; Supervision; Validation.

IG: Formal analysis; Investigation; Visualization; Writing-original draft.

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ORIGINAL RESEARCH

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Analysis of the Accuracy and Quality of Information in YouTube Videos on Female Urethroplasty

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Abstract

Objective

Bladder outlet obstruction is a relatively rare problem in women. Experience in diagnosing and managing female urethral stricture disease is limited. The literature emphasizes that urethral dilatation is usually not successful, but surgical reconstruction is successful. Physicians and patients want to obtain information from social media because surgery cannot be performed in every center. With this study, we aimed to make an integrative review of the quality of the information in YouTube videos in the field of female urethroplasty.

Material and Method

Using the keyword female urethroplasty, we searched https://www.youtube.com/ for the last 5 years. Video features, quality, and reliability analysis were performed. The reliability and quality of the medical information in the videos were determined by the JAMA (Journal of American Medical Association) scoring system, the Global Quality Score (GQS), and the DISCERN questionnaire.

Results

39 videos out of a total of 49 were included in the study. India was the country with the most video uploads (43.6%). There was no significant correlation between DISCERN score and the number of views and video comment count (rho;-0.101 p=0.539, rho;0.018 p=0.924, respectively). The DISCERN score, GQS, and JAMA scores of videos from Academic institutions or societies were found to be higher than personal videos (p=0.037, p=0.037, p=0.001, respectively).

Conclusion

Although there are limited videos about female urethroplasty on YouTube, the accuracy and reliability of the medical information in the most watched videos are low.

Keywords: Female urethroplasty, Social media, Urethroplasty, YouTube

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Introduction

In women, lower urinary tract symptoms (LUTS) primarily arise from storage problems, and bladder outlet obstruction (BOO) is a relatively rare issue in women (1). Female urethral stricture disease (FUSD) is a rare entity reported in approximately 1% to 4.9% of women presenting with lower urinary tract symptoms (LUTS) (2, 3). Even those in the field of education have very limited experience in diagnosing and managing female urethral strictures (4, 5). While endoscopic and open surgical techniques are available for the treatment of recurrent female urethral strictures, the literature emphasizes that urethral dilation is usually unsuccessful, while surgical reconstruction is successful (4). Many patients consult by searching the internet to manage their health issues, determine whether they need professional assistance, and verify information provided during clinical consultations. In recent years, the increased accessibility to the internet has contributed to the rise in the use of web resources for accessing medical information (6). According to a survey conducted by the American Urological Association, 74% of urologists reported using social media platforms such as Facebook and YouTube, with 33% stating that it influenced their clinical practices (7). Particularly, YouTube has emerged as a potential resource for obtaining and disseminating health-related information, surpassing traditional text-based information sources (8). While a recent survey indicated that 86% of individuals who use the internet for health information believe that online health information is reliable, it is important to note that the quality of health-related information on the internet varies significantly and is often uncontrolled and unregulated (9). The aim of this study was to conduct a comprehensive review of the quality of information currently available in YouTube videos related to female urethroplasty.

Material and Method

On February 16, 2023, we searched https://www.youtube.com/ using the keyword "female urethroplasty" and listed the videos added in the last 5 years. Videos that were not related to female urethroplasty, not in English and those that were for commercial/advertisement purposes were excluded from the study.

Ethics Approval

Since all the videos were publicly available on the social media website (YouTube.com) and no human or animal participants were involved in the study, ethical approval was not required.

Video Characteristics, Quality, and Reliability Analysis

The videos were categorized based on the following criteria: video length (seconds), time since upload on YouTube (days), number of views, view rate (number of views/number of days since upload), number of comments, video source, target audience, language format, and categorization of video content.

The categorization of the video source was divided into two groups: academic institutions or associations, and personal uploaders. The target audience of the videos was categorized into two groups: physicians and patients. The categorization of video content was divided into three groups: theoretical, practical, and theoretical+practical. The rate of video likes wasn't calculated because YouTube removed the public dislike count from all videos in November 2021. So Video Power Index wasn't calculated like the rate of video likes.

Once included videos were listed, they were independently analyzed and scored by researchers trained in female urethroplasty. In video scoring, a common score was given by achieving moderate consensus.

The evaluated videos were assessed using the JAMA (Journal of the American Medical Association) scoring system, which was developed by Silberg et al. (10) to assess the quality of online information based on four criteria: authorship, attribution, disclosure, and currency. Additionally, the videos were evaluated using the Global Quality Score (GQS), a Likert scale ranging from 0 to 5, which was developed by Singh et al. (8) to analyze the usefulness of web publications for patients. The GQS scored the videos based on their educational value. These evaluation methods were utilized to determine the accuracy, reliability, and overall quality of the medical information presented in the videos. These two scoring systems allow for a non-specific evaluation of health-related websites (11). The quality and reliability of treatment options information provided by patients and information providers in health-related videos were assessed using the DISCERN questionnaire, developed by Charnock et al. (12) The DISCERN questionnaire consists of 15 questions, with each question being scored on a scale of 1 to 5.

Statistical Analysis

In the statistical analysis of the study results, the Statistical Package for the Social Sciences version 22.0 software (SPSS Inc., Chicago, IL, USA) was used. Median, minimum, maximum, number, and

percentage were used as descriptive methods. The Shapiro-Wilk test was performed to evaluate the normality of the distribution. The chi-square test was used for comparing categorical variables. The Kruskal-Wallis test was used to compare the means, and the Mann-Whitney U test was used to determine the group that caused the difference. Pearson and Spearman rho correlation analyses were used to evaluate the correlation between parameters. A significance level of $p<0.05$ was considered statistically significant.

Results

Between February 16, 2023, and February 16, 2018, a total of 49 videos were listed on YouTube using the keyword "Female Urethroplasty." Out of these, 8 videos were in a language other than English and were excluded from the study. Additionally, two videos were excluded because they were commercial. The study included a total of 39 videos, as there were no specific lower or upper limits applied to the video duration. The total duration of all videos combined was 50,860 seconds, with a total of 91,632 views and 842 likes. It is worth noting that no dislikes were observed for any of the videos. 97.4% (n=38) of the videos are targeted to physicians. The country with the highest number of video uploads is India with 43.6% (n=17), while 9 videos (23.1%) did not have a specified country identification. Classification and descriptive statistics of videos are presented in Table 1.

The correlation analysis between DISCERN score and GQS, JAMA score, and video features to measure the reliability and quality of information about treatment options for patients and information providers in health-related videos is shown in Table 2. A moderate

Table 1 Classification and descriptive statistics of videos

Video length (second) [median, (min.-max.)]		483 (197-11845)
Time since upload on YouTube (day) [median, (min.-max.)]		880.50 (21-1859)
Number of views [median, (min.-max.)]		821.50 (80*33012)
Video comment count [median, (min.-max.)]		1 (0-7)
Video source	Academic institutions or societies (n; %)	6 (15.4)
	Personal (n; %)	33 (84.6)
Video content	Only theoretical information (n; %)	3 (7.7)
	Only practical information (n; %)	28 (71.8)
	Theoretical + practical information (n; %)	8 (20.5)
Language format	English audio (n; %)	28 (71.8)
	English subtitles (n; %)	11 (28.2)
Target audience	Physicians (n; %)	38 (97.4)
	Patients (n; %)	1 (2.6)
Videos country origin	India (n; %)	17 (43.6)
	United States of America (n; %)	4 (10.3)
	France (n; %)	4 (10.3)
	Other country (n; %)	5 (12.8)
	Unknown origin (n; %)	9 (23)
Video view rate [median, (min.-max.)]		1.51 (0.11-29.98)
DISCERN Score [median, (min.-max.)]		30 (21-61)
GQS [median, (min.-max.)]		2 (1-5)
JAMA Score [median, (min.-max.)]		1 (0-3)

GQS: Global Quality Score, JAMA; Journal of American Medical Association

Table 2 Correlation analysis between DISCERN score and video parameters, GQS, and JAMA scores.

			GQS	JAMA Score	Video length	Number of views	Video view rate	Time since uploaded on YouTube	Video comment count	Video source	Video content	Language format	Target audience
Spearman's rho	DISCERN Score	Correlation Coefficient	0.660	0.579	0.431	-0.101	0.393	-0.603	0.018	0.339	0.579	0.596	0.166
		Sig. (2-tailed)	**<0.001**	**<0.001**	**0.006**	0.539	**0.013**	**<0.001**	0.924	**0.035**	**<0.001**	**<0.001**	0.312

GQS: Global Quality Score, JAMA; Journal of American Medical Association

Table 3 Analysis of videos in terms of evaluator scores and video parameters in the target audience, video source, video content, and language format classification

	Video source		p	Video content			p	Language format		p
	Academic institutions or societies	Personal		Only theoretical information	Only practical information	Theoretical + practical information		English audio	English subtitles	
DİSCERN Score [median, (min.-max.)]	48 (25-61)	29 (21/48)	**0.037**	25 (23-37)	28.50 (21-48)	40.50 (34-61)	**0.001**	33.50 (21-61)	25 (21-31)	**<0.001**
GQS [median, (min.-max.)]	3 (2-5)	2 (1-4)	**0.037**	2 (1-3)	2 (1-4)	3 (2-5)	0.134	3 (1-5)	2 (1-3)	**0.025**
JAMA score [median, (min.-max.)]	1.50 (1-3)	1 (0-2)	**0.001**	1 (1-1)	1 (0-2)	1 (1-3)	**0.024**	1 (0-3)	1 (1-1)	0.375
Video length (second) [median, (min.-max.)]	2930.50 (350-11845)	400 (132-5386)	**0.049**	350 (243-890)	390 (112-1859)	551 (213-1039)	**0.001**	483 (234-11845)	390 (132-5386)	0.391
Time since upload on YouTube (day) [median, (min.-max.)]	370.50 (213-1296)	726 (21-1859)	0.185	491 (21-1296)	805.50 (112-1859)	551 (213-1039)	0.383	491 (21-1859)	1240 (598-1812)	**<0.001**
Number of views [median, (min.-max.)]	728 (273-3819)	821 (60-33012)	0.669	80 (60-359)	1045 (110-33012)	821.50 (272-3819)	**0.039**	688 (60-33012)	1227 (110-3853)	0.212
Video views rate [median, (min.-max.)]	2.19 (0.28-3.94)	1.50 (0.11-29.98)	0.371	0.27 (012-3.81)	1.42 (0.11-29.98)	1.85 (0.26-3.94)	0.440	1.69 (0.12-29.98	1.09 (0.11-3.08)	0.086
Videos comment count [median, (min.-max.)]	0 (0-1)	1 (0-7)	0.163	0 (0-0)	1 (0-7)	0.50 (0-2)	0.256	0 (0-7)	1 (0-6)	0.560

GQS: Global Quality Score, JAMA; Journal of American Medical Association

correlation was found between DISCERN score and the other video evaluation parameters, GQS, and JAMA score (rho: 0.660, p<0.001; rho: 0.579, p<0.001, respectively). There were no significant correlations observed between DISCERN score and the number of views and video comment count (rho: -0.101, p=0.539; rho: 0.018, p=0.924, respectively).

The evaluation scores for video sources, video content, and language format categories are presented in Table 3. Videos originating from academic institutions or societies exhibited higher DISCERN scores, GQS scores, and JAMA scores compared to personal videos (p=0.037, p=0.037, p=0.001, respectively). However, a similar trend was not observed for the

number of views, video view rate, and video comment count (p=0.669, p=0.371, and p=0.163, respectively). Regarding video content, theoretical and practical videos obtained higher DISCERN scores than solely practical videos, while only practical videos garnered more views (p=0.001, p=0.039, respectively). English audio format videos demonstrated a higher DISCERN score (p<0.001).

Discussion

There is currently no consensus on the definition of female urethral stricture (FUS), and widely accepted diagnostic methods or criteria are lacking (1, 3). Even those who receive training in the management and treatment of female urethral stricture disease (FUSD) have limited experience (4, 5). The current literature indicates that video-based education can significantly contribute to the development of clinical and surgical skills in various medical specialties and postgraduate training (13). Furthermore, it has been observed that video-based education often yields more favorable outcomes compared to traditional written text, while maintaining a similar content structure (14). In this context, individuals frequently strive to acquire information by following national or international associations and renowned experts in the field. After receiving a diagnosis, patients also engage in online research, although not to the same extent as healthcare professionals. YouTube, as a popular video-sharing platform, is favored for its easy accessibility, free usage, and wide user database. It allows viewers to interact with content creators, making it a preferred platform for accessing health-related information. Both patients and medical professionals increasingly rely on the internet and video-sharing websites like YouTube to gather information about health issues (15, 16). YouTube has become a valuable resource for acquiring information about medical conditions and providing educational content to patients. However, the lack of established uploading criteria can significantly impact the quality and accuracy of instructional videos, posing a risk of misinformation dissemination (17). This is likely due to the absence of established upload criteria, which greatly influences the quality and accuracy of instructional videos.

In recent years, the quality of information on social media platforms related to female urology has been subject to increasing investigation. The reliability of YouTube information on bladder pain syndrome has been assessed using video quality criteria, and it has been found to be reliable (18).

There is limited research available on the evaluation of videos related to female urethroplasty, with only one publication found in the PubMed database. Sahin et al. (19) conducted a study employing the Global Quality Score (GQS) and an original checklist score named Female Urethroplasty Control Score (FUCS) to assess the quality and stages of female urethroplasty. The study revealed that video content from academic sources had higher GQS and FUCS scores compared to videos from urologists (19). While Sahin et al. (19) study did not utilize the DISCERN score, which is more suitable for evaluating health-related information, our study, like theirs, found higher GQS scores in videos from academic institutions or societies. Additionally, our study observed higher DISCERN and JAMA scores in videos from academic sources, contributing to the existing literature. Although the literature suggests that videos published by hospitals and clinicians tend to have higher DISCERN values, it is also noted that the reliability of videos does not differ based on the source of upload (15, 20-23). In our study, significant correlations were found between DISCERN scores and several parameters, including academic institutions or societies as the video source, theoretical+practical information as the video content, and English audio language format. These findings suggest that videos originating from academic sources, containing both theoretical and practical information, and presented in English audio format are associated with higher DISCERN scores, indicating better quality and reliability. Importantly, our study demonstrated no significant correlation between the number of views, video comment count, and DISCERN scores.

The total viewing duration of 50,860 seconds, total view count of 91,632, and total likes count of 842 for the 39 videos included in the study indicate a significant interest in this field. The high view count and positive engagement suggest that the topic of the videos, in this case, female urethroplasty, is of interest to the audience and highlights the relevance and potential impact of the content in this domain. Our study demonstrates that videos related to female urethroplasty, published on YouTube using the keyword "female urethroplasty" in the last 5 years, have shown low scores in terms of GQS, JAMA score, and DISCERN score. Indeed, our study also emphasizes the need for an increase in academic institutions or societies as sources of videos in this field. However, promoting digital health literacy and the World Health Organization's call for creating a new space dedicated to verified health information would be beneficial (19).

Some limitations of this study should be acknowledged. First, the study focused solely on the YouTube platform, which may not provide a comprehensive representation

of all available health-related videos online. Other social media platforms and websites that host health-related content were not included, which could limit the generalizability of the findings. Additionally, the study evaluated a limited number of videos, which may not capture the full range of content and quality available on the platform. A larger sample size could provide a more comprehensive understanding of the landscape of health-related videos. A strength of the study is the use of established evaluation criteria to assess the accuracy and reliability of medical information in the scored videos. By applying standardized assessment tools, such as the GQS, JAMA score, and DISCERN score, the study provides a systematic approach to evaluating the content of the videos.

Conclusion

The study highlights the limited availability of videos related to female urethroplasty on YouTube, as well as the low accuracy and reliability of medical information in those videos. This underscores the need for an increase in the production of online videos prepared by academic institutions or reputable societies that are both informative and easily understandable.

Conflict of Interest Statement
The authors declared no potential conflicts of interest concerning the research, authorship, and/or publication of this article.

Ethical Approval
Ethical approval was not obtained as all videos were publicly available on the social media website (YouTube.com) and no human or animal participants were included in the study.

Consent to Participate and Publish
All patients included in this research gave written informed consent to publish the data contained within this study.

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

Authors Contributions
AB: Conceptualization; Data Curation; Formal Analysis.

YÖ: Investigation; Methodology; Project Administration.

AŞ: Resources; Validation Visualization.

KK: Writing – Review & Editing.

SÇ: Supervision.

GB: Writing – Original Draft.

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ORIGINAL RESEARCH

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Panthotenic Acid Derrivate Dexpanthenol Mitigates the Effects of Lung Ischemia-Reperfusion Induced Cardiac Damage by its Anti-Inflammatory Action

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Abstract

Objective
Pulmonary ischemia-reperfusion (IR) injury causes cardiac damage through inflammation related to hypoxic conditions. Dexpanthenol (DEX) has an anti-inflammatory action in various tissues such as lung, liver, and kidney. This study aimed to show the effects of DEX on myocardial damage secondary to pulmonary IR injury.

Material and Method
Thirty two rats were randomly divided into four groups as sham, IR, DEX (500 mg/kg, intraperitoneally, single dose), and IR+DEX. After left thoracotomy, non-traumatic vascular clamping was applied for 60 minutes, followed by 60 minutes of reperfusion to create a lung IR model. After sacrification, heart tissues were collected and placed in formaldehyde solution for histopathological and immunohistochemical analyses. Hyperemia, hemorrhage, and degeneration were examined, Immunostainings of cyclooxygenase-1 (COX-1), hypoxia-inducible factor 2 alpha (HIF-2α), and interferon alpha (IFα) were performed.

Results
Cardiomyocytes in the sham group appeared elongated, branching, and of normal size with well-defined intercalated discs. Delicate endomysium sheaths surrounding the cardiac cells were observed, along with a dense capillary network surrounding the cells. In contrast, the IR group exhibited alterations in cardiac tissue, including hyperemia, hemorrhage, and disruption of the cross-striated banding pattern of the cardiac cells. Also; COX-1, EPAS-1/HIF-2α, and IFα expressions were elevated in the IR group. Treatment with DEX resulted in a reduction of these pathological outcomes.

Conclusion
In the context of pulmonary IR, damage is likely to occur not only in lung tissue but also in other organs. This is attributed to the dissemination of immunomodulatory cytokines developed within the tissue to other organs through the bloodstream. DEX is a derivative of pantothenic acid, recognized for its tissue-protective effects. In this study, it was histopathologically and immunohistochemically shown that DEX could be protective against lung IR-induced cardiac damage.

Keywords: Cardiac damage, Dexpanthenol, Ischemia-reperfusion, Lung ischemia

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Introduction

Pulmonary ischemia reperfusion (IR) injury is a serious condition that occurs especially in cases of pulmonary embolism cardiopulmonary bypass, circulatory arrest, and lung transplantation (1). Both lung IR injury and its complications may cause significant mortality and morbidity rates (2, 3). For example, severe primary graft dysfunction is observed in approximately 30% of transplant patients due to IR injury during lung transplantation, (4).

The abrupt onset of ischemia in the pulmonary artery creates resistance against right ventricular contraction. Since the heart and lungs are connected by large vessels, it is anticipated that any vascular pathology impacting one will invariably influence the other. In circumstances where there is an elevation in pulmonary artery pressure, such as in cases of pulmonary embolism, probably, there will also be an increase in pressure within the right ventricle of the heart, leading to cardiac damage (5). Another damage mechanism in cardiac tissue is the reflection of oxidative stress and inflammation that develops in other tissues (1).

When oxygenation and blood supply are impaired, hypoxia-related damage begins to develop in the tissue. Cells' energy production slows down, protein synthesis decreases, and it becomes unable to provide the resources necessary to sustain life. Disruption of ATP metabolism results in hypoxanthine accumulation and the formation of reactive oxygen species (ROS) (6). The ischemic damage ultimately results in cell death accompanied by the release of damage-associated molecules. These molecules stimulate cytokine synthesis, such as interferon alpha (IFα) and other interleukines, and the inflammatory response that can cause secondary organ injuries such as cardiac injury (1, 7) (Figure 1).

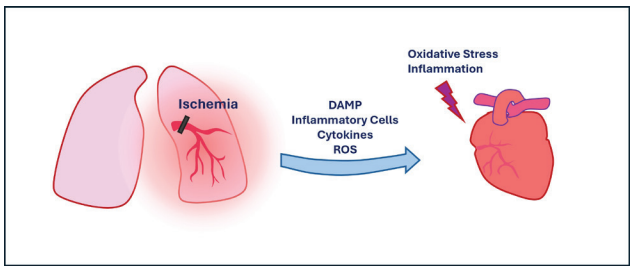

Figure 1
The mechanism of lung ischemia reperfusion induced cardiac injury. DAMP: Damage associated molecular pattern, ROS: Reactive oxygen species

Although reestablishment of blood flow and oxygenation forms the basis of recovery, it also mediates the formation of another condition called reperfusion injury. Oxygen rushing into the tissue during reperfusion becomes the substrate for the production of reactive oxygen radicals resulting from intracellular metabolic disorders. While it provides the oxygen needed by the tissue, on the other hand, it increases the formation of ROS that cause damage (6). Some studies have suggested that reperfusion injury is mediated by inflammatory cell activation in the reperfused tissue (8, 9). Exacerbation of inflammatory response in ischemic lung injury can lead to major organ dysfunction and multiple organ failure (10).

In the tissue where secondary damage develops, some pathways are activated at the cellular level, in addition the to changes mentioned above. Inflammation and ROS induced oxidative stress stimulate cyclooxygenase (COX) enzymes and hypoxia-inducible factor (HIF) derivatives involved in the production of prostaglandins, which reinforce or reduce inflammation with local hormonal effects (11, 12).

Dexpanthenol (DEX) is a derivative of vitamin B_5, used in wound healing due to its tissue-protective effect. DEX has been stated to be protective for many tissues from neurons to the liver due to its antioxidant, antiinflammatory, and antiapoptotic properties (13-16). Studies have shown that DEX reduces oxidative stress which develops through various mechanisms including IR-induced damage, by preserving levels of glutathione, myeloperoxidase, and catalase. (16). Additionally, DEX has been found to lower inflammatory cytokine levels and reduce both apoptosis and inflammation by influencing the endoplasmic reticulum stress pathway and decreasing caspase levels (15, 17, 18). There are also studies showing that DEX may be protective in cardiac damage developing with different pathological mechanisms or in IR injury of several organs by the antioxidant and anti-inflammatory pathways (19-22). However reflecting the importance of our study, there is no data on its use in lung IR injury induced cardiac damage, yet.

As lung IR injury involves several pathological mechanisms, various alternatives that provide mitochondrial protection or nitric oxide synthetase regulation, or reduce the inflammation have been included in the treatment (3). Nevertheless, there is still a need for new molecules, especially to use in combination therapies to prevent or reduce lung IR injury. Considering the therapeutic properties, we

assume that DEX may be beneficial. So, the present study was designed to deepen our understanding of the possible protective effects of DEX on lung IR induced cardiac injury.

Material and Method

Ethics and Experimental Model

Thirty-two adult male Wistar Albino rats obtained from the Suleyman Demirel University Experimental Animals Laboratory weighing range of 300-350 g were used in the experiment. Rats were kept at 21-22 degrees Celcius, 12 hours of light, and 12 hours of darkness. An ad libutium feeding regimen was applied. Rats were randomly divided into four groups, with eight rats in each group. The groups were assigned as follows:

Sham group: 1 ml/kg saline was administered intraperitoneally (i.p.) to the rats. After 30 minutes, a thoracotomy procedure was performed under anesthesia, but the IR model was not performed and hilus was visualized.

IR group: 1 ml/kg saline was administered i.p. to the rats. After 30 minutes, a thoracotomy procedure was performed under anesthesia. After the left thoracotomy, a non-traumatic vascular clamp was placed on the hilus for 60 minutes of ischemia. Then, 60 minutes of reperfusion was performed (16).

IR+DEX group: 500 mg/kg DEX (Bepanthen® 500mg/2ml flk, Bayer, Turkiye) as a single dose was administered i.p. to rats. After 30 minutes, a thoracotomy procedure was performed under anesthesia. After left thoracotomy, a non-traumatic vascular clamp was placed on the hilus for 60 minutes of ischemia. Then, 60 minutes of reperfusion was performed (16).

DEX group: 500 mg/kg DEX as a single dose was administered i.p. to rats. After 30 minutes, a thoracotomy procedure was performed under anesthesia, but the IR model was not performed.

Following a 12-hour fasting period, experimental animals were subjected to i.p. anesthesia with Ketamine (80-100 mg/kg) / Xylazine (8-10 mg/kg). Subsequently, the thoracic region was shaved, and a left thoracotomy was performed under anesthesia. After identification of the left lung hilum and trachea, non-traumatic vascular clamping was applied for 60 minutes, followed by 60 minutes of reperfusion. Once reperfusion was confirmed by visualization of blood flow, the animals were euthanized. Surgical exsanguination was carried out via abdominal incision. After sacrification, heart tissues were collected and placed in formaldehyde solution for histopathological and immunohistochemical analyses.

Histopathological Method

Heart samples were collected and preserved in a 10% neutral formalin solution. The heart samples were then embedded in paraffin wax following standard tissue processing using a fully automated tissue processing device (Leica ASP300S, Wetzlar, Germany). Subsequently, 5 µm thick sections were cut from the paraffin blocks using a fully automated rotary microtome (Leica RM2155, Leica Microsystems, Wetzlar, Germany). These sections underwent staining with hematoxylin-eosin (HE), followed by cover slipping, and examination under light microscopy.

Histological lesions in the hearts were graded semi-quantitatively using an ordinal grading system. This evaluation included assessing hyperemia, hemorrhage, inflammatory cell infiltrations, and degenerative necrotic changes in myocardial cells. Descriptions of normal (score = 0) to severe (score = 3) affections were assigned (Table 1) (23).

Table 1 Histopathological and immunohistochemical graduation of analysis

Scores	Histopathological graduation	Immunohischemical graduation
0	Normal	No expression
1	Mild: Mild hyperemia with no additional findings	Focal and weak staining
2	Moderate: Moderate hyperemia with mild hemorrhage	Diffuse and weak staining
3	Severe: Presence of degeneration in addition to marked hyperemia and hemorrhage	Diffuse and marked staining

Immunohistochemical Examination

For immunohistochemical analysis, three series of slices were cut from the paraffin blocks and mounted on slides coated with poly-L-lysine. Then sections were subjected to immunohistochemical staining following the manufacturer's instructions to assess the expression of COX-1, Endothelial PAS domain-containing protein 1 (EPAS-1)/HIF-2α, and IFα using the streptavidin-biotin method. Primary antibodies used were COX-1 [COX-1 Antibody (17): sc-19998 (Santa Cruz, Texas, USA)], HIF-2 α [EPAS-1/HIF-2α Antibody (190b): sc-13596 (Santa Cruz, Texas, USA)], and IFα [IFα Antibody (PA5-119649)] (ThermoFisher Scientific, MA, USA) at a [1/100] dilution. Immunohistochemistry was performed on the sections using streptavidin-alkaline phosphatase conjugate and a biotinylated secondary antibody after a 60-minute incubation with the primary antibodies. Mouse and Rabbit Specific HRP/DAB IHC Detection Kit - Micro-polymer (ab236466) from Abcam (Cambridge, UK) was used as the secondary antibody, and diaminobenzidine (DAB) was employed as the chromogen. For negative controls, antigen dilution solution was applied instead of primary antibodies. Each evaluation was conducted on blinded samples by a specialized pathologist.

At an objective magnification of X40, the immunohistochemical expressions were scored on a scale of 0-3. Accordingly, 0 indicates no expression, 1 indicates focal and weak staining, 2 indicates diffuse and weak staining, and 3 indicates diffuse and marked staining (Table 1) (23) The Image J 1.46r software (National Institutes of Health, Bethesda MD) was used to determine the positive immunohistochemical reaction. 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

Statistical analyzes include histopathological evaluation scores and staining levels of immunological markers. For this purpose, non-parametric Kruskall Wallis test was used for multiple group comparisons and Mann Whitney U test was used for two group comparisons - by using a a package program. The level of significance was considered at $p<0.05$.

Results

Histopathological Findings

Microscopic examination revealed no pathological findings in the myocardial tissue of the sham and DEX groups. Cardiomyocytes in these groups showed signs of elongation, branching, and normal size with well-defined intercalated discs. Delicate endomysium sheaths surrounding the cardiac cells were observed, along with a dense capillary network surrounding the cells. In contrast, the IR group exhibited alterations in cardiac tissue, including hyperemia, hemorrhage, and disruption of the cross-striated banding pattern of the cardiac cells. Treatment with DEX resulted in the amelioration of these pathological findings. The differences in the IR group compared to the sham and in the IR+DEX group compared to the IR were both statically significant ($p<0.001$ and $p<0.01$, respectively) (Figure 2).

Immunohistochemical Findings

Immunohistochemical investigations showed very low or non-existent expression of COX-1, EPAS-1/HIF-2α, and IFα in the sham group. In contrast, the myocardial cells of the IR group exhibited moderate to markedly elevated levels of COX-1, EPAS-1/HIF-2α, and IFα expressions. Treatment with DEX resulted in a reduction of these pathological outcomes (Figures 3, 4, 5).

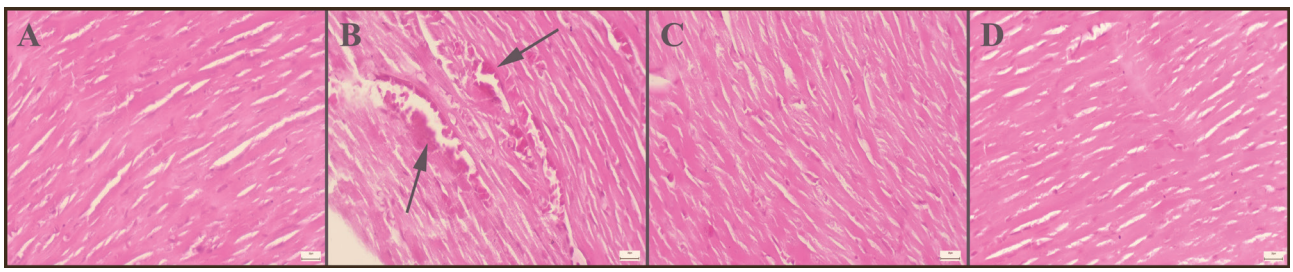

Figure 2

Representative histopathological figures A: Normal myocardial tissue histology in sham group, B: Severe hyperemia and hemorrhage (arrows) in IR group, C: Marked amelioration in IR+DEX group, D: Normal myocardium histology in DEX group, HE, scale bars=20μm

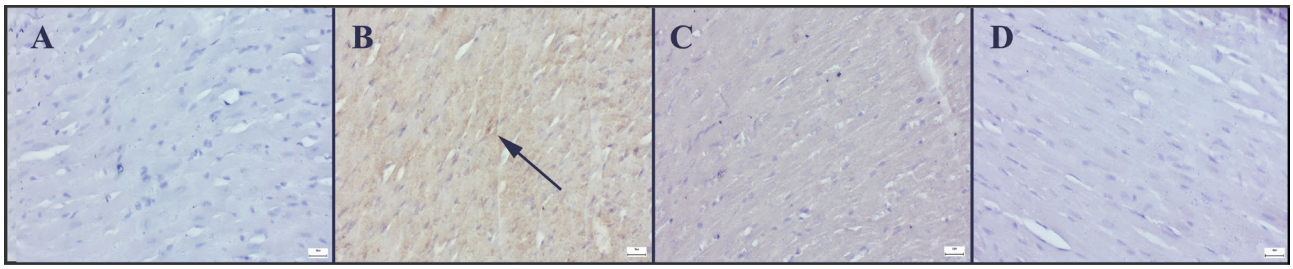

Figure 3
mmunohistochemically COX-1 expressions of heart tissues of the groups. A: Negative expression in sham group, B: Increased expressions in myocardial cells (arrows) in IR group, C: Markedly decreased expression in IR+DEX group, D: Negative expression in DEX group, Scale bars=20μm, streptavidin biotin peroxidase method.

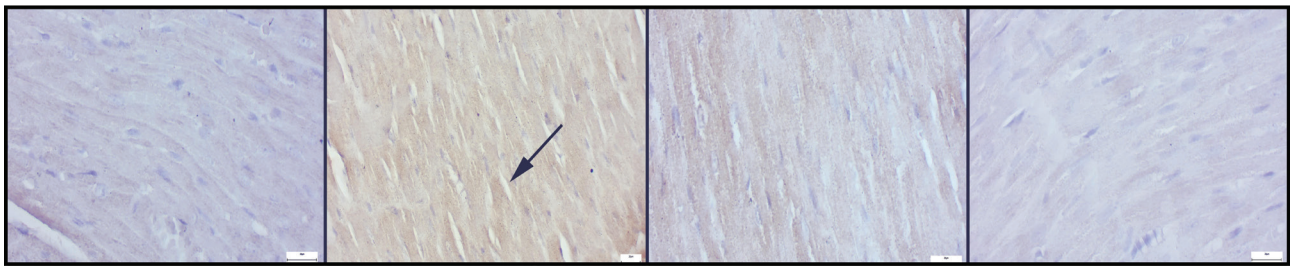

Figure 4
Immunohistochemically EPAS-1/HIF-2α expressions of heart tissues among the groups. A: Negative expression in sham group, B: Increased expressions in myocardial cells (arrows) in IR group, C: Markedly decreased expression in IR+DEX group, D: Negative expression in DEX group, Scale bars=20μm, streptavidin biotin peroxidase method.

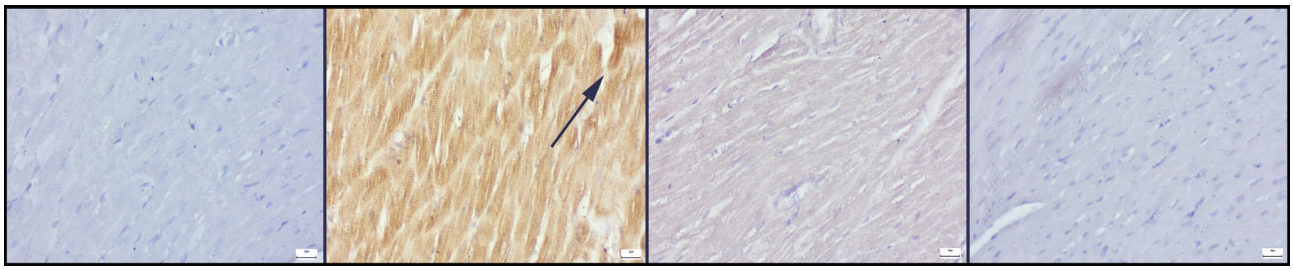

Figure 5
Immunohistochemically IFα expressions of heart tissues of the groups. A: Negative expression in sham group, B: Increased expressions in myocardial cells (arrows) in IR group, C: Markedly decreased expression in IR+-DEX group, D: Negative expression in DEX group, Scale bars=20μm, streptavidin biotin peroxidase method.

In immunohistochemical scoring, a statistically significant increase in COX-1, EPAS-1/HIF-2α, and IFα scores was observed in the IR group compared to the sham group ($p<0.001$ for all). These changes were significantly reversed in the DEX+IR group compared to the IR group ($p<0.01$ for all). One of the interesting findings of the statistical analysis was that the difference between the IR+DEX group and the sham group in terms of EPAS-1/HIF-2α was not statistically significant. Distribution of the data in groups as median and mode within parenthesis and the p values of the comparisons were shown in Table 2.

Table 2 The statical analyses of histopathological and immunohistochemical scores

Marker/Group	Sham (n=8)	IR (n=8)	IR+DEX (n=8)	DEX (n=8)
COX	0 (0)[a,b]	2.5 (3)[a,c]	1 (1)[b,c]	0 (0)
HIF-2α	0.5 (0)[a]	3 (3)[a,c]	1(1)[c]	0 (0)
IF α	0 (0)[a,b]	3 (3)[a,c]	1 (1)[b,c]	0 (0)
Histopathologic evaluation	0 (0)[a]	2 (2)[a,c]	0.5 (0)[c]	0 (0)

IR: ischaemia reperfusion, DEX: dexpanthenol, COX: cyclooxygenase, HIF-2a: hypoxia-inducible factor 2 alpha, IFa: interferon alpha, a: p<0.001, b: p=0.05, c: p< 0.01. data are expresses as mean and mode within parantheses.

Discussion

IR injury consist of ischaemia-induced tissue damage and reperfusion-induced oxidative damage. Tissue damage increases with the contribution of inflammation secondary to IR. Due to cytokines and ROS released into the circulation, damage may develops in organs other than the ones in which IR injury develops (24).

In our study, the presence of hyperemia, hemorrhage, and disruption in the cross-striated banding pattern of cardiac cells indicates the development of inflammation-related damage in the heart during lung IR injury. Disruption of the cross-striated banding pattern of the cardiac cells leads to impaired cardiac muscle contraction. Irregularity of muscle contraction may also be a symptom of arrhythmias caused by inflammation (25, 26). Regression of these findings with DEX treatment demonstrates a reduction in inflammation and is an important finding of our study.
The HIF pathway is one of the systems that protects the organism under hypoxic conditions. (27). HIF-2α predominantly controls a wide range of transcription factors and coregulators, contributing to its diverse functions in hypoxic conditions. HIF-1α stands as the most extensively studied isoform, while HIF-3α emerges as the most novel isoform. Among them, HIF-2α exhibits a more tissue-specific manner and is highly expressed in cardiac, endothelial, and hepatic tissues, etc. It was shown that an increase in HIF-2α led to enhanced vascular permeability in kidney tissue with IR, thereby prolonging inflammatory cell migration and inflammation (28). Additionally, in inflamed tissue, hypoxia is not surprising because of increased metabolic demand (29). Thus, HIF-2α expressions could be induced by hypoxia and/or inflammation directly and/or indirectly. In our study, lung IR-induced hypoxic conditions in cardiac cells were proven by increased HIF-2α levels, and decreases were shown by DEX. Importantly, it is the first report that DEX reduced HIF-2α levels in lung IR-induced cardiac damage. Considering the previous reports on the anti-inflammatory effect of DEX, it is possible that DEX decreased HIF-2α levels by its anti-inflammatory properties. One of the interesting results of our study was that the difference between sham and IR+DEX groups was not statically significant in terms of HIF-2α. It can be concluded that DEX improved the response to hypoxia in damaged tissue to the point that it approached the sham group. These results deserve more detailed studies.

It is known that COX enzymes play a role in the synthesis of prostaglandins from fatty acids such as arachidonic acid located in the cell membrane. Prostaglandins, synthesized within the cell, are predominantly locally acting products with an important role in inflammation management (12). Although COX-1 is considered as a structural enzyme and COX-2 is mostly associated with inflammatory processes, it was reported in a study that COX-1 and COX-2 were increased together in an IR injury model (30, 31). In addition, some studies have suggested the need to focus on endothelial COX-1 due to its possible contribution to vascular dysfunction (32). COX-1 activation leads to vasoactive prostaglandin and thromboxan A2 production which means further vascular constriction (33). Considering these facts, COX-1 was evaluated in this study, and the increase in COX-1 in the IR group is considered as another indicator of inflammation developing in the tissue. A decrease in COX-1 immunoexpression with DEX treatment, along with other findings, indicates the regression of inflammation. It is also known that inhibition of COX-1 reduces the impairment of endothelial dysfunction in inflamed tissue (34). HIF-2α and COX-1 are both involved in cardiac endothelial vascular changes in the case of inflammation. The decreases in the immunoexpression of HIF-2α and COX-1 with DEX could both reduce inflammation and protect the cardiac endothelium from the inflammation-related changes.

IFα is another cytokine that regulates and modulates the activation of the immune system. Its role in inflammation is somewhat intricate. As an immunoregulatory cytokine, IFα typically limits inflammation, yet elevated levels of IFα activity can, in certain instances, exacerbate inflammation and increase tissue damage (35). In our study, the observed increase in IFα levels in the IR group indicates the activation of the immune system. This immune activation signifies the occurrence of inflammation in the cardiac tissue. Conversely, in the treatment group, the lower IFα activity compared to the IR group could be interpreted as DEX reduced inflammation initiated in the cardiac tissue.

There are some limitations of the current study. Firstly, due to the acute design of the study, DEX was administrated on one day and in a single dose. The effect of repeated or different doses of DEX should be evaluated in further studies. Secondly, we used only histopathological and immunohistochemical analyses to evaluate the DEX effect on cardiac tissues. The results should be confirmed by quantitative analyses such as PCR, Elisa, or Western Blot as well as qualitative analyses. Lastly, we monitored the inflammatory changes in cardiac tissue through the immunoexpressions of the final proteins of some pathways. To conclude to how DEX affects on inflammation, the related pathways should be evaluated.

In the context of pulmonary IR, the damage is likely to occur not only in lung tissue but also in other organs, secondary. This is attributed to the dissemination of immunomodulatory cytokines developed within the tissue through the bloodstream to other organs. DEX, a derivative of pantothenic acid recognized in the literature for its tissue-protective effects and known for anti-inflammatory properties, mitigates inflammation in cardiac damage resulting from lung IR injury, as evidenced by these alterations and changes in immunological markers.

Considering the importance of the IR injury in various tissues, it is important to have a sufficent number of treatment alternatives. The fact that the treatment alternative is an easily available, inexpensive and relatively safe molecule such as DEX will provide a significant advantage for such an important clinical situation. Thus, the protective effects of DEX in IR injuries should also be studied in clinical settings.

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

Ethical Approval
The experimental design adhered to the guidelines for animal research set forth by the National Institutes of Health, and received approval from the Committee on Animal Research at Suleyman Demirel University prior to commencement of the study (approval no: 11.07.2024/08-309).

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Availability of Data and Materials
The data is available upon reasonable requests from the Corresponding Author.

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

MA: Investigation; Formal analysis; Writing-original draft.

OO: Data curation; Formal analysis; Writing-original draft; Writing- review & editing.

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ORIGINAL RESEARCH

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Chronic Obstructive Pulmonary Disease (COPD) and Magnesium Levels: Relationship Analysis

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Abstract

Objective

Identifying modifiable risk factors is very important for preventing acute chronic obstructive pulmonary disease (COPD) severity. This study was conducted to determine the role of serum magnesium levels in acute COPD exacerbation.

Material and Method

A total of 104 COPD patients with a mean age of 66.63 ± 8.79 years and female 17.3%(18) males 82.69%(86) were included in our study. Chest X-ray, respiratory function test, and biochemical blood tests were performed on the cases. The relationship between magnesium level and COPD severity was evaluated using appropriate statistical methods.

Results

Demographic data of the patients were collected, biochemical evaluation was performed, and the relationship between magnesium levels and respiratory parameters was investigated. Serum magnesium was found to be low in patients (25%). COPD evaluation test was compared with mMMR and CAT, dyspnea symptoms were observed to be more in hypomagnesemia cases, and the difference was found to be statistically significant (p:0.029, p:0.030, respectively). In terms of respiratory function tests, FEF25-75 was found to be lower and statistically significant in hypomagnesemia patients. Disease duration was found to be statistically significant in hypomagnesemia cases (p:0.033). When compared with male and female patients, smoking was observed more in male individuals, and this parameter was also found to be statistically significant (p:0.044).

Conclusion

It was concluded that hypomagnesemia may increase respiratory distress in COPD patients and contribute to COPD exacerbations.

Keywords: Chronic Obstructive Pulmonary Disease, Magnesium, Respiratory function test

Introduction

Chronic obstructive pulmonary disease (COPD) is a significant and prevalent respiratory condition that continues to present a growing global health challenge (1). Epidemiological data indicate that the worldwide prevalence of COPD is approximately 10%, with rates increasing with advancing age (2). The World Health Organization (WHO) projects that this trend will persist, leading to a further increase in cases by

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2030 (3). COPD is associated with high morbidity and mortality rates, which makes it a critical public health concern. Its etiology and pathogenesis involve a complex interplay between genetic predisposition and environmental factors, including tobacco smoke, air pollution, occupational exposure, and other environmental hazards (4).

Magnesium plays a crucial role in maintaining muscle strength and optimizing exercise performance, primarily through its effects on blocking calcium channels and inhibiting acetylcholine release (5). As a vital mineral, magnesium is involved in several physiological functions including smooth muscle relaxation, bronchodilation, and immune system regulation (6,7). It has been postulated to exert a protective effect against respiratory conditions owing to its contributions to processes such as bronchodilation, mast cell stabilization, regulation of neurohumoral mediators, and enhancement of mucociliary clearance in the airway smooth muscles (8,9). Moreover, magnesium demonstrates potent vasodilatory and bronchodilatory properties while also modulating the release of acetylcholine and histamine. Its anti-inflammatory actions make it a potential therapeutic agent for respiratory diseases such as asthma and COPD (7). While some studies indicate that hypomagnesemia may result in increased muscle contraction, others have highlighted the role of magnesium in alleviating bronchial muscle tension through its involvement in oxidative stress defense mechanisms and the regulation of leukocyte activation (10,11).

Material and Method

Purpose and Type of Research

This study aimed to assess the correlation between chronic obstructive pulmonary disease (COPD) severity and serum magnesium concentration in COPD patients presenting to the chest disease outpatient clinic.

Research Population and Sample

This study investigated the association between the severity of COPD and serum magnesium levels in patients diagnosed with COPD. Patients diagnosed with COPD for at least two years were included in the study. Patients with gastrointestinal disorders, pancreatitis, renal diseases, endocrine or metabolic conditions, hepatic dysfunction, or the use of thiazide diuretics were excluded. The study adhered to the principles of the Declaration of Helsinki and participation was voluntary. Data regarding patient demographics (age, disease duration, body mass index [BMI]), clinical details (comorbidities, smoking habits, and long-term oxygen therapy [LTOT] use), and medical records were collected through in-person interviews and patient files. Comorbidities, such as systemic arterial hypertension, heart disease, obstructive sleep apnea (OSA), and malignancies, were considered based on previous diagnoses. Diagnostics included plain chest radiographs (anterior, posterior, and lateral views), pulmonary function tests (PFT) using the BTL "CardioPoint-Spiro" module, and biochemical markers. COPD was classified according to severity: Mild: FEV1 ≥ 80%; Moderate: FEV1=50-80%, Severe: FEV1=30-50%, and Very Severe: FEV1 ≤ 30%. Pulmonary function tests were conducted, and patients with an FEV1/FVC ratio <70% and an FEV1 value ≤ 79% were included. Disease severity was assessed using the COPD Assessment Test (CAT) and the Modified Medical Research Council (mMRC) dyspnea scale. Biochemical markers such as calcium (Ca), magnesium (Mg), sodium (Na), potassium (K), and C-reactive protein (CRP) were measured using radioimmunoassays. Serum magnesium levels <1,7 mg/dL were defined as hypomagnesemia (12,13). Serum magnesium levels were assessed during acute COPD exacerbations and analyzed for respiratory distress, disease duration, and hospitalization frequency. The association between serum magnesium levels, COPD assessment tools (CAT and mMRC), respiratory parameters, and biochemical markers was investigated using the appropriate statistical methods.

Statistical Analysis

Data analysis was conducted using the SPSS software (version 22.0; IBM®, Chicago, USA). Normality was assessed using visual (histograms and probability plots) and analytical (Shapiro-Wilk test) methods. The descriptive statistics are reported as follows: Mean ± standard deviation for normally distributed continuous variables. Median (min-max) for non-normally distributed data. Frequency and Percentage of Categorical Variables. Comparisons between groups were performed using the independent t-test for normally distributed variables and Mann-Whitney U test for non-normally distributed variables. The chi-square test was used for nominal data. Linear regression analyses were conducted to examine the relationship between magnesium levels and respiratory parameters (FEF 25-75, FEV1, CAT score, and mMRC). Multivariate regression analyses were performed to assess the impact of magnesium levels on age, respiratory function, and biochemical markers. Statistical significance was set at $P < 0.05$. This study did not utilize artificial intelligence (AI) tools, including large language models (LLMs) or chatbots, at any stage of production.

Results

Data from all patients with COPD presenting to the chest disease outpatient clinic with acute exacerbations were analyzed. The study cohort comprised 104 patients (78 males (75%) and 26 females (25%). The mean age of the patients was 66.63 ± 8.79 years. Comorbidities were observed in 58% of the participants. Regarding smoking status, 15 patients (8%) were never-smokers, 53 patients (50.96%) were former smokers, and 36 patients (34.61%) were current smokers. Furthermore, 19% of patients received long-term oxygen therapy (LTOT). The frequency of acute exacerbations within a year was 68.26%, with 60.57% of these patients requiring inpatient treatment. When classified according to the GOLD (Global Initiative for Chronic Obstructive Lung Disease) criteria, the highest patient distribution was observed in GOLD Stage 3. Table 1 summarizes the demographic data, and the GOLD classification distribution is shown in Figure 1. Magnesium Levels and Their Impact In previous studies, serum magnesium (Mg) levels of <1,7 mg/dL

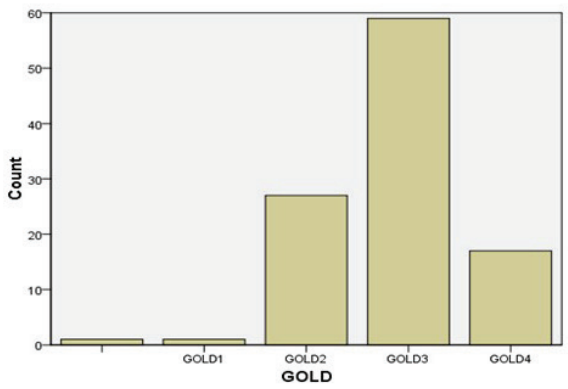

Figure 1
Distribution according to stages
GOLD:Global Initiative for Chronic Obstructive Lung Disease

were classified as hypomagnesemia (12,13). Based on this threshold, the patients in our study were categorized into hypomagnesemic (25%) and normomagnesemic (75%) groups. Serum magnesium levels were evaluated in relation to respiratory parameters, dyspnea scales, and biochemical markers. A negative correlation was observed between magnesium levels and mMRC (Modified Medical Research Council Dyspnea Scale) and CAT (COPD Assessment Test) scores, indicating that lower magnesium levels were associated with more severe symptoms. Conversely, a positive correlation was found between magnesium levels and FEF 25-75 (mid-expiratory flow rate), suggesting improved respiratory function with higher magnesium levels (Figures 2, 3, and 4; Table 2). FEF 25-75 is a marker of small airway obstruction and

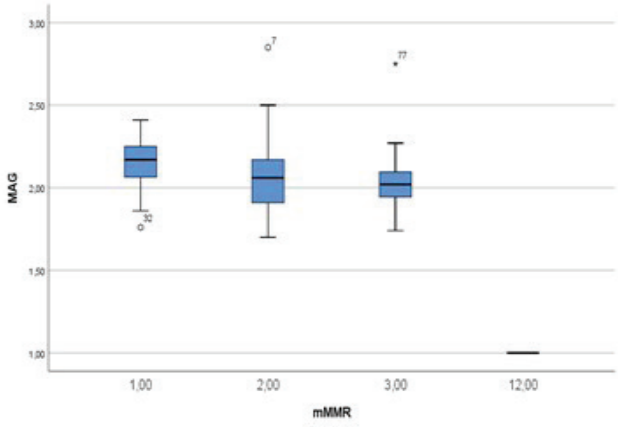

Figure 2
Relationship between magnesium and mMRC
MRC: Modified Medical Research Council
MAG:Magnezyum

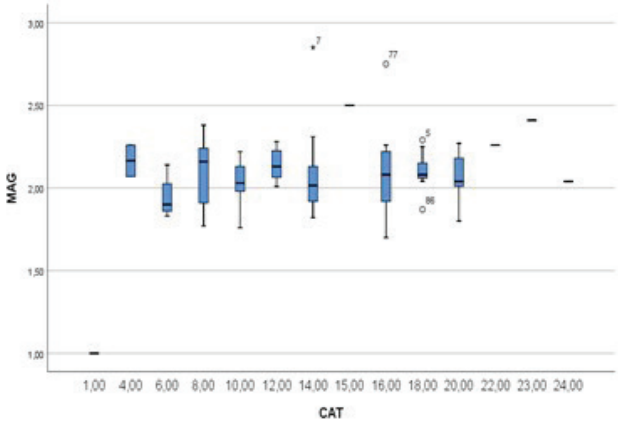

Figure 3
Relationship between magnesium and CAT
CAT: The COPD Assessment Test

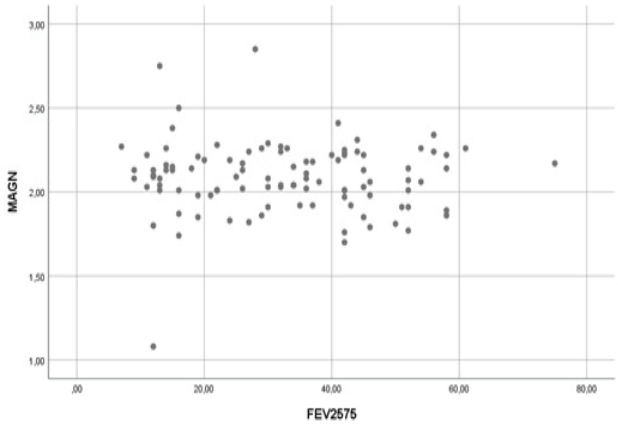

Figure 4
Relationship between magnesium and FEF25-75

Table 1 Demographic and clinical data of patients

All patients	**(min-mx) n mean %**
Age (mean ± SD) years	(44-89) 66.63 ±8.79
Sex (%) Female Male	 n:18 (%17.30) n:86 (%82.69)
Body mass index (BMI) (mean ± SD)	26.16 ± 5.89 (16.3-44.7)
Duration of COPD (years) (mean ± SD)	(2-20) 8.67 ± 4.585
The number of comorbidities median (min-max) no comorbidities comorbidity 1 comorbidity 2 comorbidity 3	2 (0-4) n:46(%41.3) n:48(%45) n:9(%5) n:1 (%2.5)
Smoking (pack/year) median (min-max) current smoker old smoker never smoker	40 (1-100) n:36(%34.61) n:53(%50.96) n:15(%14.42)
Number of patients who are NONE LTOT Number of patients who are on LTOT	n: 85 (%57.5) n:19(%36.3)
No hospitalizations in 1 year 1 hospital stay in 1 year 2 hospital stays in 1 year Number of hospitalizations in 1 year ≥3	n:41 (%39.42) n:32 (%30.76) n:20 (%19.23) n:9 (%8.65)
1 year No exacerbation 0 Number of exacerbation in 1 year: 1 Number of exacerbation in 1 year: 2 Number of exacerbation in 1 year: 3 Number of exacerbation ≥4 per year	n:33 (%31.73) n:37 (%35.57) n:20(%19.23) n:9 (%8.65) n:5 (%4.80)
GOLD1 Mild GOLD2 Moderate GOLD 3 Severe GOLD 4 Very Severe	n:1(%0.96) n:27 (%25.96) n:59 (%56.73) n:17 (%16.34)

GOLD:Global Initiative for Chronic Obstructive Lung Disease, LTOT:long term oxygen therapy BMI: Body mass index
Comorbidity: The coexistence of two or more diseases or disorders.

indicates that it is a candidate for COPD in the early period before COPD occurs. This allows us to take precautions against COPD.

Additional Findings A statistically significant negative correlation was identified between disease duration and magnesium levels (p=0.033). The group with more years of smoking has lower magnesium. The result was not statistically significant (p=0.55) CRP value was found to be higher in the group with low magnesium value. It was not statistically significant. (p=0,868) (Table 2). Sex comparisons revealed that smoking rates and serum calcium levels were significantly higher in males than in females (p=0.044 and p=0.001, respectively) (Table 3). Our findings suggest that magnesium may play a protective role in patients with COPD, potentially mitigating disease severity. These results underscore the importance of Mg in the management of COPD, warranting further research to explore its therapeutic implications.

Discussion

Magnesium, a crucial intracellular cation, plays a significant role in regulating bronchial tone and respiratory muscle function. Its deficiency has been associated with bronchospasm and respiratory distress, underscoring its importance

Table 2 Parameters of patients according to magnesium value

	n	low magnesium	n	Normal Magnesium	p
Age	26	65,23±10,38		67,10±8,21	0,106
Cigarette	26	19.74±9.80	78	18.85±10.517	0.55
BDI	26	26.15±5.51	78	26.16±6.02	0.819
Comorbidity	26	0.70±0.80	78	0.66±0.79	0.932
hospitalization	26	1.35±1.02	78	1.27±0.211	0.698
disease year	26	10.73±6.32	78	9.01±4.65	0.033
Attack	26	2.833±1.418	78	2.769±1.557	0.624
CAT	26	13.92±4.739	78	12.230±4.607	0.030
MRC	26	2.079±0.627	78	1.576±0.724	0.029
Calcium	26	9.117±0.552	78	9.278±0.498	0.718
CRP	26	17.471±30.171	78	16.874±29.708	0.868
WBC	26	9.936±5.271	78	9.897±2.821	0.109
neutrophil	26	69.250±13.422	78	64.910±1.219	0.068
Calcium	26	9.11±0.55	78	9.27±0.49	0.718
FEV1	26	42.423±13.546	78	45.579±13.676	0.753
FEV1/FVC	26	67.076±5.999	78	64.679±6.657	0.845
FVC	26	70.42±18.47	78	67.32±19.46	0.693
FEF25-75	26	31.34±15.26	78	35.53±14.69	0.013

FEF25-75: Mid-expiratory flow rate, FEV1: 1. Forced expiratory volume per second,
FVC: Forced vital capacity, CAT: The COPD Assessment Test, MRC:Modified Medical Research Council
Comorbidity: The coexistence of two or more diseases or disorders.

Table 3 Distribution of patients' parameters according to gender

	n	Male	n	Female	p
Age	87	66.72±8.94	18	65.77±8.14	0.617
Cigarette	87	20.17±9.32	18	15.28±12.65	0.044
comorbidity	87	0.67±0.78	18	0.67±0.79	0.663
Attack	87	2.92±1.44	18	2.22±1.53	0.724
Magnesium	87	2.093±0.19	18	1.99±0.29	0.411
Calcium	87	9.26±0.45	18	8.72±183	0.001
Hospitalization	87	1.46±1.00	18	0.83±0.98	0.652

Comorbidity: The coexistence of two or more diseases or disorders.

in chronic respiratory conditions, such as COPD (14). Magnesium also contributes to membrane stabilization and supports various physiological processes, including modulation of inflammation and smooth muscle relaxation (13). Previous studies suggested a potential correlation between insufficient magnesium intake and the development of asthma or COPD (9). The present study, with a mean patient age of 66.63 ± 8.79 years, aligns with findings by Singh et al., where the mean patient age was reported as 60.4 ± 6.5 years (15). The male-to-female ratio in this study (82.69% male, 17.30% female) contrasts with Singh et al.'s study, in which the proportions were 58% and 42%, respectively (15). The prevalence of hypomagnesemia varies considerably across studies. Rajjab et al. reported hypomagnesemia in 33.76% of patients with COPD (16), while Kumar et al. and Makwana et al. found higher rates of 45% and 57%, respectively (13,17). In the current study, 25% of COPD patients exhibited hypomagnesemia during acute exacerbations, which was relatively low but still significant. The wide variation in hypomagnesemia prevalence may reflect differences in the study populations, dietary magnesium intake, and comorbid conditions. Consistent with other studies, this study observed that hypomagnesemia was more prevalent in advanced COPD stages (GOLD stages 3 and 4). This finding corroborates those of Shah et al., who associated hypomagnesemia with advanced disease stages and frequent exacerbations (18). In the present cohort, 59% of the patients were in GOLD Stage 3, and hypomagnesemia was correlated with worsening COPD severity indicators, such as higher CAT and mMRC scores ($p = 0.030$, $p = 0.029$). Different studies have found that magnesium levels are low in advanced stages and acute exacerbations of COPD. (19,20). The association between low magnesium levels and increased frequency of exacerbations has been highlighted in previous studies. They reported a negative correlation between FEV1 and magnesium levels, with lower magnesium levels being associated with higher rates of exacerbations (21,22). Similarly, in this study, a negative correlation between magnesium and FEV1 was observed, although this was not statistically significant. However, a significant positive correlation was found between magnesium levels and FEF 25-75, suggesting a primary impact of hypomagnesemia obstruction ($p = 0.013$).

Several factors may contribute to hypomagnesemia in COPD patients. These include heavy smoking, poor dietary intake, and the use of medications such as corticosteroids and beta-agonists, which can promote magnesium depletion (23). In our study, 93% of the patients were current or former smokers and all were on corticosteroids and beta-mimetics. Although cigarette pack-years were higher among hypomagnesemia patients, the difference was not statistically significant ($p = 0.55$). Underweight and malnutrition are commonly reported in patients with COPD. Previous studies have reported malnutrition rates of 20-40% in patients with COPD (24). In our study, 8.65% of patients were underweight, a lower proportion than anticipated, with a mean BMI of 26.16 ± 5.89, indicating that most patients were of normal weight or overweight. No significant relationship was observed between BMI and magnesium levels. Magnesium deficiency may exacerbate systemic inflammation by promoting histamine release and neutrophil activation (25). Although our study found a negative correlation between magnesium and CRP levels, consistent with other findings (26), this was not statistically significant. Low magnesium levels are associated with prolonged hospital stays and increased mortality in patients with COPD (13,15). Although our study observed a higher attack frequency and hospitalizations among hypomagnesemic patients, these findings were not statistically significant ($p = 0.624$, $p = 0.698$). Our results suggest that magnesium deficiency in COPD exacerbations is associated with worsened dyspnea (MRC) and higher symptom scores (CAT), potentially indicating its role in disease progression and symptom severity. These findings underscore the importance of regular monitoring of magnesium levels in patients with COPD, particularly during exacerbations, as a potential marker for disease severity and prognosis. The protective role of magnesium in respiratory health emphasizes its importance in COPD management. Addressing hypomagnesemia through nutritional or pharmacological interventions may improve outcomes in patients with COPD, especially those with frequent exacerbations or advanced disease stages. Further studies are warranted to explore Mg supplementation as a therapeutic option for COPD.

Conclusion

This study highlights the importance of monitoring serum magnesium levels in patients with COPD during acute exacerbations, especially upon admission and throughout hospitalization. Our findings suggest that in addition to standard bronchodilator therapy, magnesium supplementation may safely and effectively reduce dyspnea in patients with COPD exacerbations. Our results also indicated that both pharmacological and non-pharmacological magnesium support could play a role in decreasing the frequency of COPD exacerbations and reducing the length of hospital stay. This study contributes to the growing literature on the role of magnesium in COPD management. However,

further multicenter studies with larger sample sizes and extended follow-up periods are necessary to establish the therapeutic efficacy of magnesium in COPD treatment and to provide more comprehensive guidelines for its use in clinical practice.

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

Ethical Approval
Ethics Committee approval for this study was obtained from Harran University, as evidenced by a letter dated 12.12.2022- HRÜ/22.24.28. This study was conducted in accordance with the principles outlined in the Declaration of Helsinki.

Consent to Participate and Publish
Written informed consent for participation and publication was obtained from all the participants or their legal guardians included in the study.

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

Authors Contributions

İMH: Data curation; Formal analysis; Investigation; Methodology; Visualization; Conceptualization; Funding acquisition; Data curation; Resources; Supervision; Writing-original draft. Writing-review & editing.

FG; Conceptualization; Validation Supervision; Methodology Writing-review & editing.

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ORIGINAL RESEARCH

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Effect of Ascorbic Acid on SH-SY5Y Cells at Different pH Levels

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Abstract

Objective

Cancer is a complex disease, and many different methods are used to treat it. Ascorbic acid is one of the nutritional supplements used to prevent the proliferation of cancer cells. Therefore, the effect of ascorbic acid at different pH levels on SH-SY5Y cells was investigated.

Material and Method

The cytotoxic effect of ascorbic acid at different pH levels and concentrations, and its effect on cell proliferation and gene expression were studied on SH-SY5Y cells.

Results

The pH and ascorbic acid levels that decreased the viability of the SH-SY5Y cell line and inhibited migration in wound healing were pH:8 and IC_{50}:4.15. In addition, it was determined that p53 expression level increased ($p<0.05$) and MDM2 and AKT1 expression levels decreased ($p<0.05$) at the pH and IC50 values mentioned above.

Conclusion

The findings of this study show that AA applied at high pH affects the viability of SH-SY5Y cells, inhibits their migration capacity, and alters the expression levels of p53, MDM2, and AKT1 genes.

Keywords: Ascorbic acid, Migration, pH, Proliferation, Viability

Introduction

Cancer is characterized as a complex disease that can be altered by genomic and epigenomic factors that occur with the change of gene expression in cells, causing cells to live longer and proliferate (1). There is substantial evidence that ascorbic acid (AA) has anticancer properties and has been proposed as a potential anticancer agent (2-4). The pharmacological use dose of AA has been redesigned for the treatment of treatment-resistant cancer cells in combination with radiotherapy, monotherapy, and chemotherapeutic

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drugs in various cancers, including breast cancer (5), colorectal cancer (6) melanoma (7), gastric cancer (8) and pancreatic cancer (9). A dose of 10 mM AA has been observed to cause apoptosis in neuroblastoma and melanoma cells and has been shown to act as an important modulator of the growth of murine myeloma cells in an in vitro colony assay (10). Some studies with vitamin C have shown that it causes cytotoxicity through the depletion of adenosine triphosphate in cancer cells (9, 11). Therefore, it can be proposed that vitamin C exerts an effect on the intracellular metabolism of malignant cells by disrupting the redox balance of H_2O_2; this represents a promising area for further investigation.

The process of metabolic reprogramming that occurs in cancerous cells is frequently accompanied by an increase in the acidity of the extracellular matrix. Measurement of pH in tumour tissue using microelectrode, magnetic resonance or fluorescence results in an extracellular pH ranging from 6.5 to 6.9. A notable characteristic of many tumours is the variation in pH levels across their structure These levels are usually higher near the surface of the tumour and lower in the middle. Surfaces formed by highly metastatic cells have a pH ranging from 6.1 to 6.4, whereas, for not metastatic tumours, the pH ranges from 6.7 to 6.9 (12).

The level of p53 in cells is positively correlated with the level of DNA damage and can vary. The presence of low levels of p53 leads to the arrest of the cell cycle, while high levels of this protein lead to apoptosis. Activation of PI3K/AKT leads to inhibition of p53 through activation of MDM2, another tumour suppressor. MDM2 is an oncoprotein that regulates tumour formation. The p53-induced expression of PTEN results in the formation of a p53/PTEN interaction, which in turn suppresses cell survival through the inhibition of the PI3K/AKT signaling pathway. PTEN forms a complex with p53 and affects the transcriptional activity of p53 by controlling its DNA binding. MDM2 is phosphorylated for nuclear translocation by the AKT kinase (13). Furthermore, it is known that p53 and PTEN interact and regulate each other at the transcriptional and protein levels. This could be an important control mechanism for switching between survival and death. PTEN stabilizes the p53 protein in two ways: by triggering the AKT-MDM2 complex and by increasing the acetylation of the p53 protein. It is therefore likely that the decreased p53 activity observed in PTEN-lacking tumor cells is due to this mechanism. As noted above, the PTEN and p53 proteins interact with each other and represent among the most significant control mechanisms for cells to switch between survival and death (14).

In addition to all this information, although the use of ascorbic acid as a natural antioxidant is widespread, it is unclear how it will affect SH-SY5Y cells at different pH levels. In the present study, we aimed to evaluate the effect of dose-dependent ascorbic acid on cytotoxicity, migration, and p53, MDM2, PTEN, and AKT1 expression in SH-SY5Y cells at different pH levels.

Material and Method

Cell Culture

SH-SY5Y (ATCC, USA) cells were incubated with Dulbecco's Modified Eagle Medium (Capricorn, Germany) supplemented with 10% fetal bovine serum (Sigma-Aldrich, USA) and 100 IU/mL penicillin, 10 µg/mL streptomycin (Sigma-Aldrich, USA).

MTT Assay

In our study, an MTT assay was performed according to the study of Riss et al. (15). The cells were seeded in 96-well plates at a seeding density of 10,000 cells/well, and then left to grow for 24 hours before treatment with ascorbic acid (Sigma) and different pH. Cells were then treated with 0, 2, 4, 6, 8, 16 mM AA and 6, 7, 8 pH for 24 hours. The pH of the cell culture medium was adjusted four times a day with HCl and NaOH to maintain a constant pH value (12). A multiscan plate reader (Synergy HTX BioTek, USA) was used to record optical densities at 570 nm. The percentage of viability of the cells was calculated according to the method of Yeap et al. (16).

Cell Migration Assay in Vitro

The cell migration assay was employed to ascertain fundamental cell migration characteristics, including speed, persistence, and polarity. A total of $1x10^4$cells were seeded into each well of a 6 well plate. The cells were incubated at 37°C in 5% CO_2 for 24 hours to allow them to adhere to the surface and form confluent monolayers. These confluent monolayers were then scratched with a sterile pipette tip. This left a ~ 0.4 mm wide scratch. To remove detached cells, the wells were washed with a culture medium (17). The culture medium was refreshed four times for 24 h with media containing IC_{50}= 5.46 mM, 3.66 mM, 4.15 mM AA, and 6,7,8 pH, respectively. The pH of the cell culture medium was adjusted with HCl and NaOH to maintain a constant pH value (12). Each wound size was visualized using an inverted microscope. The wound closure rate (%) was calculated using ImageJ software (18).

Real Time PCR Expression Analysis

The IC_{50} concentrations determined for pH=6, pH=7,

and pH=8 were applied to SH-SY5Y cells for 24 h, respectively. The real-time PCR expression analysis was conducted in accordance with the manufacturer's methodology. The primer sequences used were as follows; p53 F: TCTACAAGCAGTCACAGCACAT, p53 R: CAACCTCAGGCGGCTCATAG MDM2 F: TGGCGTGCCAAGCTTCTCTGT, MDM2 R: ACCTGAGTCCGATGATTCCTGCT, PTEN F: CGACGGGAAGACAAGTTCAT, R: AGGTTTCCTCTGGTCCTGGT, AKT1 F: TCTATGGCGCTGAGATTGTG, R: CTTAATGTGCCCGTCCTTGT, ACTB F: CATGTACGTTGCTATCCAGGC, R: CTCCTTAATGTCACGCACGAT. The results were normalized using the ACTB gene expression data. The CT values of the target genes were determined, and the relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ (Livak method).

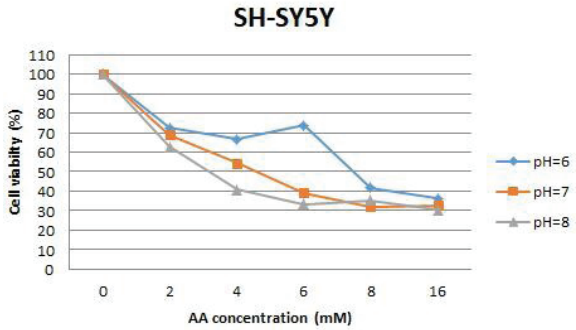
Statistical Analysis of Data

The statistical analysis was conducted using GraphPad Prism v.8 (San Diego, CA, USA). To evaluate the differences between the groups, the Student's t-test was employed. The level of significance was set at $p < 0.05$.

Results

MTT Assay

The effect of AA and different pH values on the viability of SH-SY5Y cells was evaluated by MTT assay. Six different concentrations of AA (0, 2, 4, 6, 8, 16 mM) and three different pH (6, 7, 8) media were used in this study. Results showed that the combination of pH 8 and AA (IC50) provided the most effective reduction in proliferation. (Fig. 1). IC50 values determined depending on pH change were 5.46 mM for pH=6, 3.66 mM for pH=7, and 4.15 mM for pH=8.



Cell	IC50 (AA, mM)		
	pH=6	pH=7	pH=8
SH-SY5Y	5.46	3.66	4.15

Figure 1
Cell viability and inhibition of SH-SY5Y cells due to different AA and pH levels. Values are mean ± SD of triplicate value. *p<0.05 vs. control group.

Migration Assay

Cell migration assay was performed to determine the effect of different AA and pH levels on the migration of SH-SY5Y cells. The migration of SH-SY5Y cells was inhibited by 31.57% for pH=6, 77.27% for pH=7, and 89.51% for pH=8, respectively, compared to the control group (Fig. 2).

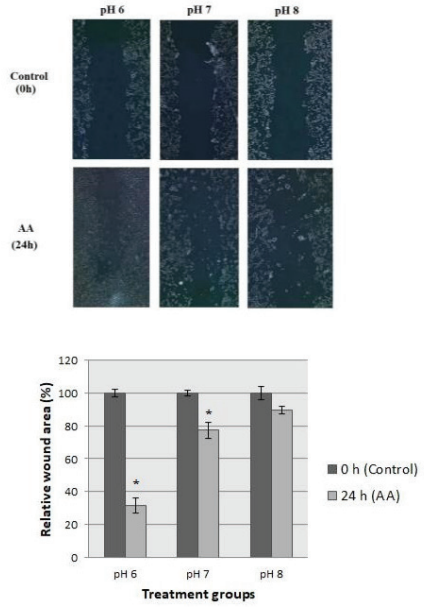


Figure 2
The SH-SY5Y cells were cultivated until they reached confluence, after which a scratch was made to create a wound. The combination treatment has been observed to have a more pronounced effect on cell migration. The initial scratch and 24-hour observation results are presented as the gap area. Each bar represents the mean ± SD. (*p<0.05, compared to control group).

Expression Analysis

In the context of p53, MDM2, and AKT1 expression results, a statistically significant outcome was observed in the comparison between pH 8 and pH 8 + AA ($p < 0,05$) Conversely, no statistically significant outcome was observed in the remaining experimental comparisons ($p > 0.05$) (Fig. 3).

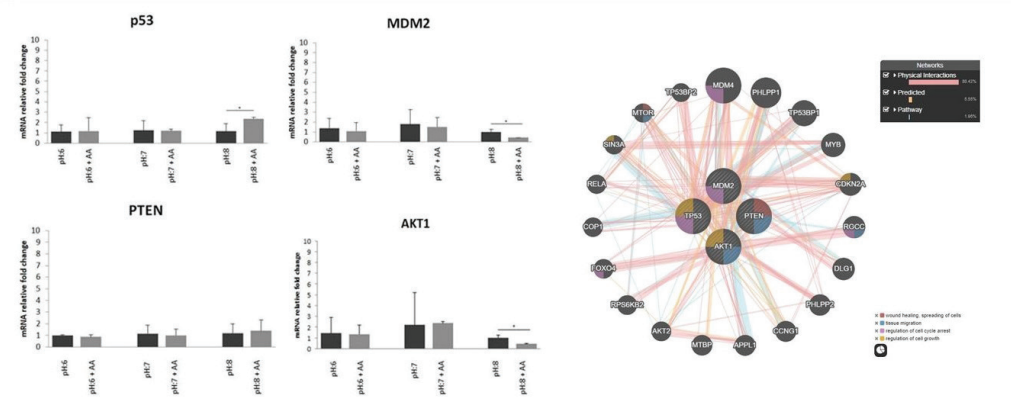


Figure 3

Relative mRNA expression results and statistical comparison between groups. Values are expressed as mean ± SD. * p<0.05. Gene network and function info from the Genmania database (https://genemania.org/).

Discussion

High-dose vitamin C is a common treatment option among complementary and alternative medicine practitioners for a range of various diseases. Except for the known complications of vitamin C in individuals with renal failure or glucose-6-phosphate dehydrogenase deficiency, high-dose intravenous vitamin C appears to be a safe intervention. However, it is emphasized that complementary and alternative medicine practitioners should be informed about the use of vitamin C in patients with cancer, and chronic and incurable diseases and should be careful about unexpected harm, drug interaction, or benefit (19).

Recent study information on the physiological properties of Vit-C, its pharmacokinetics, and results from preclinical reports indicate that high-dose Vit-C can be used effectively in the treatment of various tumor types (20). Tumour cells have been observed to rapidly consume glucose for glycolysis, resulting in the rapid production of lactate. This process enables tumour cells to obtain the energy they require to sustain their proliferation, regardless of the oxygen content present. Therefore, the higher metabolic rate of tumor cells has been considered as the main cause of the acidic tumor microenvironment (21). Tumours have a distinctive microenvironment, characterized by elevated temperatures, high expression of specific enzymes, a tendency towards a reduction in redox potential, and an acidic pH of approximately 6.5 (22).

Cancer cells can evade acid stress by activating and expressing proton and lactate transporters and exchangers. Therefore, they have an extracellular acidic and intracellular alkaline pH gradient. The alteration in the acid-base balance of tumour cells has been linked to an increase in several key characteristics, including proliferation, evasion of apoptosis, metastatic potential, aggressiveness, invasiveness, treatment resistance, and immune evasion. Reversing the pH gradient may be one of the most promising anticancer strategies paving the way for the development of new and innovative therapies. These include tumor-targeted pH-sensitive antibodies and pH-sensitive nanoparticle conjugates with anticancer drugs. An alternative approach is the oral or parenteral use of buffer systems, such as sodium bicarbonate, to neutralize tumour acidity. While buffering therapy does not pose any problems against standard treatment methods, it makes it possible to use different combinations to increase the effectiveness of the treatment (23).

The anticancer potential of pharmacological AA has been established in several cancer cells. It is known that pH may be a critical effect factor for multiple anticancer therapies. As a result of the investigation of the therapeutic effect of AA on cell lines PC3 and DU145 cultured at different pHs, it was shown that acidic pH inhibited AA uptake in PCa cells and weakened the cytotoxic activity of pharmacological ascorbic acid (12). In other studies, high doses of L-ascorbic acid reduced the viability of the HT29 cell line in vitro (24), and the combination of AA and selenium had an additional chemopreventive effect on the HCT116 and SH-SY5Y cell lines (1). It was observed that a 99.6% pure titanium plate was coated with 596.29 nmol ascorbic acid after application of 5 M NaOH and applied to hBCCs, MDA-MB-231 cells.

The results obtained after this application showed that it synergistically inhibited the proliferation, spreading, and migration of the cells (25).

AA contributes to its anticancer effects by regulating several key processes, including cell growth and differentiation, DNA methylation, the activity of the Ten-eleven translocation family protein (TET), hypoxia-inducible factor-1α (HIF-1α) signaling, and the regulation of gene expression, including transcription factors such as p53, NF-κB, and AP-1, as well as vascular endothelial growth factor (VEGF). These processes can modulate the expression of tumour suppressor genes and oncogenes (26). p53, one of the tumour suppressor proteins, has an important role in the regulation of factors such as cell cycle, apoptosis, aging, cell proliferation, and differentiation. In addition, it has been shown that its expression level changes in various cancer types and it is known to have an important effect (27).

MDM2 functions as an inhibitor of the p53 protein, thereby modulating the p53 signaling pathway (28). MDM2 inhibition has been shown to induce p53-mediated reversal of the Bcl2/Bax ratio and lead to cell death in PTEN-deficient colorectal cancer cells. PTEN is an important negative effector of the PI3K signaling pathway that can inhibit the activation of Akt and other downstream kinases (29). PTEN is a tumour suppressor gene that can halt cell proliferation and encourage cellular apoptosis (28). AKT, the cell survival oncoprotein (also referred to as protein kinase B), has a pivotal function in several processes that promote cell survival, proliferation, growth, and migration. This gene is over-activated in human cancers and is known to be closely associated with poor prognosis and treatment resistance (30).

In this study, the combination of pH=8 and 4.15 mM applied to SH-SY5Y cells was found to be the optimum treatment affecting the mechanism of these cells. Furthermore, p53 expression increased, while MDM2 and AKT expression decreased in this combination ($p < 0.05$). PTEN expression increased, but there was no statistical change.

The maintenance of acid-base balance (pH) is a fundamental requirement for the survival of cells. Due to the rapid proliferation of cancer cells, CO_2 and lactic acid production as a result of intensive respiratory requirements disrupt the acid-base balance of the cells and lead to a change in pH. A more profound comprehension of metabolism and pH regulation in cancer cells is imperative for the advancement of diagnostic instruments and novel therapeutic interventions, thereby enhancing the well-being of cancer patients. This study shows that AA applied at low pH has little effect on the proliferation of SH-SY5Y cells, but at high pH, the acid-base balance of the cells is disturbed and AA decreases proliferation in these cells. However, although it was observed that AA has anticancer effects in SH-SY5Y cells at different pHs in vitro, it should be noted that this effect cannot be compared with in vivo and should be supported by in vivo studies.

Conflict of Interest Statement
There is no conflict of interest.

Ethical Approval
Not applicable.

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

Author Contributions
OS: Conceptualization, methodology, investigation, administration, visualization, data curation, formal analysis writing-original draft.

PAK: Conceptualization, methodology, investigation, supervision, administration, writing—review a editing.

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REVIEWS

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Mitochondria and Cancer

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Abstract

Mitochondria generate energy through cellular respiration and regulate various cellular processes such as heat production, generation and detoxification of reactive oxygen species, metabolism, apoptosis, and calcium homeostasis. In human cells, large numbers of mitochondria are present, each containing multiple copies of mitochondrial DNA. Variations in mitochondrial DNA have been associated with the onset and progression of various diseases, including neurological, cardiovascular, and metabolic disorders and also several cancers. These variants can be important drivers of cancer and may play a crucial role in tumor development. Additionally, mitochondrial copy number changes and structural variations, such as deletions can be associated with different types of cancer. Therefore, understanding the fundamental mechanisms is highly crucial. The molecular genetic correlations of mitochondrial DNA alterations and cancer, emphasize the importance of mitochondrial integrity in maintaining cellular homeostasis. Gaining knowledge of these associations can help us comprehend cancer processes as well as potential routes for targeted treatments and prevention, while further investigation is still required.

Keywords: Mitochondria, cancer, mitochondrial genome, mitochondrial DNA variations

Structure and Function of Mitochondria

Mitochondria are organelles found in eukaryotic cells, which are responsible for generating energy in the form of adenosine triphosphate (ATP) through a process called cellular respiration (1). They are commonly referred to as the "powerhouses" of the cell because of their role in producing ATP, which is essential for a wide range of cellular functions, including metabolism, growth, and movement (2).

Organization of mitochondria is in the form of four morphologically and functionally distinct parts: (i) the outer membrane, permeable to ions and small molecules, whose traffic is mediated by specific

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transporters and channels; (ii) the intermembrane space, the region between the matrix and the cytosol, where important processes such as the exchange of proteins, lipids, metal ions and initiation of the apoptotic pathway occur; (iii) the inner membrane, comprised of respiratory complexes in its inward folds (cristae), which surrounds the matrix and enables the transport of ions, metabolites, proteins through specialized transporters; (iv) the matrix, containing mitochondrial DNA (mtDNA) and proteins, which are associated with important biochemical pathways such as the citric acid cycle and beta-oxidation of fatty acids (3-6). The process of cellular respiration involves a series of chemical reactions that take place within these compartments, which ultimately result in the production of ATP (7).

The oxidative phosphorylation system includes five protein complexes and two electron carriers embedded in the inner mitochondrial membrane (5). During respiration, electrons from nicotinamide adenine dinucleotide + hydrogen (NADH) and succinate are transferred to ubiquinone via complexes I and II, then pass through complex III, cytochrome c, and end at complex IV. The energy from electron transfer through complexes I, III, and IV pumps protons from the mitochondrial matrix to the intermembrane space which in turn activates ATP synthesis in complex V (7-11). The structure of mitochondria and energy metabolism is summarized in Figures 1a and 1b, respectively.

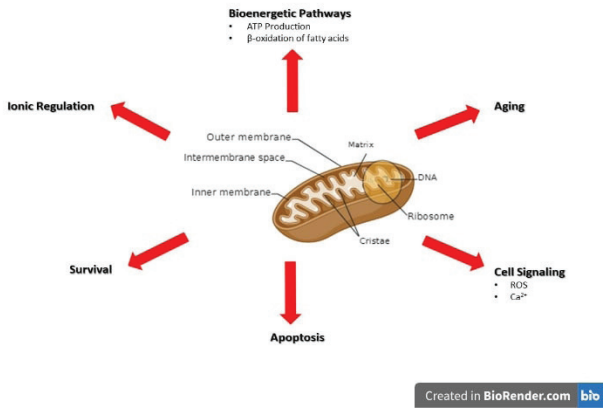

Figure 1A
Represents morphological and functional organization of mitochondria.

In addition to producing ATP, mitochondria are also involved in several other important cellular processes such as heat production, generation and detoxification of reactive oxygen species (ROS), regulation of

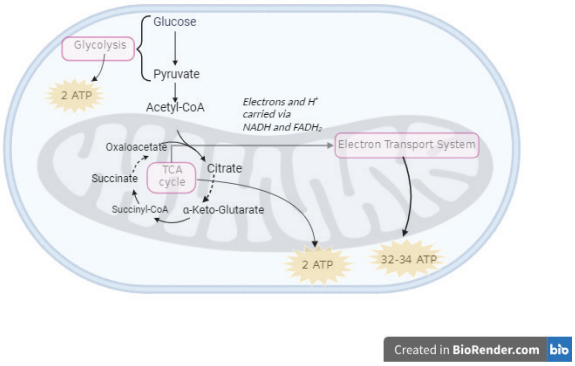

Figure 1B
Illustrates and summarizes the energy metabolism pathways (Glycolysis, Tricarboxylic acid (TCA) cycle and Electron transport system (ETS), FADH2: Flavin adenine dinucleotide) Adapted from: Libretext 2020 120 & Koklesova 2022 121 (Created in Biorender.com)

intracellular calcium (important for muscle contraction and other cellular processes), lipid metabolism, synthesis of steroid hormone, certain amino acids and heme (9). Mitochondria also play a role in apoptosis (programmed cell death), which is an important process for removing damaged or unwanted cells from the body. Mitochondria release certain proteins that trigger the apoptotic pathway when a cell is damaged or no longer needed in mammalian cells (12).

Mitochondria have their DNA, known as the mitochondrial genome or mtDNA, which is separate from the cell's nuclear DNA (nDNA), and are believed to have originated from free-living bacteria that were engulfed by ancestral eukaryotic cells in a process called endosymbiosis (13). This is supported by the fact that mitochondria have their ribosomes, and the structure of their DNA is similar to that of bacteria (14).

Mitochondrial Genome

Margit Nass and Sylvan Nass first described and isolated mitochondrial DNA in 1963 (15). However, the first complete mtDNA sequence was published 18 years later in 1981 as the mtDNA Cambridge reference sequence (CRS) (16, 17). Currently, the revised CRS (rCRS—revised Cambridge Reference Sequence), a modified version of the sequence presented by Anderson et al., is used for nucleotide numbering of the mitochondrial genome (16-19).

The mtDNA is a circular double-stranded DNA molecule that is typically between 16,000 and 20,000

base pairs long, depending on the organism (20, 21). In humans, the mitochondrial genome contains no histones and is only 16,569 base pairs long (2, 7). There are no introns in the mitochondrial genome and all genes are adjacent to each other with few exceptions. It consists of a total of 37 genes, including 13 polypeptides encoding four of the five complexes (complexes I, III, IV, and V) that make up the oxidative phosphorylation system discussed previously, as well as 22 transfer RNAs (tRNA) and 12S and 16S ribosomal RNAs (rRNA) required for mitochondrial protein synthesis. Along with mitochondrial genes, nuclear genes also play a role in the assembly mechanism of oxidative phosphorylation complexes (5, 7, 11, 22).

The two mtDNA chains, named light (L) and heavy (H), are quite different in their base composition. The heavy chain is rich in purines and the light chain is rich in pyrimidines. The distribution of genes in the two chains is asymmetrical. The L-chain contains only the ND6 gene and some t-RNA-encoding genes, while the 12S and 16S ribosomal RNAs and tRNAs and most of the genes encoding proteins are located on the H-chain (13, 23, 24). The approximately 1 kb long non-coding region (Displacement loop, D-loop) contains the H-chain replication origin and promoters required for the transcription of both chains. The mitochondrial DNA is illustrated in Figure 2.

The genetic code of mitochondrial DNA shows some differences compared to the universal genetic code. The “UGA” stop codon in the human nuclear code encodes tryptophan, and the “AUA” (isoleucine) is encoded as methionine in the mitochondrial genome. “AGA” and “AGG”, which encode arginine, are arguably known as non-standard stop codons in mitochondria (19).

Mitochondrial DNA has a 10-20 times faster evolution rate than the nDNA and is therefore, more susceptible to mutations (25). Lack of protective histones, lack of intronic regions, ineffective repair mechanisms, high replication speed in mtDNA, and low fidelity of mtDNA polymerase are the reasons for the higher incidence of mtDNA mutations (25-27).

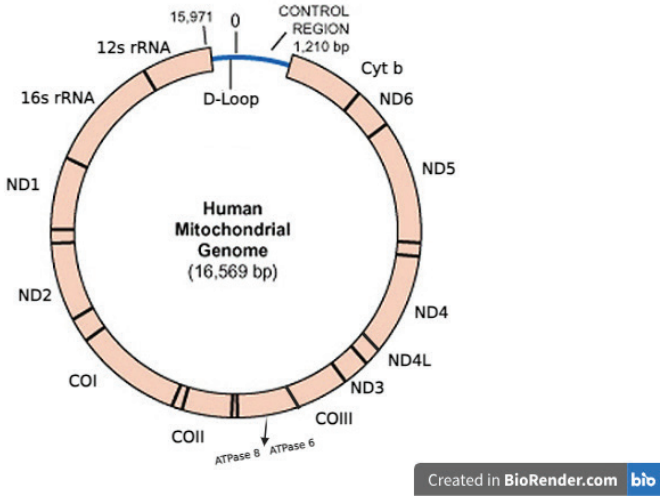

Figure 2
Mitochondrial DNA
The mitochondrial genome is represented in the figure. Cyt b: Cytochrome B; ND6: Mitochondrially Encoded NADH:Ubiquinone Oxidoreductase Core Subunit 6; ND5: Mitochondrially Encoded NADH:Ubiquinone Oxidoreductase Core Subunit 5; ND4: Mitochondrially Encoded NADH:Ubiquinone Oxidoreductase Core Subunit 4; ND4L: Mitochondrially Encoded NADH:Ubiquinone Oxidoreductase Core Subunit 4L; ND3: Mitochondrially Encoded NADH:Ubiquinone Oxidoreductase Core Subunit 3; COIII (or MTCO3): Mitochondrially Encoded Cytochrome C Oxidase III; ATPase 6 (or MT-ATP6): Mitochondrially Encoded ATP Synthase Membrane Subunit 6; ATPase 8 (or MT-ATP8): Mitochondrially Encoded ATP Synthase Membrane Subunit 8; COII (or MTCO2): Mitochondrially Encoded Cytochrome C Oxidase II; COI: or MTCO1): Mitochondrially Encoded Cytochrome C Oxidase I; ND2: Mitochondrially Encoded NADH:Ubiquinone Oxidoreductase Core Subunit 2; ND1: Mitochondrially Encoded NADH:Ubiquinone Oxidoreductase Core Subunit 1; 16s rRNA: 16S ribosomal RNA; 12s rRNA: 12S ribosomal RNA. Adapted from: Cold Spring Harbor Laboratory's DNA Learning Center 2024 122 (Created in Biorender.com)

Unlike the nuclear genome, which is inherited according to Mendelian inheritance laws, the mitochondrial genome shows matrilineal inheritance (28). This is because the developing embryo receives the majority of its cytoplasm and organelles, including its mitochondria, from the egg cell (29).

Heteroplasmy and Threshold Effect

There are hundreds to several thousand mitochondria in every human cell. Each mitochondrion contains up to ten copies of mtDNA packaged in nucleoprotein structures called nucleoids (30). Cells and tissues that need more energy usually have more mtDNA. Mostly, all mtDNA copies are identical and this is called homoplasmy (31, 32). However, errors that occur during mtDNA replication or repair can result in the formation of a mutant mtDNA molecule, and these can proliferate clonally by unknown mechanisms, eventually resulting in a metastable state called heteroplasmy (33, 34). In heteroplasmy, mutant and wild-type genomes coexist at different rates in the same organelle/cell/tissue (35). It has been shown that a low level of heteroplasmy can also occur in normal cells, and therefore the mutation load of mtDNA must exceed the minimum critical biochemical threshold (usually 70-90%) for mitochondrial dysfunction to occur in a tissue (31, 32). Since the energy requirements of tissues and organs are different from each other, the symptomatic effect of mutant mtDNA ratio differs according to organs (33).

In some cases, heteroplasmy can be benign and have no noticeable effect on the organism. In other cases, it can lead to mitochondrial diseases or disorders, which can affect a wide range of functions in the body that rely on energy production (36). As a result, mutations in the mitochondrial genome have been implicated in a variety of diseases, including neurodegenerative disorders, metabolic disorders, and aging. Additionally, mitochondrial haplotypes refer to a set of genetic variations or polymorphisms that are inherited together on the mtDNA from a single parent or ancestor and are used to trace maternal lineages and evolutionary population history (37-40). The analysis of mitochondrial haplogroups has been used to investigate a range of topics, including human migration patterns, genetic diversity within populations, and the association between specific haplogroups and disease susceptibility, including cancer (41-43).

Mitochondrial Variants and Cancer Relationship

In literature, mitochondrial mutations have been associated with different mitochondrial diseases that mostly affect the nervous system and muscle tissues (44, 45). Primary mtDNA diseases are mostly due to maternally inherited point mutations and large deletions that usually occur de novo during embryonic development (46-55). Recently, it was shown that mitochondrial dysfunction plays a key role in diseases such as Alzheimer's, major depressive disorder, and coronary artery disease (56-61). However, precise mechanisms of pathogenesis are still unknown.

Contrary to conventional wisdom, functional mitochondria are essential for the cancer cell. Although mutations in mitochondrial genes are common in cancer cells, they do not generally inactivate mitochondrial energy metabolism but rather alter the mitochondrial bioenergetic and biosynthetic state (62). It has been reported that the rate of individuals with somatic mutations in the nuclear and/or mitochondrial genome may differ between 13% and 63% depending on the type of cancer (63, 64). Additionally, it is possible to identify mtDNA variations in a single tumor type or different cancer types (65).

Somatic mutations that may be associated with tumorigenesis have been reported in many mitochondrial genes, particularly those encoding the mitochondrial respiratory chain proteins (64, 66, 67). These mutations include both synonymous and non-synonymous somatic mtDNA alterations (63, 68). In general, the most common variations associated with carcinogenesis are in complex I genes (69). In contrast, the number of somatic variations reported for complex III (cytochrome b, mt-CYB gene), which is solely encoded by mtDNA, is scarce, except for bladder cancer (70). Among the protein-coding genes, complex I and IV mutations are thought to be more potent in inducing carcinogenesis (67, 71).

Both the coding and non-coding sections of the mtDNA have been found to include mutations in all forms of cancers including glioblastoma (72, 73). Strong selection is applied to tumor cells as a result of metabolic dysregulation and its aftereffects. Therefore, it appears that obtaining somatic mtDNA mutations that affect oxidative phosphorylation is another way to promote tumor growth (63).

Various cancers have been associated with mtDNA mutations in D-loop and other mitochondrial genes. Particularly those in the genes ND4 and ND5 that encode the subunits of Complex I of the respiratory chain, have been linked to several malignancies, including those of the liver and kidney (76). These alterations frequently increase cellular proliferation and apoptosis resistance, advancing cancer. Moreover, conflicting data links the ND3 G10398A

mutation to an increased risk of cancer, particularly breast cancer (77, 78) and the T16519C mtDNA control region variant is associated with endometrial cancer (79). Also, mutations in ND5, ATP6, and ATP8 are frequently observed in breast cancer (66). Additionally, a synonymous T6777C SNP in cytochrome c oxidase subunit 1 (CO1), seems to lower the incidence of ovarian cancer along with variants in several nDNA mitochondrial genes (80, 81). In colorectal cancers, rRNA point mutations are more common than tRNA and both non-synonymous and synonymous mutations can be observed in all mitochondrial genes; controversially, in stomach and lung cancers, point and indel mutations are detected in tRNA (82, 83). Furthermore, D310 instability is also commonly detected, especially in bladder, breast, colorectal, head and neck, and lung cancers (66). Finally, the mtDNA control region variant C150 has been associated with an increased risk of human papillomavirus (HPV) infection and cervical cancer (64, 84).

The polymorphic D-Loop region is thought to be critical for modulating mtDNA transcription and replication. D-loop polymorphisms have been linked to an increased risk of numerous cancers, including breast, cervical, skin, liver, stomach, and colon (85). In addition, our study suggested that some unique mitochondrial variations may be evaluated as prospective cancer biomarkers for the risk and progression of brain tumors and that the D-loop individual variations in mtDNA may play a crucial role in glioma biology (86). Therefore, in cancer research, the analysis of the non-coding D-loop control region as well as the coding regions of the mitochondrial genome is also important (87, 88). Somatic mutations of the D-loop region are detected more frequently in advanced cancers (89). In several tumors, increased mutation numbers have been associated with poor prognosis (90).

Although mutations in tRNAs encoded by mitochondria have been reported frequently in other respiratory chain diseases, the number of variations associated with cancer is quite low (22). This is because tRNA mutations affect secondary structures and alter mitochondrial function by causing instability in the stem and loop regions (68, 91). Similarly, the impacts of rRNA mutations are substantially more severe than alterations in protein-coding genes, making rRNA modifications uncommon (22).

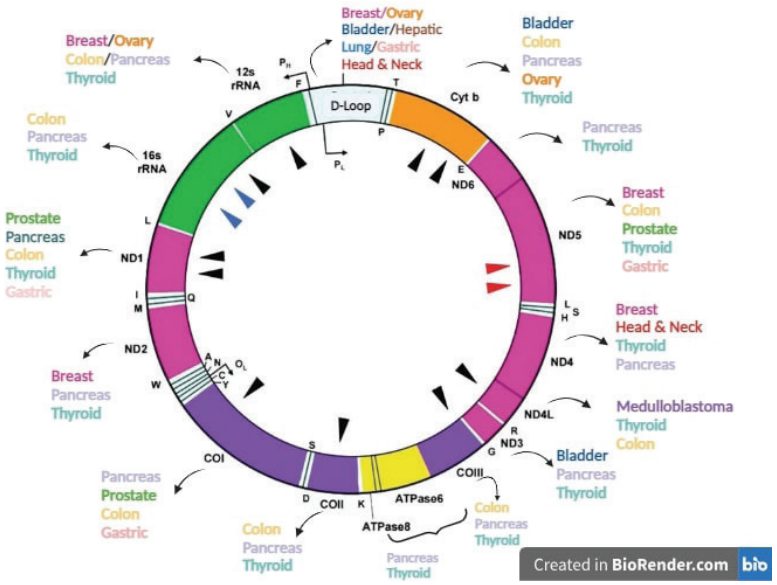

Figure 3

Mitochondrial DNA and related cancers

Human mitochondrial DNA is a ~16 kbp circular, double-stranded DNA containing 37 genes, encoding 13 electron transfer complex (ETC) component proteins, 2 ribosomal RNAs, and 22 transfer RNAs. The mutations of ETC coding regions, D-loop and rRNA genes in mtDNA were commonly found in various cancers. mtDNA mutations in each region and related cancers are illustrated. Black arrowheads represent the mtDNA somatic mutations by homoplasmic alterations; blue arrowheads are rarer heteroplasmic substitutions and red arrowheads are mixed homoplasmic/heteroplasmic variants. Adapted from: Errichiello et.al 2018 123 (Created in Biorender.com)

Furthermore, deletions detected in mtDNA have also been associated with different cancers. A 21 bp deletion causing increased cell growth due to overexpression of the mt-CYB gene has been found in bladder cancer (92, 93). The other most frequently detected structural variation is a 4977 bp deletion that has been found in different cancer types such as breast, colorectal, stomach and head & neck, comprising five tRNA genes and seven protein-coding genes (34, 66, 94). Figure 3 summarizes the mtDNA variations related to several cancers.

Mitochondrial copy number may also vary in several cancers. It has been shown that the mtDNA copy number is increased in some cancers (such as thyroid, pancreas and prostate) and decreased in others (for instance bladder, breast, colorectal and stomach), but the findings of several investigations contradict one another (64, 95). The exact mechanism of copy number variations (CNV) is still unknown. However, it is considered that the increase may be due to compensation for impaired oxidative phosphorylation, while the decrease in CNV might be caused by mutations in the D-loop region, which plays a role in replication (64, 88, 89).

Apart from mutations, structural variations and copy number changes in mitochondria, mutations in nuclear genes that are part of the mitochondrial proteome can also cause copy number and stability changes in mtDNA and thus play a role in the formation and development of cancer (96-98).

Overall, while more research is needed to understand the relationship between mtDNA mutations and cancer fully, there is growing evidence that these mutations may be important drivers of tumorigenesis and could serve as potential targets for cancer prevention and treatment.

Mitochondrial Bioenergetics and Cancer Relationship

Common disorders caused by defects in mitochondrial function are known to influence energy production in cells and can produce a wide range of symptoms across different organs. (99). Moreover, using Genome-Wide Association Studies (GWAS), mitochondrial variations were investigated to identify their possible contribution to cancer risk (100). To determine the causal link between mitochondrial-related genetic variations and various cancer types, Mendelian Randomization (MR) methodology was applied to the variants, which helps in overcoming reverse causality and confounding variables that frequently restrict observational studies. As a result, strong evidence has been discovered correlating a fundamental enzyme for the production of isoprenoid, Farnesyl Diphosphate Synthase (FDPS) expression level with the risk of breast cancer (101, 102). On the other hand, the NOP2/Sun RNA Methyltransferase 4 (NSUN4) (takes part in the assembly of the mitochondrial ribosome) expression level is associated with prostate and breast cancers (100).

Cancer cells use glycolysis and the mitochondrial oxidative phosphorylation system (OXPHOS) as their principal energy sources. There is often a shift in energy metabolism from oxidative phosphorylation to glycolysis, a process known as the Warburg effect (103). This change enables cancer cells to survive and proliferate even in the absence of oxygen, a condition known as hypoxia. The specific mechanisms underlying this shift are not fully understood, however, it is thought to be linked to mutations in genes involved in mitochondrial metabolism and changes in signaling pathways (104).

There may be two classes of mutations in cancer cell mtDNA: mutations that impair OXPHOS and serve to stimulate neoplastic transformation, and those that facilitate cancer cell adaption to changing bioenergetic environments (62). Thus, tumor growth can be inhibited by modifying the production of metabolites in mitochondria or the OXPHOS genes (105). Instead of using glycolysis, a wide variety of cancer cell types rely on OXPHOS to increase their potential for tumorigenicity (106). Cancer cells upregulate the OXPHOS and TCA cycles to produce more ATP than the surrounding normal cells and develop resistance to chemotherapy (105, 107). OXPHOS allows mitochondria to produce ATP primarily by using pyruvate produced during glycolysis. Thus, mitochondrial malfunction in cancer cells can cause an increase in ROS production, contributing to genomic instability and cancer progression.

The absence of histones, inefficient DNA repair mechanisms, and proximity to ROS generated by the OXPHOS system all contribute to the high mutation rate observed in mtDNA, which is approximately 10–17 times higher than that of the nuclear genome (35). Furthermore, altered mitochondrial function can affect the expression of genes implicated in apoptosis, conferring resistance to chemotherapy and radiation therapy (108).

In summary, mitochondrial bioenergetics plays a critical role in cancer development and progression. Understanding the mechanisms underlying mitochondrial dysfunction in cancer cells and developing

strategies to target these pathways could lead to new and effective cancer treatments.

Future Aspect: Mitochondria-targeted Approaches in Cancer

Targeting mitochondrial bioenergetics has emerged as a potential therapeutic strategy for cancer treatment. For instance, extracellular citrate is imported by cancer cells to stimulate their proliferation, and it is oxidized in the mitochondrial TCA cycle to make ATP. Similar to citrate, isocitrate is an intermediate metabolite in the citric acid cycle that is present in both the cytosolic component and the mitochondria (105). On the other hand, for the past 100 years, metformin has been used to treat diabetes. Clinical investigations conducted in the past few years have demonstrated its efficacy against cancer (109). Because insulin stimulates the growth of breast cancer cells, metformin lowers insulin levels in breast cancer patients to diminish tumor cell proliferation. At the same time, it suppresses tumor progression by blocking complex I and PI3K pathway (105). On the other hand; in the form of ammonium cations, rhodamine can selectively target mitochondria because of the inner mitochondrial membrane's (IMM) negative potential, shown in MCF-7 cells- which is a widely used human breast cancer cell line (110).

Furthermore, Atovaquone is an approved antimicrobial medication that has lately shown anti-cancer activity and potential in clinical trials treating ovarian cancer (108). It reduces ATP synthesis by blocking mitochondrial complex III and increasing ROS levels, which in turn limits tumor cell proliferation (103).

Dichloroacetic acid is a novel anti-cancer drug that inhibits the TCA cycle and has been demonstrated in clinical trials to have both synergistic and inhibitory effects on liver cancer cells (109). Additionally, IACS-010759 is a small molecule of therapeutic grade that inhibits complex I of the mitochondrial electron transport chain, which is effective in treating acute myeloid leukemia (AML) and brain malignancies (103). Nevirolol is a third-generation beta-1 adrenoceptor inhibitor. Not only was it initially used to treat heart failure and hypertension, but it can also be used as a novel anti-cancer drug to treat cancer patients (111).

A recent technique known as " RNA polymerase mitochondria (POLRMT) targeting" suppresses mitochondrial transcription, depriving tumor cells of an energy source (112). Small compounds that are lipophilic and positively charged, peptide carriers, or metal complexes like ruthenium or iridium can all be used as mitochondrial targeting agents (111).

At present, methods for delivering medications that target mitochondria include surface modification of nanocarriers or chemical ligation of active pharmaceuticals by pro-mitochondrial agents (111). In contrast to traditional methods of delivering drugs to the mitochondria, mitochondria-targeted nanosystems provide the following advantages: delivering conventional medications via nanomaterials can improve drug solubility, extend drug half-life in vivo and enhance bioavailability, reduce side effects, and increase drug concentration and therapeutic index at the tumor site.

The primary method of delivering anti-cancer medications or nanoparticles to mitochondria is destroying mitochondria using mitochondria-cytotoxic peptides or peptide assemblies and combining them with chemotherapy or photothermal-promoted morphology transformation (PMT) (113). Furthermore, using nanoscale tubes, researchers proved that cancer cells can take over the mitochondria of immune cells. This discovery demonstrates how cancer cells rely on healthy cells for survival and proliferation (35).

Moreover, triphenylphosphonium (TPP) can preferentially target mammalian cells' mitochondria (111, 114). TPP-based anti-cancer drugs primarily target cancers with high membrane potential and deliver the medication to the tumor cell mitochondria for treatment. TPP's lipid solubility allows it to cross biological membranes easily. Currently, TPP is used in two ways: directly coupled with pharmaceutical compounds or modified to target mitochondrial nanosystems (111). Other TPP derivatives, alone or in combination with other therapeutic compounds, have shown promising anti-cancer properties. For example, dodecyl TPP inhibited the proliferation of suspended breast cancer stem cells in a dose-dependent manner (111, 115). However, difficulties can arise since TPP does not target all tumor cells due to its limited applicability as a mitochondrial targeting agent for tumor cells.

Furthermore, drug combinations incorporating functional peptides that target the mitochondria can increase tumor cell targeting, but they do not completely protect normal cells. Peptide-drug conjugates (PDCs) appear to respond mostly to single-factor stimuli (113). Although some studies have shown that functional peptides are biocompatible, there is still dispute about their tumor degradation rate and long-term safety. The U.S. Food and Drug Administration (FDA) has approved two PDCs for use in clinical trials: LUTATHERA (Novartis Pharmaceuticals Corporation; Basel, Switzerland)

which treats somatostatin receptor-positive pancreatic and gastrointestinal neurosecretory cancers, and PEPAXTO (Oncopeptides AB; Stockholm, Sweden) which treats recurrent bone marrow cancer (115, 117, 121).

Finally, Photodynamic therapy (PDT), photothermal therapy (PTT), chemodynamic therapy (CDT) and sonodynamic therapy (SDT) have been highly discussed in recent years (118, 119). PDT and PTT are non-invasive, easy to control, and possess low side effects but face problems of reduced depth of penetration and toxicity. On the other hand, CDT employs endogenous hydrogen peroxide but is interfered with by glutathione in tumor cells. In addition, SDT utilizes ultrasound for deeper penetration, however, the process is ineffective in hypoxic conditions. The combination of all these treatment modalities increases the overall efficacy and decreases the risk of tumor recurrence (118, 119). In summary, various mitochondria-targeted cancer therapies have been explored, each with its own benefits and limitations.

Conclusion

Mutations and alterations in mtDNA have been linked to various forms of cancer, as these genetic changes can disrupt normal mitochondrial function, leading to increased oxidative stress and impaired cellular energy metabolism. The molecular genetic associations between mtDNA mutations and cancer highlight the importance of mitochondrial integrity in maintaining cellular homeostasis. Understanding these connections provides valuable insights into the mechanisms of tumorigenesis and opens potential avenues for targeted therapies and diagnostic tools in oncology. Overall, while more research is needed to fully understand the relationship between mtDNA mutations and cancer, there is growing evidence that these mutations may be important drivers of tumorigenesis and could serve as potential targets for cancer prevention and treatment.

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

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Authors Contributions
GG: Conceptualization; Formal analysis; Investigation; Visualization; Writing – original draft; Writing – review & editing (crticial review)

ŞKY: Conceptualization; Data curation; Formal analysis; Investigation; Validation; Visualization; Writing – original draft; Writing – review & editing (crticial review)

CBA: Conceptualization; Data curation; Investigation; Project administration; Validation Supervision; Writing – original draft; Writing – review & editing (crticial review)

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