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ABOUT THE JOURNAL

About the Health Sci. Q.

Health Sciences Quarterly (Health Sci. Q.) journal as known by the name of "Journal of Scientific Perspectives" until April 2021 which has been published since 2017 is an international peer-reviewed journal of HOLISTENCE ACADEMY. It is published quarterly in January, April, July, and October. All manuscripts submitted for publication are evaluated by the editor-in-chief, section editor, editorial board, and referees. In addition, the journal provides a medium for highlighting selected articles reporting highly significant original findings, as Editor's Choice Manuscripts.

Aims and Scope

Health Sciences Quarterly (Health Sci. Q.) is an open-access journal that publishes original research papers, case reports, and reviews, clinical studies covering a wide range of subjects in life sciences and medicine as well as clinical and experimental investigations only in English.

Researchers in health sciences will find much of great use and interest in the Health Sci. Q.

HSQ aims to supply scientists of health with resources in order to provide the scientific knowledge through the publication of peer-reviewed, high quality, scientific papers and other material on all topics related to Medicine, Pharmacy and pharmaceutical sciences, Dentistry, Nursing, Bioethics, History of medicine, Health economics, Pharmacoeconomics, Medical education, Public health, and Epidemiology.

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ORIGINAL ARTICLE

The relationship of nurses' best practice examples with organizational resilience in combating the COVID-19 pandemic: The case of a state hospital

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Abstract

The purpose of this research, it is to determine the relationship between nurses' best practice examples and organizational resilience in the COVID-19 process. This is a mixed methodology research (qualitative and quantitative). The sample of the study consists of 193 nurses. 3 themes, 6 categories and 27 codes representing best practice examples were identified in the qualitative phase of the research. In the quantitative phase of the research; while the nurses' strategic awareness score is 3925, adaptability score is 5361 and integrity score is 4397, the total score is 13683. The research identified 27 examples of best practice. It was found that the level of organizational resilience of nurses was high and there was a significant relationship between the level of education of nurses and the level of strategic awareness and between time in the profession and the level of strategic awareness, adaptability and integrity. A similar relationship was found between nurses' best practice examples and organizational resilience levels during the COVID-19 pandemic.

Keywords: Resilience, COVID-19, nursing, best practices

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Introduction

Recent global events such as pandemics, crises, and emergencies can hinder a system's ability to deliver its products or services effectively [1]. COVID-19 pandemic is not limited to a single hospital or country; it is testing the resilience of health systems worldwide [2].

In an uncertain environment, a resilient organization can achieve more than mere restoration [3]. In addition to learning from mistakes and using them for preventive purposes, it is possible to predict with a proactive perspective, to be prepared for the future, and to make daily work valuable by making use of established collaborations [4]. By providing organizational learning, it makes it possible to establish an infrastructure and adapt easily to possible sudden situations, and even manage possible conflicts and problems and make it possible to take advantage of these situations by turning them into opportunities [5].

Organizational resilience in healthcare institutions

Resilience in health systems is multisectoral (health, social sciences, economics, etc.); multi-level (micro/individual, medium/organizational, macro/political and national); and more than one population (individuals, communities, decision makers, professionals, scientists, etc.) [6].

When health organizations are considered as a system, its durability is also affected by different factors. Organizational resilience in health institutions occurs at three basic levels: individual, team and organizational level [7].

Considering that health systems are a complex system, it is thought that the best way is to integrate the bottom-up system by developing resilience in caregivers instead of trying to control the health system in order to ensure resilience in health organizations [8]. The relationship between organizational resilience and individual resilience reflects the typical interaction between system and subsystem [9]. Instead of blindly complying with all the rules given to individuals from top to bottom; training should be given to develop their own capacities and how to adapt themselves to the system in

situations of unpredictability and uncertainty in the complexity of the system, and individuals should be empowered to be resilient [10]. In addition, resilience should be contributed through organizational learning by sharing excellent practices instead of bad results [11].

The following best practices regarding organizational resilience in healthcare institutions can be given as examples.

By accepting the Pediatric Intensive Care (PICU) unit of Birmingham Children's Hospital as a pilot project, an initiative called Learning from Excellence (LfE) has been developed, which aims to provide a tool to identify and capture learning from peer-reported excellence or positive deviation [12]. With this initiative, staffs in the unit were asked to voluntarily report their excellent practices, thus creating an excellent practice pool. As a result of this initiative, it has been seen that LfE (http://www.learningfromexcellence.com) learning by sharing excellent practices is as valuable as reflecting on individual mistakes and has the potential to have a positive impact on workplace culture and morale. New ideas have been reached [12]. This application has been used as a tool to increase resilience by sharing excellent practices during the COVID-19 pandemic (https://learningfromexcellence.com/covid19/.).

Nursing and organizational resilience

Nurses and nursing services are considered as an important element in the formation of resilient organizations in the health system in the fight against the COVID-19 pandemic. Nurses are recognized as one of the key elements in the management of hospitals and the health system around the world [13]. Nurses are healthcare professionals who are at the center of COVID-19 prevention and response efforts and provide front-line care for complex COVID-19 cases requiring hospitalization [14]. Nurses are the largest component of the healthcare workforce, with more than 20 million worldwide. Nurses have experienced many changes in the fields of education, management, research and practice from past to present. They had to deal effectively with the changes and difficulties they encountered in every field. Thanks to these

experiences, they learned the importance of adaptability. They have adapted and developed to the needs of the future with what they have learned from experiences, successes and mistakes [15].

For this reason, it is clear that the health sector, and especially nurses, is largely responsible for the implementation of measures to prevent the transmission of COVID-19. Nurses, one of the most trusted healthcare professional groups, also play a strategic role in providing community education on disease prevention and reducing the spread of misinformation about the pandemic [14].

The purpose of this research; It is to determine the relationship between nurses' best practice examples and organizational resilience in the COVID-19 process.

Research questions

The answers to the following questions were sought for the purpose of the research;

1. What are best practice examples of nurses during the COVID-19 pandemic? (Qualitative research question)

2. What is the organizational resilience of nurses during the COVID-19 pandemic? (Quantitative research question)

3. Is there a relationship between nurses' best practice examples and organizational resilience during the COVID-19 pandemic? (Mixed methods research question)

In the first part of this research, the concept of organizational resilience is explained and its importance in the management of the COVID-19 pandemic and its relationship with the nursing profession is explained. In the second part, information about the data collection tools, analysis and software used for the design of the research is given. In the third part, the results of the analysis and the analysis results of the two different methods used are combined and interpreted. In the fourth part, the current findings and the studies in the field are evaluated. In the fifth part, suggestions are given within the framework of the results obtained for the decision makers.

Materials and Methods

Ethical Approval

The ethical and scientific aspects of the study were approved by the Non-Interventional Clinical Research Ethics Committee of Istanbul Medipol University with the letter numbered 10840098-772.02-E.63216 dated 30/11/2020. In order to carry out the study in terms of its subject, the study permit was obtained from the Ministry of Health of the Republic of Türkiye COVID-19 Scientific Research Evaluation Commission and Tekirdağ Provincial Health Directorate with the letter dated 29/12/2020 and numbered 12641312-044. All participants were informed about the research and were given a duly signed Informed Consent Form (ICF) after agreeing to participate in the research. The confidentiality and anonymity of the participants was guaranteed through the use of the codename "Interviewee" followed by a number indicating the sequence in which the interviews were conducted.

Study Design

This research is a mixed method research using a multi-stage mixed method design. Mixed method research is a research method in which inferences are made by using qualitative and quantitative methods together [16-17]. In this study, mixed method was preferred because a single data source was insufficient to explain the research question. In this way, in the qualitative phase of the research, manager nurses' knowledge of best practice examples during the COVID-19 pandemic was examined in depth, and then in the quantitative phase, the answers given by many nurses for organizational resilience were evaluated.

As a matter of fact, in accordance with the research design, the researchers collected and analyzed the quantitative and qualitative data simultaneously. The findings obtained from these two stages were associated in the interpretation stage of the research. The rationale for this approach is to correlate the results obtained from the qualitative method, in which the participants' views are investigated in depth, and the quantitative method, which provides a general understanding of the research questions [16-20].

Population, Location and Selection Criteria

The research was carried out between 01/12/2020 and 01/12/2021 at the Ministry of Health of the Republic of Türkiye, Tekirdağ Provincial Health Directorate Çorlu State Hospital. The population of the research consists of 415 nurses working in Çorlu State Hospital within the specified date range.

Maximum variation sampling, which is one of the purposive sampling methods, was used to determine the qualitative phase sample of the research. For this reason, a total of 10 people, including 8 responsible nurses (middle level managers), 1 assistant head nurse (senior manager) and 1 head nurse (senior manager) working in the relevant services during the COVID-19 pandemic period, were included in the group. It is stated that it is an appropriate size for 6-8 people to participate in the focus group discussions, chosen among those with common experience on the subject [20]. The qualitative phase of the research was completed after it was decided that the data collected in the focus group interview with ten nurse managers had reached sufficient saturation for the research.

Simple random sampling method, one of the probability sampling methods, was used in the quantitative phase of the research. Quantitative stage sampling using the Power Analysis and Sample Size (PASS) program; α=0.05, 5% sampling error condition was determined as 200 nurses. Data was collected from 10 nurses in the qualitative phase and from 196 nurses in the quantitative phase of the research. After the missing, erroneous, etc. incomplete data were eliminated from the data collected in the quantitative phase, 183 nurses were included in the study.

Data Collection

In the qualitative phase of the research, data were collected through the focus group interview technique, one of the qualitative research methods, in order to discover the best practice examples of nurses during the COVID-19 pandemic. Focus group interview refers to a group interview technique that focuses on a specific subject among qualitative research methods. The main purpose of this data collection

technique, which prioritizes the subjectivity and discourse of the interviewees, is to obtain high-quality data from a social content in which the participants freely express their own views, taking into account the opinions of others [20-21]. The interview was conducted using a semi-structured questionnaire. The semi-structured questionnaire was created with the literature on the research question and the opinions of experts in the field of health management. The interview was held in the meeting room of the hospital, which the participants knew beforehand. The meeting room was made ready before the meeting in order to increase the quality of the meeting and recording. The interview was recorded on a voice recorder via a desk microphone, with the consent of the participants. The interview was conducted by a researcher and two observers. Participants, who were informed about the research topic and method, were asked to introduce themselves with their socio-demographic characteristics. During the interview, each participant was given sufficient time to express himself, the interview was continued until there was no new information, and it was concluded with additional contributions. After the interview, the audio recordings were arranged and backed up by reviewing the researcher/observer notes.

In the quantitative phase of the study, data were collected using a two-part questionnaire in order to determine the organizational resilience levels of nurses during the COVID-19 pandemic. The questionnaire consists of the Personal Information Form (9 questions) and the Organizational Resilience Scale (22 questions) to obtain the socio-demographic information of the research group developed by the researchers. Organizational Resilience Scale was developed in 2013 by Lee et al. and adapted into Turkish in 2018 by Öztürk as consisting of 22 questions specific to the health sector. Organizational Resilience Scale is Likert type; it consists of three sub-dimensions: strategic awareness, adaptation capacity and integrity [22]. Sub-dimension total score and scale total score are used in calculating scale scores. The maximum total score of the scale (22 Items), which consists of 5-point Likert items, is 110. Strategic Awareness (7 Items) total score is maximum 35, Adaptation Capacity (9

Items) total score is maximum 45, Integrity (6 Items) total score is maximum 30. The fact that the sub-dimension scores and the total score of the scale are as high as possible indicates that the organizational resilience is that high [11]. The questionnaires were collected by the researchers using face-to-face interview technique with the nurses who voluntarily accepted to participate in the study and gave verbal and written consent.

Data Analysis

The data obtained by the focus group interview method, which was carried out in the qualitative phase of the research, were analyzed with the directed content analysis method, which allows summarizing and interpreting according to the previously determined themes [20]. All of the raw data collected with the help of a voice recorder in the focus group interview were listened to by the researchers and then transferred to the Microsoft Word program and converted into text. In order to prevent possible data loss and to reduce errors, the texts were monitored simultaneously with the voice recordings and the accuracy of the process was checked and controlled. Strategic awareness, adaptation capacity and integrity, which are the concepts that form the basis of the research, were determined as the themes. After this stage, the coding process was started by the researcher. The reliability of the coding was ensured by re-coding independently from the first coding by three researchers. The MAXQDA 2020 Analytics Pro Program, qualitative data analysis software, was used in order to organize the data and collect it in one place, to analyze it, to create notes and summaries, and to standardize the processes. The researchers decided that the codes obtained in line with the themes determined in the analysis of the focus group interview conducted with 10 people had reached sufficient saturation for the research [20].

In the quantitative phase of the research, the data obtained by the questionnaire technique were analyzed using the SPSS 21.0 package program. Descriptive statistical methods were used in the analysis of the data. T test or Mann Whitney U test was used for comparisons of two groups by determining whether the data showed normal distribution, and Analysis of Variance (ANOVA) or *Kruskall Wallis* test was used for comparisons of three groups. Scheffe or Tamhane T2 test was used for subgroup comparisons. Interpretation of the obtained results was evaluated at 95% confidence interval and $p<0.05$ significance level.

In the last stage; the findings obtained as a result of qualitative data analysis and the findings obtained as a result of quantitative data analysis were integrated with mixed method data analysis [16-17,19]. In the research, best practice examples obtained as a result of qualitative data analysis were evaluated in terms of socio-demographic variables and organizational resilience sub-dimensions obtained as a result of the analysis of quantitative data.

Limitations of the Research

Research; 10 nurses working in the units related to the COVID-19 pandemic as middle and senior managers and 193 nurses working in Çorlu State Hospital. The findings obtained in terms of the field practices related to COVID-19 and organizational resilience field knowledge; It is limited to the data that the participants can share and want to share, and the knowledge, skills and equipment of the researchers.

Results

In this part of the research, the findings obtained by qualitative and quantitative research methods, respectively and the findings related to the combination of the two stages are given.

Results regarding the qualitative stage

In the Focus Group Meeting, the participants were visited beforehand and their consent was obtained by informing them about the research topic. The availability of each participant was learned for the day and time of the interview, and the most ideal day and time was determined for researchers and participants. The interview was held around a round table in the hospital meeting room, as the participants felt comfortable and provided the appropriate conditions. The interview lasted 88.40 minutes with a total of 10 participants, 2 of whom were senior managers and 8 were middle managers.

Table 1 includes the themes, categories and codes obtained from the data of the focus group interview method. The obtained data were coded and analyzed and the codes were collected under certain categories and themes by inductive method. In this context, 3 themes, 6 categories and 27 codes representing best practice examples were determined. According to Table 1, the highest number of codes are in the theme of adaptation capacity (n:17), the theme of integrity (n:6) and the theme of strategic awareness (n:4).

Table 1. Code list of the focus group discussion.

Themes	Categories	Codes
Integrity	Effectiveness of Organizational Networks	My Intensive Care Application Applications of Providing Psychological Support to the Patient Patient-Family Communication Practices Interprofessional Cooperation
	Employee Participation	Selfless work Motivation Applications for Nurses
Strategic Awareness	Organizational Analytical Capability	Awareness Meetings Governance Practices Case Studies Suggestions for Inferences
Adaptation Capacity	Innovation Culture	How to Use the Sphygmomanometer Using Stretch Wrapped Phones Protective Equipment Production New Methods in Patient Care and Treatment Nasal Parts Production for Ventilator Telephone Application to Patient Rooms Glass Partition Application for Nurses Patient Washing Stretcher Construction Mirror Application for Nurses Washing Machine Application Adaptation to Learning and Innovation Emergency Trolley Use Arrangement of Patient Room and Room Visits
	Organizational Health	Dirty, Semi-Clean, Clean Area Application Regulation of Nurse Working System Contagion in Nurses
	Role and Responsibility	In-Service Trainings

Figure 1. Word cloud of codes representing examples of best practice.

Figure 1 shows the word cloud shaped according to the frequency of occurrence of best practice examples in the codes. The most frequently repeated example of best practice is the word "Governance Practices" (n:27). Apart from Governance Practices, the words "Case Studies" (n:25) and "Awareness Meetings" (n:22) stand out as the most frequently used words. The least repeated best practice examples are "Patient Washing Stretcher Construction" (n:4), "Nasal Parts Production for Ventilator" (n:3), "Glass Partition Application for Nurses" (n:3).

Results Regarding the Quantitative Stage

The skewness and kurtosis coefficients were examined in order to understand whether the research data conformed to the normal distribution. It has been observed that the research data are in accordance with the normal distribution (Skewness: -0,314; Kurtosis: 0,163). The fact that the skewness and kurtosis coefficients are between +1,96 and -1,96 in the assumption of normality indicates that the data are normally distributed [23]. In this study, in which 183 people participated, the scale total score is maximum 20130, Strategic Awareness total score is maximum 6405, Adaptation Capacity total score is maximum 8235, and

Integrity total score is maximum 5490.

Table 2 contains statistics on the organizational resilience scale. According to **Table 2**, the strategic awareness score is 3925, the adaptation capacity score is 5361, the integrity score is 4397, and the total score is 13683. According to the relevant results, the highest scores are respectively integrity (0,80), adaptation capacity (0,65), and strategic awareness (0,61).

As a result of the analysis made with the socio-demographic characteristics of the participants, a significant difference was found in the Level of Education and Time in the Profession groups, while no significant difference was found in the other groups.

According to the results of the *Kruskal Wallis* H test, which was conducted to determine whether the participants' level of participation in the scales differs according to the education level groups; The difference between the strategic awareness levels of the participants according to the education level groups was found to be statistically significant at the 95% confidence level ($\chi2$=8,876; df=3; *p*=0,031; *p*<0,05). Mann Whitney U test was used to determine which group caused the significant difference. According

Table 2. Analysis results of the organizational resilience scale.

	min	max	$\overline{x}$	df	Total	Proportion**
Strategic Awareness (7 Items)	9,00	35,00	21,4481	5,12656	3925,00	**0,61**
Adaptation Capacity (9 Items)	12,00	45,00	29,2951	7,47893	5361,00	**0,65**
Integrity (6 Items)	10,00	30,00	24,0273	6,25593	4397,00	**0,80**
TOTAL (22 items)	38,00	110,00	74,7705	16,95799	13683,00	

*N = 183

** For standardized comparison between sub-dimensions this calculation (Total value of sub-dimension of scale/ Maximum value of sub-dimension of scale) was performed.

Table 3. Analysis results of the differences of the participants by education level groups.

Scales	Education level	n	Mean Rank	χ^2	df	*p*	U-test
Strategic Awareness	High School (1)	10	90,25				
	Associate Degree (2)	24	65,94	8,876	3	0,031*	2-3
	Bachelor (3)	131	98,60				
	Master (4)	18	79,67				

*p<0.05

to the results of the U test; It was determined that there was a significant difference between the 2nd Group (Associate Degree) and the 3rd Group (Bachelor) of education levels. The strategic awareness level of the participants with undergraduate education (Mean Rank=98,60) is higher than those with associate degree education (Mean Rank=65,94).

According to the results of the Kruskal Wallis H test, which was conducted to determine whether the participation levels of the participants in the scales differ according to the duration groups in the profession; Strategic Awareness ($\chi2$=9,584; df=3; p=0,022; p<0,05), Adaptation Capacity ($\chi2$=7,929; df=3; p=0,047; p<0,05) and Integrity of the participants ($\chi2$=10,387; df=3; p=0,047; p<0,05) levels were found to be statistically significant at the 95% confidence level. Mann Whitney U test was used to determine which group caused the significant difference. According to the results of the U test; duration in the profession

in the levels of Strategic Awareness, Adaptation Capacity and Integrity of the participants The 1st Group (less than 10 years), 2nd Group (11-20 years) and 3rd Group (21-30 years) and 4th Group (31-40 years) was determined to be a significant difference. The strategic awareness level of the participants who have less than 10 years (Mean Rank=86,72), 11-20 years (Mean Rank=94,61) and 21-30 years (Mean Rank=94,93) is lower than those in the profession for 31-40 years (Mean Rank=154,25). The adaptation capacity level of the participants whose professional life is less than 10 years (Mean Rank=87,76), 11-20 years (Mean Rank=94,46) and 21-30 years (Mean Rank=92,13) is lower than those professional life is 31-40 years (Mean Rank=149,75). The holistic level of the participants whose professional duration is less than 10 years (Mean Rank=88,41), 11-20 years (Mean Rank=93,29) and 21-30 years (Mean Rank=89,32) is lower than those professional life is 31-40 years (Mean Rank=159, 58).

Table 4. Analysis results of participants' differences in occupation by duration.

Scales	Time in Profession	n	Mean Rank	χ^2	df	p	U-test
Strategic Awareness	less than 10 years [1]	10 7	86,72	9,584	3	0,022*	1-4 2-4 3-4
	11-20 years [2]	42	94,61				
	21-30 years [3]	28	94,93				
	31-40 years [4]	6	154,25				
Adaptation Capacity	less than 10 years [1]	10 7	87,76	7,929	3	0,047*	1-4 2-4 3-4
	11-20 years [2]	42	94,46				
	21-30 years [3]	28	92,13				
	31-40 years [4]	6	149,75				
Integrity	less than 10 years [1]	10 7	88,41	10,387	3	0,016*	1-4 2-4 3-4
	11-20 years [2]	42	93,29				
	21-30 years [3]	28	89,32				
	31-40 years [4]	6	159,58				

Table 5. Table on education level and strategic awareness themes- governance practices code.

Themes	Categories	Codes	Interviewee	Interviewer's Statement
Strategic Awareness	Organizational Analytical Capability	Governance Practices	Interviewee 4	*(…) It affects graduation... undergraduate graduates can say they know everything... individuality is at the forefront... High school and associate degree graduates... are more open to communication and suggestions (...)*
			Interviewee 7	*(…) It also stems from the professional level of education, but of course, administrative (…)*

Combining the Two Stages

This part of the research was formed as a result of combining the findings related to the qualitative phase with the findings related to the quantitative phase.

In the qualitative phase of the research, the coding in which the best practice examples are included, respectively Adaptation Capacity (n:17), Integrity (n:6) and Strategic Awareness (n:4). According to the results obtained in the quantitative phase of the research, the highest scores are respectively integrity (0,80), adaptation capacity (0,65), and strategic awareness (0,61).

In the quantitative phase of the research; the difference between the strategic awareness levels of the participants according to the education

Table 6. Table on regarding duration in the profession and the integrity themes-motivational practices for nurses' code.

Themes	Categories	Codes	Interviewee	Interviewer's Statement
Integrity	Employee Participation	Motivational Practices for Nurses	Interviewee 5	*(…) as if we have never worked before, as if we started working with Covid (…)*
			Interviewee 3	*(…) a professional position is a loss of a year, I mean the loss of a degree, we get the same money, or for example, you enter taxes at the end of the year (…)*
			Interviewee 6	*(…) new friends say that I stay below the minimum wage, and on the contrary, it causes psychological destruction (…)*

Table 7. Table on regarding duration in the profession and the integrity themes- interprofessional collaboration code.

Themes	Categories	Codes	Interviewee	Interviewer's Statement
Integrity	Effectiveness of Organizational Networks	Interprofessional Collaboration	Interviewee 2	*(…) most branches started to work in covid wards, so an ENT specialist has to think about a patient in general now while only looking at the ear (…)*
			Interviewee 7	*(…) my communication between the first physicians I knew and the physicians I know now is not the same (…)*

Table 8. Table on regarding duration in the profession and the strategic awareness themes- governance practices code.

Themes	Categories	Codes	Interviewee	Interviewer's Statement
Strategic Awareness	Organizational Analytical Capability	Governance Practices	Interviewee 4	*(…) I've been working for 18 years, sometimes even though she was a matron, she went to the intensive care unit and cared for the patient (…)*

Table 9. Table on regarding duration in the profession and the adaptation capacity themes- in-service trainings code.

Themes	Categories	Codes	Interviewee	Interviewer's Statement
Adaptation Capacity	Role and Responsibility	In-Service Trainings	Interviewee 2	*(…) most branches started to work in covid wards, so an ENT specialist has to think about a patient in general now while only looking at the ear (…)*
			Interviewee 8	*(…) my communication between the first physicians I knew and the physicians I know now is not the same (…)*

level groups was statistically significant at the 95% confidence level; It was determined that the participants with undergraduate education level were lower than those with associate degree.

In the qualitative phase of the research, this issue is associated with "arrogance" and "administrator" in the Strategic Awareness Themes- Governance Practices code. Interviewee statements in **Table 5** show this.

Also, the qualitative phase results show that, the level of education and the administrator are also important in creating strategic awareness.

In the quantitative phase, the difference in the levels of Strategic Awareness, Adaptation Capacity and Integrity of the participants according to the duration in the profession was statistically significant at the 95% confidence level; It has been determined that the participants with less than 10 years, 11-20 years and 21-30 years in the profession are lower than those who have 31-40 years in the profession. In the qualitative phase, this topic is associated with "applause campaign" and "income" in the Integrity Themes - Motivational Practices for Nurses code. Interviewee statements in **Table 6** show this.

The qualitative phase results show that, the time in the profession increases many admirable works are done and these contribute to integrity by motivating nurses. But inconsistencies between time in the profession and income negatively affect integrity by breaking motivation.

Additionally, this topic is associated with "physicians" in the Integrity Themes- Interprofessional Collaboration code. Interviewee statements in **Table 7** show this.

According to the results of the qualitative research, the time spent in the profession is important in a period when the responsibilities of physicians increase, as the time spent in the profession increases, co-operation is ensured and integrity increases; however, the change in the co-operative approach of physicians by adopting a self-protective attitude affects integrity.

Also, this topic is associated with "matron" in the Strategic Awareness Themes- Governance Practices code. Interviewee statements in **Table 8** show this.

Here, witnessing the best management practices of managers throughout their time in the profession increases strategic awareness.

Finally, this topic is associated with "matron" in the Adaptation Capacity Themes- In-Service Trainings code. Interviewee statements in **Table 9** show this.

The in-service training received during the career increases the adaptation capacity.

In this part of the study, nurses' Level of Education and strategic awareness levels, and Time in the Profession and strategic awareness, adaptation capacity and integrity levels were associated.

Discussion

This mixed method research was conducted with a total of 193 nurses, 10 nurses in the qualitative phase and 183 nurses in the quantitative phase. The purpose of this research, it is to determine the relationship between nurses' best practice examples and organizational resilience in the COVID-19 process. 3 themes, 6 categories and 27 codes representing best practice examples were identified in the qualitative phase of the research. In the qualitative phase of the research, the coding in which the best practice examples are included, respectively Adaptation Capacity (n:17), Integrity (n:6) and Strategic Awareness (n:4). According to the results obtained in the quantitative phase of the research, the highest scores are respectively integrity (0,80), adaptation capacity (0,65), and strategic awareness (0,61). It was a significant relationship between the level of education of nurses and the level of strategic awareness and between time in the profession and the level of strategic awareness, adaptability and integrity.

In the study conducted with health personnel during the COVID-19 pandemic, it is stated that health personnel find the organization they work in durable, supporting the results of this research [24]. Concepts that have similar meanings with the dimensions obtained in this study as integrity, strategic awareness and adaptation capacity, the characteristics of durable health care in the content analysis study; foresight, interpretation, exchanges and adaptations [25].

As a result of this research, the existing studies in the literature support the relationship between best practice examples and organizational resilience. It is stated that there is a positive relationship between the adoption of digital applications (H4.0 application) and resilience of health institutions facing devastating events such as the COVID-19 pandemic [2]. In the study in which the concept of organizational resilience is associated with the concept of innovation, it is stated that the creation of an innovation culture in organizations will increase organizational resilience [26].

In the study, called Learning from Excellence (LfE), conducted in the Pediatric Intensive Care (PICU) unit of Birmingham Children's Hospital; It is stated that learning by sharing excellent practices is as valuable as reflecting by reporting individual mistakes, has the potential to have a positive impact on workplace culture and morale, and provides access to new ideas for quality improvement [16]. This application demonstrates that it is used as a tool to increase resilience by sharing excellent practices during the COVID-19 pandemic.

Similar to this study, in the study investigating the relationship between national culture and organizational resilience, it is stated that a more durable health service delivery can be achieved with the improvement in country and organizational culture [6].

In the study in which the effect of reducing the impact of COVID-19 in health institutions with Six Sigma method on organizational resilience was investigated; it was stated that not only the employees but also the organizational structure should be considered as a whole [27]. It is stated that collective activity in the group context increases resilience [6]. It is emphasized that resilience in health services is not only a feature of individuals but also of teams and the organization in general [28].

As a result of the study carried out in educational institutions, different from this research universe, and examining the relationship between organizational resilience and organizational health in educational institutions during the COVID-19 pandemic, it was stated that organizational resilience would support organizational health [29].

Especially during the COVID-19 pandemic, resilience research in the field of health turned into practice, and in this context, centers were established in some countries and universities Centre for Resilience in Healthcare (SHARE) in Norway, Centre for Applied Resilience In Healthcare (CARe) in England means Centre for Healthcare Resilience and Implementation Science (CHRIS) in Australia) [30].

Conclusion

In the qualitative phase of the research, 3 themes, 6 categories under these themes and 27 codes under these categories were determined. With the codes determined, 27 best practice examples of nurses were discovered during the COVID-19 process. The coding with the best examples of best practice is Adaptation Capacity (n:17), Integrity (n:6) and Strategic Awareness (n:4). As a result of the quantitative phase of the research, the highest scores are respectively integrity (0,80), adaptation capacity (0,65), and strategic awareness (0,61). As a result of this research, it has been determined that the organizational resilience levels of nurses are high during the COVID-19 process.

In the study, a relationship was determined between nurses' Level of Education and Strategic Awareness levels, and between Time in the Profession and Strategic Awareness, Adaptation Capacity and İntegrity levels. According to the results of this mixed method research, a similar relationship was found between nurses' best practice examples and organizational resilience levels during the COVID-19 pandemic. Within the scope of research results, practitioners and researchers;

• *Inclusion of practices for the discovery and follow-up of best practice examples in health institutions, increasing the awareness of employees on this issue and ensuring that these practices are spread in the organizational culture,*

• *Organizing special training programs such as crisis management, health communication, interprofessional collaboration for employees in health institutions, improving the working conditions of*

employees and increasing their motivation, increasing organizational resilience and creating a resilience culture,

• Adoption and implementation of collaboration and teamwork approach between senior managers and employees in health institutions.

It is recommended that future studies be carried out with different professional groups, with different patterns and methods, apart from the COVID-19 pandemic process.

Conflict of interest

The authors declared no conflict of interest in this study.

Data availability statement

The entire dataset supporting the results of this study is available upon request to the corresponding author. (Reason for restriction: It is limited by institutional and the ethical committee permission)

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Protective properties of spectacle lenses used as ultraviolet blockers

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Abstract

In this study, we evaluated the UV transmittance of spectacle lenses in the Turkish market, which are reported to have UV blocking properties, and tested their suitability. Samples were obtained from patients who were admitted to the ophthalmology outpatient clinic of our hospital for refractive error and who wore glasses as UV block. No limitation was made regarding the spectacle size and duration of spectacle wear. Measurements were tested by the principal investigator using an ultraviolet detector. The right and left lens were measured separately. All measurements were performed at room temperature (22-25°C) in a humidity regulated (20-40%) room without direct light. UV protection level was measured for 120 spectacles used for refractive errors. Only 28 (23.3%) of 120 glasses had protection level up to 400 nm UV. Twenty-four (20%) of the glasses had a protection level of 0 even though they were labeled as having UV blocking properties. It is clear that strict regulations need to be implemented to improve the protection levels of glasses.

Keywords: Ultraviolet radiation, spectacle lenses, cataract, eyeglasses, UV blocking

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Introduction

The sun's rays have waves of ultraviolet radiation (UVR) ranging from 200-400 nm. The ozone layer can completely absorb the deleterious ultraviolet C (UVC) range and partially absorb ultraviolet B (UVB) waves. However, ultraviolet A (UVA) in the 315-400 nm range reaches the earth's surface in large amounts [1]. Long-term exposure to both UVB and UVA radiation has been reported to cause serious ocular problems such as cataracts, corneal degenerations, conjunctival degenerations and retinal degenerations [2,3]. Wide-brimmed hats and appropriately manufactured sunglasses are used for protection against ocular UVR [1,4]. In recent years, sunglasses and eyeglasses that provide protection against UV radiation are especially preferred due to the increased awareness of the harmful effects of UV radiation on the eyes [5]. However, it is not easy to find sunglasses and eyeglasses with appropriate and effective protection. One of the reasons for this is that spectacle lenses produced by different brands do not guarantee that they provide adequate protection [6]. Even in developed countries, the public is not aware of the protective properties of sunglasses against UV radiation [3]. As a result, it is often the last resort for most consumers to rely on retailers to get a good pair of UV-blocking glasses [3,7]. There are no standards for sunglass filtering properties in most countries, and there are studies in different countries on the UV transmittance of marketed sunglasses [1,3,8]. However, there is no study evaluating the protection of spectacle lenses. In this study, we evaluated the UV transmittance of spectacle lenses in the Turkish market, which are reported to have UV blocking properties, and tested their suitability.

Materials and Methods

This study was approved by Niğde Ömer Halisdemir University Ethics Committee (2023/55) and verbal and written informed consent was obtained from each patient before the study. The study was carried out according to the principles of the Declaration of Helsinki.

Procedure

Samples were obtained from patients who were admitted to the ophthalmology outpatient clinic of our hospital for refractive error and who wore glasses as UV block. Patients were asked to come to us for control within one week after the prescription of glasses. No limitation was made regarding the spectacle size and duration of spectacle wear. Measurements were tested by the principal investigator using an ultraviolet detector (anti-radiation UV sun detector, China). The right and left lens were measured separately. All measurements were performed at room temperature (22-25°C) in a humidity regulated (20-40%) room without direct light.

After cleaning the lens surface, the front surface center of the glasses was placed towards the ultraviolet radiation source. For consistency, all measurements were taken by a single investigator and recorded by averaging three readings. Glasses blocking wavelengths up to 400 nm were considered to be safe and in compliance with standards.

Statistical Analysis

Statistical analysis was done using the Statistical Package for Social Science (SPSS) by IBM, version 25. Descriptive data were analyzed using mean and frequency (percentage, %) as appropriate.

Results

UV protection level was measured for 240 lenses of a total of 120 spectacles used for refractive errors. The protection levels of all glasses were symmetrical (right and left). Of the 120 patients, 66 were female (55%) and 54 were male (45%). The mean age of the patients was 34.3 ± 16 years. Only 28 (23.3%) of 120 glasses had protection level up to 400 nm UV. Twenty-four (20%) of the glasses had a protection level of 0 even though they were labeled as having UV blocking properties. Figures showing the measurements of some lenses are shown below. (Figure 1-3). 4 lenses provided protection up to 390 nm, 4 lenses provided protection up to 380 nm and 6 glasses provided protection up to 375 nm. The other glasses had variable protection levels between 360-370 nm (Table 1). 65% of lenses provided protection below 375nm.

Table 1. UV blocking thresholds of spectacle lenses.

	Number (%)
400 nm	28 (23.3%)
390 nm	4 (3.3%)
380 nm	4 (3.3%)
375 nm	6 (5%)
370 nm	22 (18.3%)
365 nm	4 (3.3%)
360 nm	28 (23.3%)
0 nm	24 (20 %)
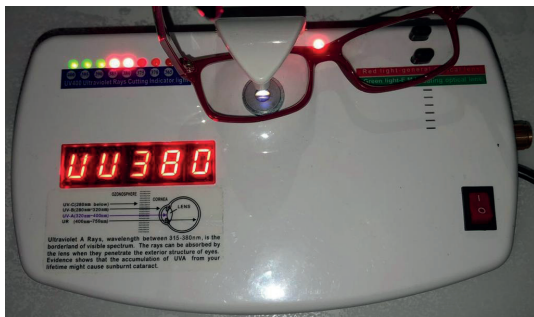
Figure 1: Measurement of a 380 nm UV blocking lens.
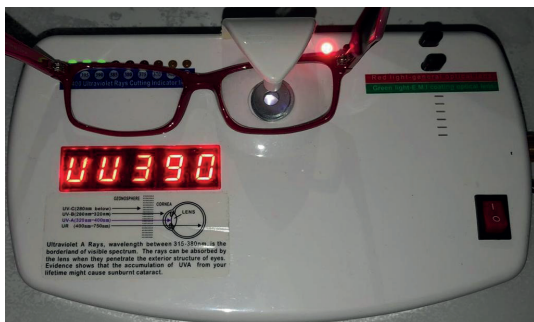
Figure 2: Measurement of a 390 nm UV blocking lens.
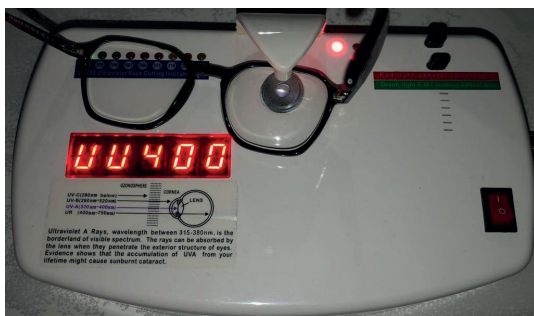
Figure 3: Measurement of a 400 nm UV blocking lens.

Discussion

Sunlight is the primary source of UV radiation. UV radiation is not included in the visible light spectrum and therefore cannot be detected. Since it cannot be perceived by the visual system, its harmful effects cannot be felt until ocular damage develops [9]. The harmful effect of UV on human health increases with cumulative exposure [10]. There are various ways to protect the eye to prevent ocular damage. Wearing a hat or umbrella, UVR-blocking glasses, sunglasses or contact lenses are the most commonly used methods [11]. The most common method is eyewear with a UV protection filter that filters out 100% of UV radiation [12]. Maximum UVR protection with sunglasses is considered mandatory, especially in the equatorial region where UVR exposure is high [1].

The reference point supported by international standards is 400 nm UVR protection level [13]. While there are many studies in the literature investigating the adequacy of the protection level of sunglasses sold in various countries, studies investigating the protection level of eyeglasses are limited. In our study, the protection levels of prescription UV blocking eyeglasses used for refractive reasons were evaluated. It was found that only 23% of the eyeglasses had 400nm protection and 20% of the eyeglasses had no protection even though they were reported to have UV blocking properties.

Although there are not many studies in the literature examining the UV protection levels of eyeglasses, there are a wide variety of examples evaluating sunglasses. In a study conducted in Ethiopia, only 73.0% of non-prescription sunglasses complied with the standards and were protective against 400nm ultraviolet radiation [1].

In the study by Bazzani et al., this rate was found to be 92.6% [14]. In addition, Adul-Kabir et al. reported that all over-the-counter sunglasses purchased from optical stores provided appropriate protection, while 53.0% of sunglasses purchased from roadside vendors did not meet the standard [15]. In a study conducted in Canada, reported that almost 100% of sunglasses whose UV levels were evaluated

complied with Canadian standards [16]. Abdulrahim et al reported that sunglasses do not provide full protection against UV radiation and that sunglasses may pose a danger to eye health [17]. When the literature studies are compared, it is seen that there are variable results depending on the development levels, socioeconomic status and living conditions of the countries.

The fact that it is one of the rare studies evaluating the degree of UV block of spectacles in the literature makes our study powerful, but there are some limitations. The glasses evaluated in our study were purchased within 1 week after prescription. However, we do not have data on how long after the lens is manufactured, it is mounted on the spectacle frame. In addition, the evaluation of UVR protection by an anti-radiation UV sun detector may not be as accurate as a spectrophotometer.

Conclusion

In conclusion, since glasses sold as UV blocking should be reassuring against UVR before they are distributed to users, information about the level of protection should be provided and the level of UV blocking should be indicated on the user card. It is clear that strict regulations need to be implemented to improve the protection levels of glasses.

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Conflict of interest

There are no conflicts of interest.

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ORIGINAL ARTICLE

Pediatric COVID-19 diagnosis: The utility of hematological and inflammatory indices

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Abstract

This study aims to systematically assess the diagnostic utility of specific hematological parameters and indices in pediatric patients for the early and accurate diagnosis of COVID-19, thereby contributing to enhanced clinical management and diagnosis of children. The study evaluated the diagnostic potential of hematological and inflammatory markers in 90 pediatric patients, including 49 with COVID-19 and 41 without. It focused on complete blood counts, systemic immune-inflammatory index (SII, calculated as platelet count multiplied by neutrophil count divided by lymphocyte count), platelet to mean platelet volume ratio (PLT/MPV), neutrophil to lymphocyte ratio, platelet to lymphocyte ratio, C-reactive protein, and procalcitonin, comparing these markers between patients with and without COVID-19. COVID-19 positive patients exhibited higher hemoglobin levels and immature granulocyte percentages, along with lower total leukocyte, neutrophil, platelet counts, and procalcitonin levels (p-values: 0.02, 0.006, 0.01, 0.002, 0.007, and 0.01, respectively). The SII and PLT/MPV ratio were significantly lower in the COVID-19 positive group (p=0.01 and p=0.006, respectively), suggesting their potential diagnostic relevance. Receiver Operating Characteristic (ROC) analysis revealed that procalcitonin, PLT/MPV, and SII had comparable diagnostic utility, with area under the curve (AUC) values indicating moderate diagnostic accuracy (procalcitonin AUC: 0.65, p=0.013; PLT/MPV AUC: 0.67, p=0.004; SII AUC: 0.65, p=0.01). Our research highlights the PLT/MPV ratio and SII as breakthrough markers for early detection of COVID-19 in children, providing a significant advance in pediatric diagnostics and enhancing our ability to meet the challenges of the pandemic.

Keywords: Hematological markers, inflammatory indices, pediatric COVID-19 diagnostics, platelet count analysis, systemic immune inflammatory index

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Introduction

The emergence of Coronavirus disease 2019 (COVID-19) as a global pandemic has been documented extensively, with its significant impact on populations across the world, including children, albeit with generally less severity compared to adults [1,2]. Symptoms in the pediatric population have been identified as ranging widely, from fever, fatigue, and dry cough to more varied symptoms such as runny nose, nasal congestion, sore throat, and gastrointestinal discomfort. The differentiation of COVID-19 from other pediatric infections is underscored by the variability of these symptoms, necessitating the evaluation of laboratory values for accurate diagnosis [3].

The importance of this study is anchored in the critical need for early and accurate diagnosis of COVID-19 among children. Laboratory parameters, including white blood cell (WBC) count, lymphocyte and neutrophil counts, platelet count (PLT), mean platelet volume (MPV), and red cell distribution width (RDW), have been highlighted as instrumental in detecting inflammatory diseases and COVID-19. Furthermore, ratios of these parameters, such as the platelet-to-lymphocyte ratio (PLR), neutrophil-to-lymphocyte ratio (NLR), platelet-to-mean platelet volume (PLT/MPV) ratio, and systemic immune inflammation index (SII), have been shown to have diagnostic utility in COVID-19 patients [4-6]. The elevation of C-reactive protein (CRP) and procalcitonin (PCT) levels, and the occurrence of anemia, thrombocytopenia, and eosinopenia in pediatric cases, have also been documented, presenting a compelling case for their inclusion in diagnostic protocols [7].

The problem of differentiating COVID-19 from other pediatric infections due to overlapping symptoms is addressed in this study through a comprehensive comparison of laboratory parameters between pediatric outpatients who are symptomatic, with distinctions made between those testing positive and negative for COVID-19. By conducting such an analysis, the study aims to clarify the diagnostic value of these parameters and indices, enhancing the accuracy and efficiency of COVID-19 diagnosis

in the pediatric population by investigating the differences in hematological values at the initial presentation.

From the perspective of ongoing research and the critical challenge posed by the pandemic, the hypothesis driving this study is that certain hematological parameters and indices can significantly improve the diagnostic process for COVID-19 in pediatric patients. The objective is to systematically evaluate these parameters to establish a more effective diagnostic framework, contributing to the broader efforts to manage and control the spread of COVID-19 among children.

Materials and Methods

Study Population

The Ethics Committee of Erciyes University granted approval for this research (2020/568). This study was conducted with pediatric patients, aged 0-18 years, who presented with symptoms indicative of COVID-19, including fever, sore throat, runny nose, and cough, at a pediatric clinic from February 1 to April 30, 2021. A total of ninety outpatients with suspected COVID-19 were recruited for the study. Exclusion criteria included patients experiencing respiratory distress or those requiring hospitalization. Our patients were from outpatient clinics. Diagnostic samples were obtained through nasal and oropharyngeal swabs, which were then analyzed for the SARS-CoV-2 virus using the quantitative real-time reverse transcription polymerase chain reaction (RT-qPCR) technique. The participants were categorized into two groups: those testing positive for COVID-19 formed the case group, while those testing negative constituted the control group. Diagnostic samples for COVID-19 were collected from all patients, including those who tested negative. If symptoms persisted, additional COVID-19 testing was conducted during subsequent visits; however, we based our analysis on the initial presentation of the patients

Data on age, gender, complete blood count (CBC), CRP, and PCT levels were systematically recorded for all participants. Comparative analysis was performed on hemogram parameters, including NLR, PLR, PLT/MPV ratio, SII, and the values of CRP and PCT, between COVID-19 positive and negative pediatric patients.

Statistical Analysis

Data analysis was performed using IBM SPSS Statistics version 22.0. The distribution of the data was assessed using histograms, q-q plots, and the *Shapiro-Wilk* normality test to determine if they followed a parametric or non-parametric distribution. Parametric data were presented as mean ± standard deviation, while non-parametric data were expressed as median (interquartile range: 25th percentile-75th percentile). The *Mann-Whitney U* test was employed for the comparison of hematologic parameters and levels of CRP and PCT between the COVID-19 positive and negative groups. Categorical data comparisons were made using the chi-square test, with *Fischer's* Exact test applied as needed. The diagnostic potential of PCT, CRP, and other significant markers was assessed through receiver operating characteristic (ROC) analysis, calculating areas under the curve (AUC), cut-off values, and standard validity indices such as the Youden index, sensitivity, and specificity. A *p*-value of <0.05 was considered statistically significant for all analyses.

Results

Demographic and Laboratory Data Analysis

In this study, 49 children tested positive for COVID-19, forming the case group, while 41 children tested negative, constituting the control group. The average age of the COVID-19 positive group was 9.3 ± 5.8 years, compared to 7.7 ± 5.5 years in the negative group, with this difference not reaching statistical significance (*p*=0.24). Gender distribution did not significantly differ between the groups (χ^2 = 0.18, *p*=0.89). Examination of complete blood count parameters and inflammatory markers revealed no significant differences in CRP, RDW, absolute lymphocyte count, MPV, and platelet distribution width between COVID-19 positive and negative patients.

In the analysis of demographic and laboratory data, the study observed significant differences in several hematological parameters between COVID-19 positive and negative pediatric patients. Hemoglobin levels were notably higher in the COVID-19 positive group (13.2 ± 1.7 g/dL) compared to the negative group (12.4 ± 1.7 g/dL, *p*=0.02). Additionally, the percentage of immature granulocytes was significantly elevated in COVID-19 positive children (0.54 ± 1.54%) versus the negative group (0.36 ± 0.23%, *p*=0.006). Conversely, total leukocyte count, absolute neutrophil count, and platelet count demonstrated a significant decrease in COVID-19 positive children, with values of 7786 ± 4420 mm^3, 4084 ± 2691 mm^3, and 247 ± 90 x103/mm^3 respectively, compared to 11232 ± 6390 mm^3, 6953 ± 5638 mm^3, and 294 ± 86 x103/mm^3 in the negative group (*p*=0.01, *p*=0.002, and *p*=0.007, respectively). Procalcitonin levels were also

Table 1. Comparison and analyses of patients' demographic and laboratory data.

Parameters	COVID-19 (+) n = 49	COVID-19 (-) n = 41	*P*
Age, years	9.3±5.8	7.7±5.5	0.24
Gender, female/male n (%)	21 (%43) / 28 (%57)	17 (%42) /24 (%58)	0.89
Hemoglobin (g/dL)	13.2±1.7	12.4±1.7	**0.02**
White Blood Cell (mm³)	7786±4420	11232±6390	**0.01**
Absolute Neutrophil Count (mm³)	4084±2691	6953±5638	**0.002**
Absolute lymphocyte count (mm³)	2735±2668	2966±2585	0.44
Immature granulocyte (%)	0.54±1.54	0.36±0.23	**0.006**
Platelet (mm³/10³)	247±90	294±86	**0.007**
Mean Platelet Volume (FL)	9.9±0.85	9.7±1.04	0.29
Red Cell Distribution Width (%)	12.8±0.97	13.6±1.87	0.056
Platelet Distribution Width (FL)	10.9±1.51	10.8±1.3	0.23
C-reactive protein (mg/dL)	5.95±9	27.9±42.7	0.06
Procalcitonin (mg/dL)	0.36±0.54	0.97±1.56	**0.01**

significantly lower in the positive group (0.36 ± 0.54 mg/dL) versus the negative group (0.97 ± 1.56 mg/dL, *p*=0.01; **Table 1**).

Hematological Indices, PLT/MPV Ratio, and SII Comparison

The study further analyzed the diagnostic value of hematological indices, including the PLT/MPV ratio and SII. The PLR and NLR rates showed no statistically significant differences between the two groups (*p*=0.61, *p*=0.09). The SII was markedly lower in COVID-19 positive children (654 ± 752) compared to the negative group (1301 ± 2249), with this difference being statistically significant (*p*=0.01). Similarly, the PLT/MPV ratio was significantly lower in the positive group (25.6 ± 10.9) than in the negative group (31.1 ± 10.9; *p*=0.006; **Table 2**).

ROC Analysis of Procalcitonin, PLT/MPV, and SII

Receiver Operating Characteristic (ROC) analysis was employed to evaluate the diagnostic efficacy of procalcitonin, PLT/MPV, and SII. The procalcitonin assay demonstrated a sensitivity of 84.7% and a specificity of 42.5%, with an area under the curve (AUC) of 0.65 (95% CI: 0.54-0.74, *p*=0.013), and a cut-off value of 0.48 mg/dl. For PLT/MPV, a sensitivity of 49%, specificity of 85%, and an AUC of 0.67 (95% CI: 0.56-0.76, *p*=0.004) were observed, with a cut-off value of 22.3. The SII showed a sensitivity of 65%, specificity of 70%, and an AUC of 0.65 (95% CI: 0.54-0.75, *p*=0.01), with a cut-off value of 462 (**Table 3**, **Figure 1**). No significant differences were found in the AUC values of procalcitonin, PLT/MPV, and SII, indicating comparable diagnostic utility among these markers.

Table 2. Comparison of complete blood cell-derived inflammation indices between COVID-19. positive and negative children.

Parameters	COVID-19 (+) n = 49	COVID-19 (-) n = 41	P
PLR	150±108	159±105	0.61
NLR	2.83±3.1	4.2±6.7	0.09
SII	654±752	1301±2249	**0.01**
PLT/MPV	25.6±10.9	31.1±10.9	**0.006**

NLR, neutrophil to lymphocyte ratio; PLR, platelet to lymphocyte ratio; SII, systemic immune inflammatory index (neutrophil*platelet to lymphocyte ratio); PLT/MPV, platelets count to mean platelet volume. All variables are reported as mean± standart deviation. Statistical significance set at 0.05. All statistically significant values are reported in bold.

Table 3. Sensitivity, specificity, positive predictive value, negative predictive value, and results of the receiver operating characteristic curve for PCT (procalcitonin), PLT/MPV (platelet/mean platelet volume) and SII (systemic immune inflammatory index) in COVID-19 children's patients.

Cut-off value	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Youden Index	AUC (95% CI)	P
PRC<0.48mg/dL	84.7	42.5	65.1	73.9	0.30	0.65 (0.54-0.74)	**0.013**
PLT/MPV <22.3	49	85	80	57	0.33	0.67 (0.56-0.76)	**0.004**
SII<462	65	70	72	63	0.36	0.65 (0.54-0.75)	**0.010**

Discussion

This study was performed to evaluate the diagnostic value of hematological parameters and indices in differentiating COVID-19 from other pediatric infections. Our hypothesis was that certain hematological markers could significantly improve the diagnostic accuracy of COVID-19 in children. Although real-time PCR analysis is considered the gold standard for COVID-19 diagnosis, it faces significant hurdles, including the need for large sample sizes, a shortage of skilled personnel, and limited laboratory capacity, all of which lead to delays in obtaining results. This situation highlights the need for alternative diagnostic approaches. Our results showed significant differences between COVID-19 positive and negative pediatric patients, particularly in the percentage of immature granulocytes, total leukocyte count, absolute neutrophil count and platelet count. In addition, the systemic immune inflammation index (SII) and the platelet-to-median platelet volume ratio (PLT/MPV) were identified as valuable diagnostic markers. These results suggest that the incorporation of these hematological parameters into diagnostic protocols may enhance the early and accurate diagnosis of COVID-19 in pediatric patients, potentially improving patient management and helping to control the spread of the virus among children. The primary aim of our research was to evaluate the diagnostic potential of hematological parameters, specifically the PLT/MPV ratio and SII, in identifying COVID-19 in children. We found that these parameters could serve as useful indicators for the diagnosis of COVID-19, highlighting the importance of rapid and accessible testing options. To our knowledge, this is the first study to investigate the diagnostic value of these parameters in a pediatric cohort.

In a study conducted with adult patients, it was found that the SII on admission independently predicts in-hospital mortality in COVID-19 patients and may assist with early risk
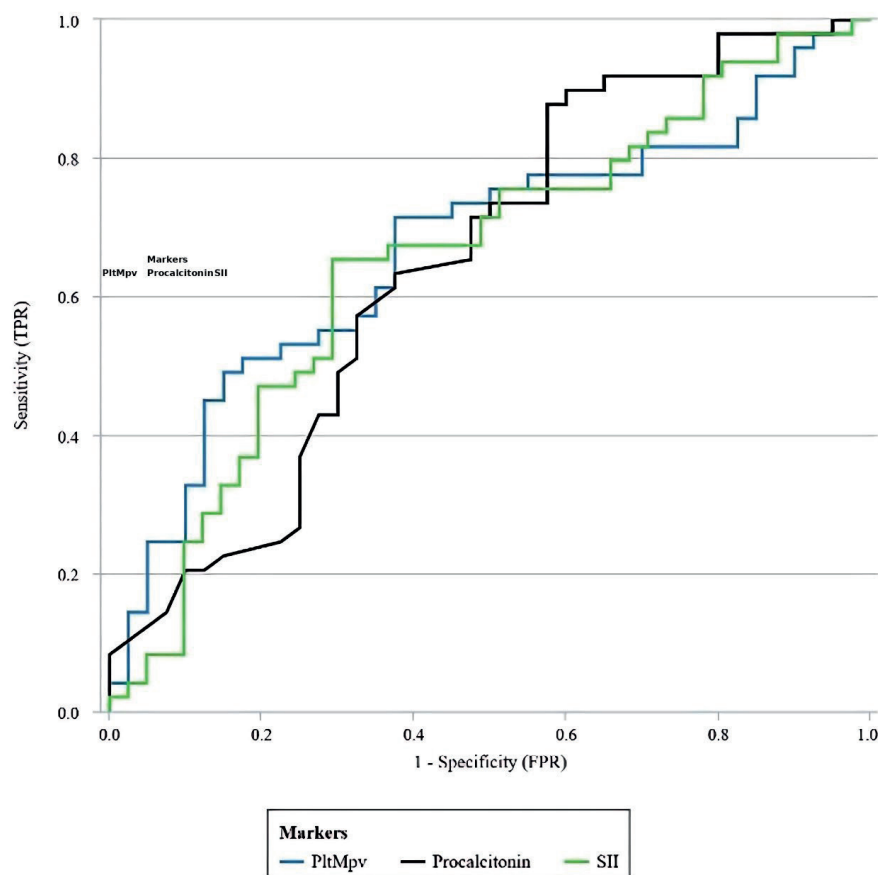

Figure 1. Sensitivity, specificity, positive predictive value, and negative predictive value of PRC, PLT/MPV, and SII parameters that were significant as a result of ROC analysis.

stratification in this group [8]. Additionally, another study aimed to examine the significance of blood cell indexes of the systemic inflammatory response, such as the neutrophil to lymphocyte to platelet ratio (NLPR), systemic immune-inflammation index (SII), and systemic inflammation response index (SIRI) in predicting intensive care unit (ICU) admission of COVID-19 patients. This study concluded that SII is a predictor of survival, while NLPR and SIRI have an additive role that needs further evaluation [9]. While SII has been identified as a predictor in several studies involving severe adult cases, our study found SII to be a significant marker in non-severe, outpatient pediatric COVID-19 cases.

In this study, we evaluated the effects on hematological parameters, PLT/MPV ratio and systemic immune-inflammatory index (SII) in COVID-19 positive children. Our results showed an age and gender distribution similar to the study by Dong et al. [10] who analyzed the epidemiology of children with COVID-19. As in the study by Ozenen et al. [11], we found that leukocytes, neutrophils, lymphocytes and platelets were decreased and hemoglobin and immature granulocytes were significantly increased in COVID-19 positive children. However, no statistically significant difference was found for lymphocyte counts, indicating that the number of lymphocytes in COVID-19 positive children was low, but this low number was not statistically significant. Our study underscores the importance of hematological markers, especially SII and PLT/MPV ratio, in diagnosing COVID-19, similar to a study on intensive care patients using indices like NLR, PLR, and SII [12]. Focusing on a pediatric outpatient cohort, our investigation highlights the versatility of these markers in various clinical settings. While our study is consistent with the findings of Cui et al. [13] regarding CRP and PCT levels, and contrasts with Ozenen et al. [11] by demonstrating decreased PCT levels in COVID-19 positive children, it suggests a nuanced interpretation that severe COVID-19 cases may have elevated CRP and PCT levels, possibly due to secondary bacterial infections. Furthermore, consistent with the observations of Yun et al. [14] and Seyhanli et al. [15] regarding MPV and PLT/MPV ratio, our study found statistically significant decreases

in these parameters in COVID-19 positive pediatric patients. This suggests a marked thrombocytic response to the virus in children, highlighting the potential diagnostic value of these parameters in the pediatric setting. The use of SII as a prognostic marker, as discussed by Usul et al., was also explored in our study and showed significantly lower SII levels in COVID-19 positive children, highlighting the role of the inflammatory response in pediatric COVID-19 cases [16]. However, our study is limited by its small sample size and the inclusion of only outpatient cases, which may limit the generalizability of our findings to a wider range of disease severity. Additionally, factors that may affect laboratory parameters, such as the use of medications (e.g., NSAIDs), nutritional deficiencies (e.g., protein energy malnutrition, anthropometric differences), and accompanying systemic diseases, were not examined in detail. Future research should aim to include a more diverse patient population, encompassing different clinical severities and inpatient scenarios, to corroborate and extend our initial findings.

Conclusion

In conclusion, our study highlights the potential of the PLT/MPV ratio and SII as cost-effective, accessible diagnostic indicators for the early detection of COVID-19 in children. These findings not only encourage further empirical research into the clinical and theoretical implications of hematological changes in COVID-19, but also provide a basis for the development of novel diagnostic approaches. Our research provides valuable insights into the use of hematological parameters in the management of COVID-19 and highlights the need for continued investigation in this important area of pediatric healthcare.

Conflict of interest

The authors have no relevant financial or non-financial interests to disclose.

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ORIGINAL ARTICLE

Silymarin promotes wound healing through regulating epithelial-mesenchymal transition in rat model: Histopathological and immunohistochemical evidences

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Abstract

The wound is the disruption of the normal structure, integrity, and function of the skin and subcutaneous tissue. It is known that current wound management strategies applied in the treatment of acute and chronic wounds both cause an increase in health costs and do not achieve the desired level of success. Therefore, more effective and easily applicable treatment methods are needed. SM is an agent known to have hepatoprotective, anticancer, antidiabetic, cardioprotective, neuroprotective, antimicrobial and antioxidant effects. And it is also used in dermatological applications. However, the therapeutic effects of Silymarin (SM) on wound healing are still unknown. In this study, the effects of SM were investigated by comparing it with dexpanthenol (Dxp), whose favorable effects on wound healing are known. Sham, Dxp, and SM groups were formed. 18 animals were used for each group. Two circular full-thickness skin wounds were taken from the nape of the neck (1.5 cm) using a six-mm punch biopsy tool. SM and Dxp was applied once daily for 15 days, in sufficient amounts to cover the entire wound and the effects of the drugs were investigated immunohistochemically on the 5th, 10th, and 15th days in rats. Inflammation, collagenization and epithelialization were evaluated in histochemical H-E and Masson trichrome staining. E-cadherin, N-cadherin, Occludin, Vimentin, FGF-1 and MMP-9 expression levels were examined immunohistochemically. Based on histological and immunohistochemical results, SM and Dxp enhanced epithelialization and reduced inflammation more than the sham group. Furthermore, there was no significant difference in the effects on the epithelial-mesenchymal transition between SM and Dxp. Results indicated that SM is a useful therapeutic agent at least as much as Dxp in wound healing. The wound re-epithelization and anti-inflammatory effects of SM may be a new approach to the treatment of wound healing.

Keywords: Silymarin, wound healing, epithelial-mesenchymal transition, cadherin, NfκB

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Abbreviations: Dexpanthenol (Dxp), Epithelial-mesenchymal transition (EMT), Fibroblast growth factor-1 (FGF-1), Hematoxylin–Eosin (H-E), Matrix metalloproteinases (MMPs), Masson's trichrome (MT), Nuclear factor kappa B (NfκB), Silymarin (SM), Standard error of the mean (SEM), Tumor necrosis factor-α (TNFα), Vascular endothelial growth factor (VEGF).

Introduction

Our skin provides a variety of important hemostatic functions between the external environment and our body, from heat regulation to perception mechanisms [1]. More specifically, the skin functions as a defense against mechanical, chemical or phototic damage to the body [2]. The skin may undergo structural deformation or damage, which is defined as a wound, in the process of performing its task. It is known that the skin has effective and fast mechanisms to correct this unfavorably picture or to close the breaches. This process, known as wound healing, could be listed as hemostasis, inflammation, proliferation, and dermal remodeling [3]. Keratinocytes are the structures responsible for the formation and protection of the epidermis, which is the outer layer of the skin. These functions of keratinocytes are completed by a differentiation process that starts at the stratum basale and ends at the stratum corneum. Keratinocytes are polarized and show cohesion with the nearby epithelium. Therefore, keratinocytes are required to go through the partial epithelial-mesenchymal transition (EMT) to repair tissue damage [4]. EMT, also known as differentiation of epithelial cells into motile mesenchymal cells, is an integral part of wound healing and pathologically contributes to fibrosis and cancer progression [5].

When we evaluate the entire wound healing process at the molecular level, the major roles of many structural factors emerge. Vascular endothelial growth factor (VEGF) is a hemostatic agent responsible for initiating angiogenesis, which is necessary for repair of damaged tissue or organ [6]. Therefore, it has been shown in many studies that VEGF expression increases during the wound healing process [7,8]. With the rapid induction of angiogenesis, the initiation of the inflammatory process is regulated by nuclear factor kappa B (NfκB) and

tumor necrosis factor-α (TNFα). The functional roles of E-Cadherin are known, especially in the migration of large epithelial layers of e skin [9]. It has been reported that the mechanism of action underlying cadherin's remodeling of cell junctions is tyrosine phosphorylation [10]. In the phase of the proliferation, keratinocytes in the neoepidermis release matrix metalloproteinases (MMPs) to aid their migration pathways [11]. MMPs, especially MMP-9 is vital for keratinocyte migration as they assist integrin receptor cleavage [4]. Also, at this phase, it has been shown that vimentin enables the union of focal adhesions formed in response to collagen binding by β1-integrin regulation [12]. Similarly, in another study, downregulation of E-Cadherin and Occludin-1 and overexpression of N-Cadherin and vimentin were shown by EMT induction [13]. In contrast, it has been shown that EMT, an important physiological process in wound healing, is suppressed by inhibition of the MAPK/ERK kinase pathway of Fibroblast growth factor-1 (FGF-1) [14].

In the treatment of the wound healing process, shortening the treatment period or accelerating the process is of great importance in terms of increasing the quality of life and patient compliance. In vitro and clinical studies have provided evidence that topically applied dexpanthenol (Dxp) promotes superficial and post-procedural wound healing [15]. On the other hand, recent findings confirmed that Dxp up-regulates genes critical to the healing process [16,17]. Although there are agents or adjuvants used in wound healing, the search for more effective and / or safe products continues. Silymarin(SM) is a polyphenolic flavonoid isolated from milk thistle (Silybum marianum (L) Gaertn) seeds [18] . The main component of SM, silybinin, is generally considered very safe [19]. Within this scope, in this study, we investigated the effects and possible effects of Silymarin (SM) obtained from Silybum marianum plant on

wound healing by comparing it with Dxp [20]. Antidiabetic, cardioprotective, neuroprotective, antimicrobial, and antioxidant effects of SM have been demonstrated to date [21–26]. With its strong free radical scavenging and antioxidant properties, silymarin has been shown to significantly reduce burn-induced oxidative skin damage in rats [27]. Sharifi et al. reported that silymarin increased epithelialization and reduced inflammation in full thickness wounds. It has also been shown that it can significantly stimulate epithelialization and reduce inflammation [28]. However, although the UVB protective property of SM has been demonstrated, there are not enough studies on its effects on wound healing [29].

Materials and Methods

Experimental Model and Protocols

This study was performed after approval of the Ethical Animal Research Committee of Afyon Kocatepe University (AKUHADYEK-49533702/97). Four-week-old male *Wistar* rats were housed under temperature and humidity-controlled rooms (20–22ºC) with a 12-h light-dark cycle. The animals were fed with a standard rodent chow diet that composed of starch, protein, fat, cellulose, standard vitamins, and salt mixture and water. After acclimation for one week, the rats (n=54) were randomly divided into three groups: Sham (n=18), Dexpanthenol (Dxp; n=18), and Silymarin (SM; n=18). Backs of the rats were shaved and cleaned with 70% ethanol; a six mm punch biopsy instrument was used; two circular full-thickness skin wounds were taken from the back of the neck (1.5 cm) [30]. The day the wound was made is considered day 0. On days 5, 10, and 15, six animals in each group were randomly selected [28]. At the end of follow-up period, the rats were anesthetized with a mixture of ketamine–xylazine (100 and 10 mg/kg, respectively, *i.p.*) and scar tissues were taken all around from biopsy sites.

Silymarin has been supplied from Sigma-Aldrich, Germany and SM cream was prepared by dissolving excipients (15 g stearic acid, 5 g glycerin, 0.72 g potassium hydroxide, 79 g water, 0.1% sodium benzoate and 1% Tween 80'). SM was applied once daily for 15 days, in sufficient amounts to cover the entire wound. Dxp was applied as Bephanthol® cream which includes lanolin and Dxp (%5) from Bayer-Germany. Rats were treated with an equal volume of Dxp once daily for 15 days.

Histopathological Evaluation

Tissue samples for histopathological analysis were separately fixed in 10% formalin and embedded in paraffin blocks, which were processed histologically. Five μm thick samples were taken on classic slides and then deparaffinized. After standard Hematoxylin–Eosin (H-E) and Masson's trichrome (MT) staining, the slices were examined with light microscopy (Nikon, Eclipse E600, Tokyo, Japan). HE staining was applied for routine histopathological evaluation. MT staining was used to determine degree of collagenization. Inflammation and collagen deposition were graded as: 0 (none), 1 (scant), 2 (moderate), and 3 (abundant). Epithelization was graded as either: 0 (none), 1 (partial), 2 (complete, but immature or thin), and 3 (complete and mature) [31]. Scored for immunohistochemical staining as follows: 0 for staining <1%, 1 for 1 to 25%, 2 for 26 to 50%, 3 for 51 to 75%, and 4 for >75% of the examined cells. Staining intensity was graded as follows: 0, negative staining; 1, weak staining; 2, moderate staining; 3, strong staining. The histological score (H-score) for each specimen was computed by the formula: H-score = proportion score × intensity score A total score of 0 to 12 was calculated and graded as negative (−, score: 0), weak (+, score: 1 to 4), moderate (++, score: 5 to 8) or strong (+++, score: 9 to 12) [32].

Immunohistochemistry

The samples mounted on poly-L-lysine coated slides were deparaffinized, and then rehydrated in descending concentrations of ethanol. For antigen retrieval, the sections were incubated in citrate buffer (pH 6) at high temperature in microwave for 20 min and then soaked with 3% H_2O_2 and methanol mixture to eliminate peroxidase activity in tissue and finally incubated with primary antibodies for N-Cadherin (ab18203,1/200), E-Cadherin (ab76055,1/200) Vimentin (ab8978,1/200), FGF-1 (sc7910, 1/100), Occludin (ab216327,1/200), Claudin-1 (RB-

9209-RT), VEGF-A (ab52917,1/200), NfκB (ab16502,1/200), TNF (ab34674,1/200), and MMP-9 (ab76003, 1/200) at 4°C overnight. The next day, the slides were incubated with HRP secondary antibody at room temperature. The slides were developed with 3-amino-9- ethylcarbazole and counter stained with Mayer's Hematoxylin. At the end, slides were mounted with water-based mounting medium. All the chemicals were purchased from Labvision Corp. (Fremont, CA, USA). The immunoreactivity of the antibodies was evaluated under light microscopy (Nikon, Eclipse E600, Tokyo, Japan).

Statistical Analysis

All data is represented as mean ± standard error of the mean (SEM) throughout the study. The data were analyzed with *Kruskal-Wallis* analysis and *Dunn's* multiple comparison tests. Graphics were drawn with Graphpad Prism 6.01 (GraphPad Software Inc., La Jolla, CA, USA). Values of $p<0.05$ were considered as significant.

Results

Histopathological Results

As shown in **Table 1** according to the **Image 1**; results of histopathological examination showed that, there were no epithelization findings on 5th day. However, epithelization accelerated in all groups at day 10 and day 15. Although

this difference was more severe in Dxp and SM groups compared to the sham group, there was no significant difference between Dxp and SM treatment types. It was observed that inflammation developed on the 5th, 10th, and 15th days in all groups due to wound formation, and the inflammation decreased as time progressed. This reduction in inflammation during the process was higher in the Dxp and SM groups than in the sham group while no significant difference between Dxp and SM treatment types. Collagenization for tissue remodeling was observed to increase over time in all groups on the 5th, 10th, and 15th days. On the other hand, it is seen that this recovery process is faster in the Dxp and SM groups, and there is no difference between the treatment effects of the Dxp and SM groups.

Immunohistochemical Results

As shown in **Figs.1**a and b, E-Cadherin and Occludin *HScores* could not be calculated because there was no staining. However, it was observed that both protein levels increased significantly with Dxp and SM treatments on the 10th and 15th days compared to the sham (**Image 2**). In all time periods, Dxp and SM treatments were found to significantly reduce N-Cadherin and Vimentin *HScores* compared to Sham (**Figs 1c and d**). On the other hand, N-Cadherin values

Table 1. Effects of SM and Dxp, on epithelization, inflammation and collagenization of the skin of rats.

Parameters		Day 5	Day 10	Day 15
Epithelization	Sham	0	1±0.81	1.6±0.54
	Dxp	0	1.8±0.44	2.5±0.57
	SM	0	1.6±0.54	2.4±0.54
Inflammation	Sham	2.2±0.83	2.25±0.5	1.2±0.83
	Dxp	2.16±0.4	0.6±0.54	0.25±0.5
	SM	2±0.81	0.8±0.83	0.2±0.44
Collagenization	Sham	0.4±0.54	1.25±0.5	2.2±0.83
	Dxp	0.66±0.51	1.8±0.44	2.75±0.5
	SM	0.75±0.5	2±0.7	2.6±0.54

increased on the 10th day compared to the 5th day and normalized on the 15th day (**Image 2**). While Vimentin levels did not change between conjugates in the process, *HScore* values of only the SM group decreased significantly. As shown in **Fig. 1e**, no differences between all groups on the 5th day of FGF1 values. On the 10th day, FGF1 *HScore* of Sham and Dxp groups were increased compared to 5th day, significantly; but SM did not change. Moreover, while MMP-9

values in the Sham group did not change depend on the time, the Dxp and SM groups increased significantly on the 10th and 15th days (**Image 2**). However, no significant difference was found between Dxp and SM groups (**Fig. 1f**). No staining Claudin-1 on the 5th day, therefore HScores did not calculate. But, on the 10th day, Dxp and SM treatment were enhanced the Claudin-1 values compared to sham; there is no differences between all groups on the 15th day
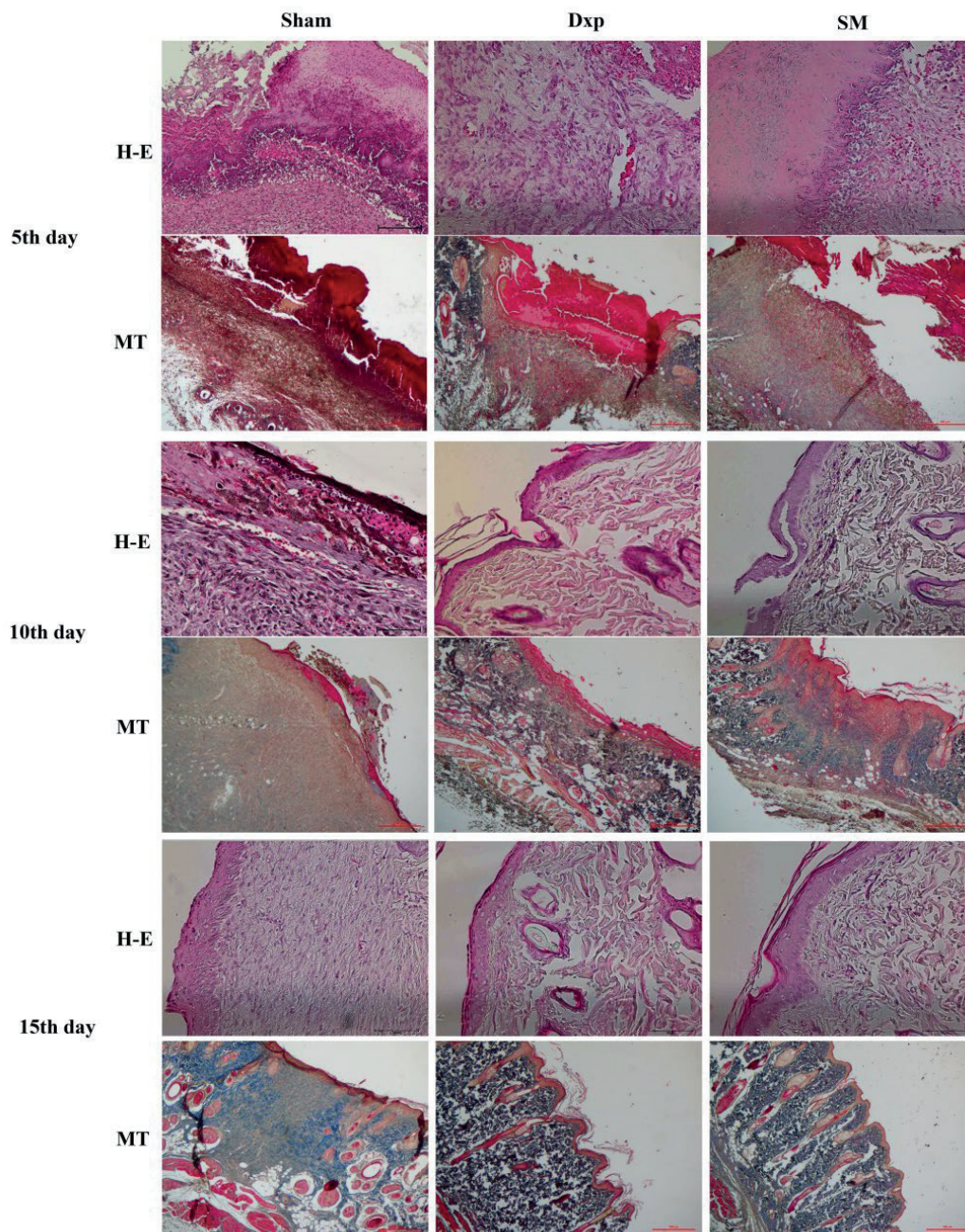

Image 1. Histopathological features of the wound sections from Sham, Dxp, and SM groups on the 5th, 10th, and 15th days. H-E staining (×200) shows epithelization and inflammation whereas MT (×40) staining shows collagenization of the wounds of the tissues.

(**Fig. 1g**). VEGF-A values of the SM treated rats were increased significantly time dependent and compared to Sham, but no differences were found in all the groups (**Fig. 1h**). Additionally, in terms of NfκB values, no significant change was observed between groups and depending on the time (**Fig. 1i**). However, TNFα values were significantly decreased on the 15th day in SM group compared to Sham and 10th day (**Fig. 1j**).

Discussion

In a clinical study, SM was shown to accelerate the wound healing process by reducing inflammation in thermal injuries [35]. In another study on rats, SM was shown to modulate skin inflammation [36]. According to the general histopathological evaluations of this study, it is seen that SM contributes positively to wound healing by reducing inflammation, increasing epithelialization and collagenization. At this stage, the wound healing effect of SM is largely parallel to that of Dxp treatment. Favorable preclinical and clinical outcomes have been reported for

FGF-1 in healing of dermal injuries and ulcers in both diabetic animal models and diabetic human patients [37]. Growth factor-based therapies can potentially accelerate the overall healing process compared to more passive-based therapies, and FGF-1 is an effective single therapeutic agent for dermal healing [14]. In our study, FGF-1 expressions increased at 10th day and tended to decrease at 15th day. It is thought that the reason for this is that the amount of FGF-1 increases during the wound healing period and starts to decrease after epithelialization is achieved. The low level of FGF1 in both Dxp and SM groups compared to the Sham group may be related to the acceleration of EMT process by Dxp and SM. Similarly, while VEGF-A is expected to induce angiogenesis necessary for the initiation of the hemostatic process [6,7]. This study results show that VEGF-A *HScore* values are hyperactivated by Dxp and SM. Vimentin is a type III intermediate filament found in mesenchymal cells of various tissue types during developmental stages and preserves cell and tissue integrity [12]. Vimentin
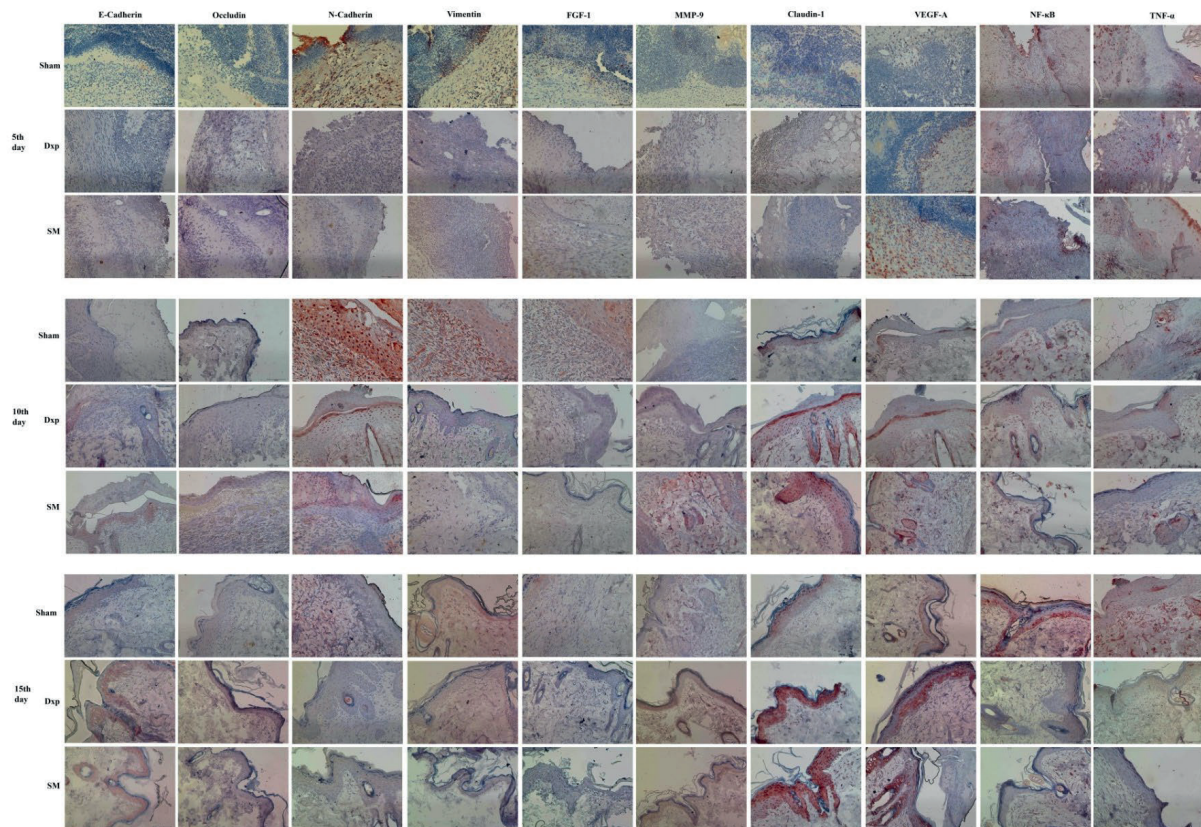

Image 2. Immunohistochemical staining (×200) of the E-Cadherin, Occludin, N-Cadherin, Vimentin, FGF1, MMP-9, Claudin-1, VEGF-A, NfκB, and TNFα proteins from Sham, Dxp, and SM groups on the 5th, 10th, and 15th days.

directly coordinates four cellular activities important in wound healing control; fibroblast proliferation, collagen deposition, keratinocyte differentiation and re-epithelialization. Loss of vimentin disrupts this coordination, leading to slow, weak and incomplete wound healing. Vimentin intermediate filaments induce changes in cell shape, adhesion, migration and signaling in EMT [5,13]. In our study, the increase in

Vimentin expression on the 10th day seems to contribute to wound healing, and its decrease in the later period of wound healing seems to be related to the shortening of the EMT process. However, MMP-9, one of the most studied MMPs, is a type IV collagenase that is expressed by keratinocytes at the anterior end of the wound and is known to promote cell migration and re-epithelialization [16]. In normal tissue,
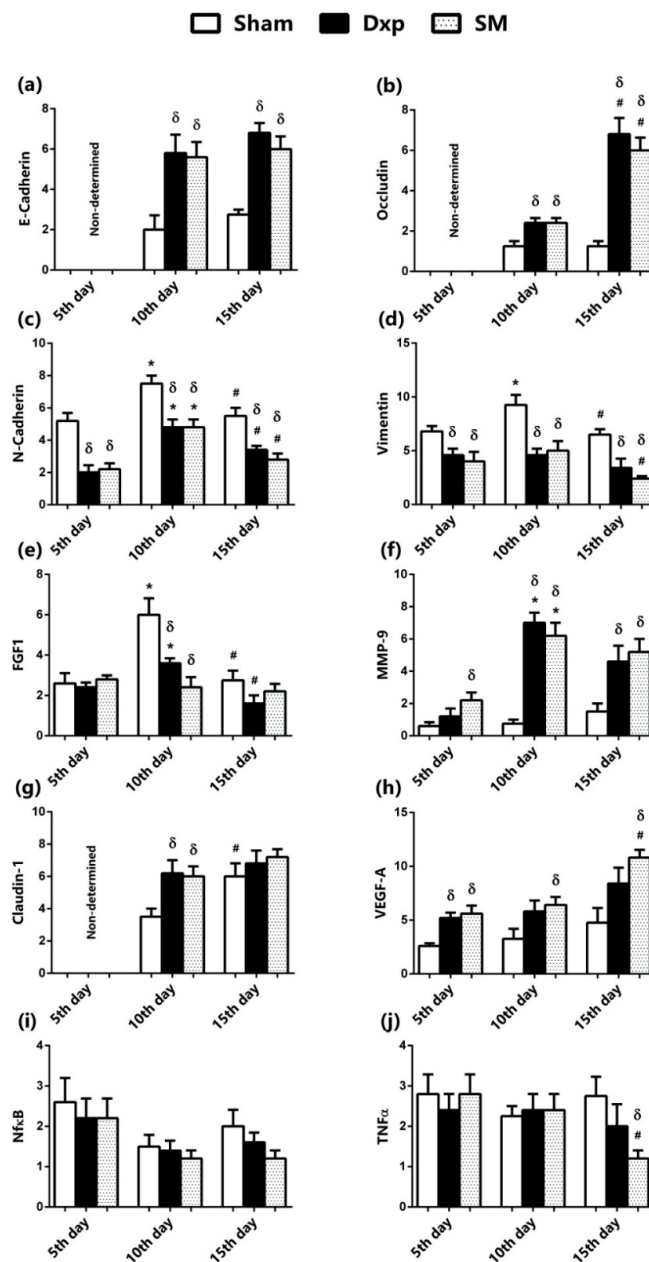
Figure 1. Immunostaining *HScore* results of the E-Cadherin (a), Occludin (b), N-Cadherin (c), Vimentin (d), FGF1 (e), MMP-9 (f), Claudin-1 (g), VEGF-A (h), NfκB (i), and TNFα (j) proteins from Sham, Dxp, and SM groups on the 5th, 10th, and 15th days. δ shows significantly difference from the sham in the same time period ($p<0.05$); * shows significantly difference in the conjugates from the 5th day ($p<0.05$); # shows significantly difference in the conjugates from the 10th day ($p<0.05$).

MMP-9 is expressed at basal levels but is rapidly up-regulated following injury. MMP-9 decreases as wounds heal [38]. If normal MMP-9 levels are suppressed, epithelization is delayed. If there is a persistent excess of MMP-9 in chronic wounds, it leads to impaired healing. Therefore, the balance of this bimodal MMP-9 action is critical to the epithelialization process [39]. In our study, while MMP-9 expressions were found to be higher on the 10th day of wound healing, a decrease was detected in the following days in the both Dxp and SM groups compared to Sham. These data indicate that our treatment agents accelerate epithelization.

It has been shown that silibinin as a natural polyphenolic flavonoid, enhanced in the breast cancer MDA-MB-231 cells E-Cadherin expression, while significantly reducing the levels of mesenchymal biomarkers such as N-Cadherin and Vimentin; as well as decreased expressions of migration-related proteins MMP-2 and MMP-9 [40]. Another study showed that silibinin counters radiation-induced invasive and migratory phenotype of prostate cancer cells and leading to inhibition of N-Cadherin and also reverses E-Cadherin downregulation [41]. In our study, it has been shown that SM contributes favorably to wound healing by reducing inflammation and increasing epithelialization and collagenization. In addition, our study results indicate that SM treatment shortens the EMT process with E-Cadherin induction and N-Cadherin suppression, in line with the literature. In guinea pigs, effects of SM were investigated on the alcohol-induced endotoxemia and results indicated that SM increased TNFα, Occludin, and Claudin mRNA expressions whereas it did not change the NfκB and levels [42]. Similarly, our findings showed that Occludin and Claudin-1 *HScore* values were enhanced. The dramatic increase in Occludin levels on the 15th day compared to the 10th day is that Occludin has an active role especially in the collagenization process. On the other hand, according to our study results, NfκB and TNFα levels did not change. These differences indicate that SM may have affected these parameters, especially while suppressing the inflammatory process. When the therapeutic effects of Dxp and SM are evaluated among all findings, it is seen that SM mimics the effects of Dxp to a great extent, and that SM is partially more effective than Dxp, especially in the processes of induction of epithelialization and suppression of inflammation.

Conclusion

EMT is an important process in wound healing. Prolongation of this process may also lead to extend the wound healing process and scar formation. Dxp-based creams, which are frequently used in daily life, contribute to wound healing processes. As a result, the cream prepared with SM kept the EMT process within certain limits and gave results similar to Dxp, which is used continuously in wound healing. This shows that the therapeutic potential of SM is promising today, when herbal medicines are being used frequently. In this context, more comprehensive studies in vitro and in vivo are needed. Longer study periods, larger sample sizes, and studies with different animal models will allow us to obtain clearer ideas about the effect of silymarin on wound healing.

Conflict of interest

The authors declare no competing interests.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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ORIGINAL ARTICLE

Apoptotic effects of *Acorus calamus* extract on prostate cancer LNCaP cells

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Abstract

Acorus calamus is a plant that is widely used in Far Eastern and Asian countries and has anti-inflammatory, anti-oxidant, anti-microbial, and anti-cancer effects. Prostate cancer is a sort of cancer that is frequently diagnosed and has a high mortality rate in men. Only a limited number of studies are available showing the effects of *Acorus calamus* on prostate cancer. To assess the effects of *Acorus calamus* extract on caspase and anti-apoptotic and pro-apoptotic markers that play a role in the apoptotic process of LNCaP cells in prostate cancer by conducting an in vitro study. LNCaP cells were incubated for 24 and 48 hours and treated with different concentrations of an ethanolic extract of *Acorus calamus* ranging from 250 to 700 µg/ml. Caspase-3, -8, and -9, Bcl-2, Bax, APAF-1, Bcl-XL, and p53 levels were measured using the ELISA method. Quantitative gene expression analyzes of Bcl-2 and Bax were performed using real-time reverse transcription–polymerase chain reaction. The Mann-Whitney U and Tukey tests were used to analyse differences between groups. $p<0.05$ was considered statistically significant. Caspase-3 and -8 and APAF-1 levels were found to be significantly higher in the 48th-hour application of 700 µg/ml of *Acorus calamus* extract than in the control group ($p<0.05$, $p<0.001$, and $p<0.001$, respectively). Bcl-2 was significantly lower and Bax/Bcl-2 expression ratio was significantly higher at all doses for 24 and 48 hours compared to the control group ($p<0.001$ for all). Although, caspase-9, Bcl-XL, and p53 were higher in experimental groups than controls, no significant difference was found. This study supported the time- and dose-dependent anti-cancer effects of *Acorus calamus* on LNCaP cells of the prostate cancer type. Further preclinical and clinical studies are requisite to support our findings.

Keywords: Acorus calamus, prostate cancer cells, LNCaP, apoptosis, caspases

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Introduction

Prostate cancer is the second most frequently type of solid tumor in men in terms of both frequency and cancer-related mortality [1]. Prostate cancer incidence is increasing under the age of 50 years, and the treatment of cases with refractory metastatic spread is especially difficult [2]. Radiotherapy and chemotherapy have serious side effects, and the cost of prostate cancer treatment is high in terms of health expenditures [3,4].

Due to drug resistance, drug toxicity, and high relapse rates, there has recently been an increasing need for natural substances and phytochemical compounds to determine safer sources to be used in the treatment of cancer [5]. In addition, with the positive results obtained in the treatment of neurodegeneration, diabetes, and cardiovascular diseases, interest in natural/herbal resources in cancer treatment is increasing, and the positive effects of phytochemicals such as galantamine, rivastigmine, and resveratrol have been demonstrated [6]. Phytochemicals isolated from plants and their extracts show activity against prostate cancer. They are considered appropriate for use in combination with existing chemotherapy, albeit not alone [7].

Randomized placebo-controlled clinical trials have been conducted with curcumin, lycopene, isoflavone, and sulforaphane in the treatment of prostate cancer, with flavone, berberine, capsaicin, silibinin, and noscapine being among the phytochemicals whose preclinical research continues [8,9]. Many of these compounds target cancer-specific pathways, such as growth-metabolic (PI3K/AKT/mTOR/CDK and AR), angiogenetic (vascular endothelial growth factor), proinflammatory (NF-kB), tumor suppressive (p53/Rb), and invasive and metastatic (WNT/beta catenin) pathways [10].

Acorus calamus (AC) is a plant native to India and used in Chinese medicine for the treatment of gastrointestinal, respiratory, neurological, kidney, liver, hypertension, metabolic disorders such as diabetes, and obesity. AC's main components are monoterpenes, sesquiterpenes, quinin, flavonoids, phenylpropanoids, and the volatile compounds alpha- and beta (β)-asarone.

AC also has good total nitric oxide and nitrite reducing activity. [11]. The anti-inflammatory, neuroprotective, anti-fungal, anti-oxidant, anti-microbial, anti-allergic, anti-ulcer, and cardioprotective effects of AC, as well as its anti-tumor and tumor protective properties, have been confirmed by in vivo and in vitro preclinical pharmacological studies [12]. It has been indicated that β-asarone, one of the main components of AC, can stimulate apoptosis in and reduce the invasive capability of cancerous cells and have antiproliferative effects in glioblastoma and gastrointestinal system cancers [13-15]. β-asarone nitro derivatives in prostate cancer (PC-3 cells), synovial cancer (SW982 cells), cervical cancer (HeLa cells), neuroblastoma (IMR-32 cells), and breast cancer (MCF-7 cells) have been shown to cause a decrease in the viability of the cancer cells [16].

In previous in vitro research, we showed the dose-response relationship of the ethanolic extract of AC with prostate cancer LNCaP cells and reported its anticancer and anti-angiogenic properties [17]. In the current study, we purposed to evaluate the effect of the AC ethanolic extract on caspases [caspase-3, -8, and -9 and apoptotic protease activating factor-1 (APAF-1)], which get involved in the apoptotic process in prostate cancer LNCaP cells, and on Bcl-2-associated X-protein (Bax), Bcl-2, Bcl-XL, and p53, which are both anti- and pro-apoptotic markers. Studies showing the effects of AC on prostate cancer are limited in literature. With this study, it is planned to support the results we found in the previous study and to present the results of their combined use with chemotherapeutic agents with in vitro studies after this study.

Materials and Methods

Cell Line and Culture Conditions

The LNCaP cell was provided from the Department of Physiology of Yeditepe University (Istanbul, Türkiye). Cells cultured in Roswell Park Memorial Institute 1640 Medium fortified with 100 U/mL penicillin G, 10% fetal bovine serum, and 100 mg/mL streptomycin. Cells maintained at 37 ^{0}C in a humidified incubator under atmospheric conditions of 5% carbon dioxide and 95% air.

Preparation of the Ethanolic Extract of AC

Whole freshly AC was obtained from the local herbal shop. The plant was processed at the Medicinal Plants, Drugs, and Scientific Research Center of Anadolu University. The entire plant was separated into smithereens and dried in the dark. The desiccated herb was ground into powder using a hand-held grinder and sifted. The crude powder was extracted with 70% ethanol in a Soxhlet apparatus for 12 h. Vacuum evaporation was used to concentrate the ethanol extract. The yield of the ethanolic extract was found to be 28.29%. Before application the ethanolic extract was freshly dissolved in dimethyl sulfoxide.

Apoptosis Assay: Caspase-3, -8, and -9, APAF-1, Bax, Bcl -2, Bcl-XL, and p53

Caspase-3, -8, and -9, Bcl-2, and p53 were assayed using their specific Invitrogen Human ELISA Kits (Thermo Fisher Scientific, USA). APAF-1, Bax, and Bcl-XL were assayed using their respective Sunred Human ELISA Kits (Shanghai Sunred Biological Technology Co., Ltd, China). The cells have been seeded at 1×10^4 cells per well into culture medium including different concentrations (0, 250, 500, and 700 μg/ml) of the ethanolic extract of AC. For the determine apoptotic effects the cells were incubated for 24 and 48 hours. In summary, following treatment, the cells underwent two washes with icy cold phosphate-buffered saline solution. They were then lysed using 500 μl of ice-cold lysis buffer, scraped, and sonicated while on ice. Then, the cell lysate was centrifuged at 4 0C for 10 minutes, and before analysis the supernatant was stored at -80 ^{0}C. Each well of the ELISA plate, 100 microliters of soluble fraction were used. The ELISA kits were performed in accordance with the manufacturer's instructions and absorbance value was measured at a wavelength of 450 nm.

Bax and Bcl-2 mRNA Amplification by Reverse Transcription Polymerase Chain Reaction

Total RNA was extracted from cultured LNCaP cells using the RNA Isolation Kit according to the suppliers' directions (A.B.T.™ RNA Purification Kit). After measuring absorbance at 260 nm on a UV spectrophotometer, RNA concentration and purity was calculated and then stored at -80 ^{0}C. The cDNA Kit was used for first-strand cDNA synthesis (VitaScript™ FirstStrand cDNA Synthesis Kit). Polymerase chain reaction (PCR) amplification was performed using the synthesised cDNA as a template. Real Time PCR was carried out (Connect Real Time PCR Detection System) with amplification of human beta actin mRNA as a housekeeping standard. The forward primer for Bax was 5'-GCCGAAATGTTTGCTGACG-3'. The reverse primer was 5'-CGCCACGGTGCTCTCC-3'. The forward primer for Bcl-2 was 5'-ACTTTGCCGAGATGTCCAGC-3' and the reverse primer was 5'-ATCCCAGCCTCCGTTATCCT-3'. The following conditions were applied: initial denaturation at 95 ^{0}C for 5 min, followed by 40 cycles of denaturation at 95 ^{0}C for 15 s, annealing at 60 ^{0}C for 30 s, and extension at 72 ^{0}C for 30 s. Automated threshold analysis was used to determine cycle thresholds. Melting curve analysis was used to confirm primer quality. Relative quantification of the gene expression was carried out by using a standard curve generated from serially diluted of control mRNA or real-time PCR amplicons. All trials were performed three times. Bax and Bcl-2 levels were standardized to beta actin to account for loading differences.

Study Groups

In our previous study, cells were treated with different concentrations (0, 250, 500, 750, 1000 and 1250 μg/mL) of AC for 24 h and 48 h. After 48 h, the XTT measurement was performed. This assay is commonly used to measure cell viability, and the percentage of cell viability was calculated using a spectrophotometer. The IC_{50} value is determined as the concentration that reduces cell viability by 50%. After 48 hours, the XTT measurement was performed, and the IC_{50} value of the AC concentration was calculated to be 732.76 μg/ml [17]. Accordingly, three concentrations (250, 500, and 700 μg/ml) were chosen to be used in experiments to find out the effects of AC on LNCaP cells. The following groups were formed by applying the specified extract concentrations to the LNCaP cells separately for 24 and 48 hours to determine the effects of the ethanolic extract on apoptotic markers.

Group 1: Control group, to which only the medium was applied (n = 6)

Group 2: 250 µg/ml extract, (24 and 48 hours, n = 6)

Group 3: 500 µg/ml extract, (24 and 48 hours, n = 6)

Group 4: 700 µg/ml extract, (24 and 48 hours, n = 6)

Statistical Analysis

All data were obtained from six independent experiments in each group and presented as mean ± standard deviation. The Shapiro-Wilk test was used to test the normality of the distribution of the continuous variables. Tukey test was used for normal distributed variables and Mann-Whitney U test was used for non-normal distributed variables. All data were analysed using the statistical programme SPSS v. 24 (SPSS Inc., USA), and $p<0.05$ considered to be statistically significant.

Results

Table 1 and **Figure 1** present the measurements of apoptotic markers in the applications of 250, 500, and 700 µg/ml of AC extract to LNCaP cells for 24 and 48 hours and their comparison between the experimental groups.

At the 24th hour of AC application, no significant difference was found between the groups in terms of caspase levels. At the 48th hour, however, the high-dose (700 µg/ml) application of AC resulted in significantly higher Caspase-3 levels compared to the control group ($p<0.05$). Caspase-8 levels increased as the AC dose increased at the 48th hour, but this increase was only significant at the highest dose (700 µg/ml) ($p<0.001$). The caspase-9 level was higher at the highest dose of AC compared to the control group, but it was not statistically significant. Similar to the caspase level, the APAF-1 level did not significantly differ between the groups at the 24th hour but was significantly higher at the 48th hour at the highest dose of AC ($p<0.001$).

When the anti-apoptotic marker Bcl-2 was examined, its levels were found to be statistically significantly lower at all doses of AC at both 24 and 48 hours compared to the control group ($p<0.001$). Regarding the other anti-apoptotic marker, Bcl-XL, there was no significant difference between groups.

While there was no significant difference in the levels of the pro-apoptotic marker Bax at the 24th hour, it was determined to be significantly higher at the 500 and 700 µg/ml doses of AC at the 48th hour compared to the control group ($p<0.05$ and $p<0.001$, respectively). Similar to Bax, another pro-apoptotic marker, p53, was found to have higher levels at all doses of AC at both the 24th and 48th hours compared to the control group, but the differences were not statistically significant.

When the Bax/Bcl-2 expression ratio was examined, there was a significant increase at low, medium, and high doses of AC at the 24th and 48th hours compared to the control group ($p<0.001$ for all, **Figure 2**).

Discussion

In the treatment of cancer, medicinal plants are used. Using plants to treat cancer continue to be investigated. In this study, the anticancer features of the ethanolic extract of the AC plant on prostate cancer LNCaP cells were investigated. It was found that AC had a greater effect on markers regulating apoptosis, especially in longer-term (48-hour) and high-dose applications.

One of the mechanisms of action of cytotoxic drugs on cancer cells is apoptosis. It is a caspase-dependent, programmed active cell death that occurs in a well-organized, non-inflammatory intrinsic and/or extrinsic pathway and takes a consequential role in the development and homeostasis of multicellular organisms [18]. In the current study, the caspase-3 and caspase-8 levels of the LNCaP cells exposed to high-dose AC extract for 48 hours significantly increased compared to the control group. Caspase-8 is an initiator caspase that is involved in the extrinsic pathway [19]. Caspase-3, on the other hand, belongs to the executioner caspase family. In recent years, in vitro studies on the effects of plant extracts on LNCaP cells have indicate promising developments [20]. In a study by Zhou et al., the caspase-3 level was found to increase in LNCaP cells treated with *Linum usitatissimum* (flax) [21]. In another study, Nikahd et al. reported an increase in caspase-3 activity in LNCaP cells, to which the extract of *Daphne pontica* was applied using the colorimetric assay method [22]. All

these studies emphasize the importance of the apoptosis pathway and the role of caspases in this pathway in the cancer treatment mechanism and support our findings.

Caspase-9 and APAF-1 play roles in the intrinsic pathway activated by mitochondrial damage in apoptosis [23] and form the apoptosome complex. In the case of intracellular stress, such as hypoxia and drug-induced DNA damage,

release of cytochrome c into the cytosol and binds to APAF-1, promoting the formation of the apoptosome [18]. APAF-1 has been shown to play a role in the development of the brain, apoptosis resistance in neurons and cardiomyocytes, and the pathogenesis of neurodegenerative diseases [24-26]. It has been reported to be involved in the therapeutic mechanisms of MCF-7 cells in breast cancer, oral cancer cells, in lung cancer, and TP53-regulated inhibitor of apoptosis-1 cells in

Table 1. Apoptotic marker measurements of experimental groups involving the application of different concentrations of *Acorus calamus* extract to LNCaP cells for 24 and 48 hours.

Experimental Groups	Mean, ng/ml (SD)							
	Caspase-3	Caspase-8	Caspase-9	APAF-1	Bcl-2	Bcl-XL	Bax	p53
Control								
24 hours	6.56 ± 0.65	2.38 ± 0.04	10.97 ± 1.18	4.64 ± 0.42	26.81 ± 6.63	7.37 ± 1.22	1.40 ± 0.14	22.43 ± 4.16
48 hours	6.79 ± 2.43	2.37 ± 0.02	11.90 ± 2.20	4.33 ± 0.47	42.53 ± 3.52	6.78 ± 0.24	1.26 ± 0.18	25.79 ± 1.49
250 μg/ml								
24 hours	5.19 ± 0.27	2.64 ± 0.31	10.65 ± 2.86	4.05 ± 0.20	8.72 ± 0.34*	9.16 ± 3.13	1.68 ± 0.13	19.75 ± 1.17
48 hours	4.92 ± 0.16	2.86 ± 0.76	7.88 ± 0.58	3.92 ± 0.29	9.29 ± 0.59*	8.22 ± 1.63	1.43 ± 0.17	26.30 ± 0.93
500 μg/ml								
24 hours	5.16 ± 0.47	3.21 ± 0.58	8.94 ± 1.39	4.46 ± 0.28	9.07 ± 1.04*	8.61 ± 1.72	1.63 ± 0.28	23.79 ± 1.29
48 hours	6.15 ± 0.36	3.08 ± 0.01	8.99 ± 0.26	5.06 ± 0.16	10.03 ± 0.38*	10.43 ± 1.89	1.83 ± 0.10**	29.94 ± 0.90
700 μg/ml								
24 hours	5.99 ± 0.46	2.58 ± 0.09	8.44 ± 0.42	4.42 ± 0.22	10.41 ± 0.49*	7.54 ± 0.55	1.58 ± 0.20	24.84 ± 2.55
48 hours	9.82 ± 0.51*	5.18 ± 0.19*	14.94 ± 0.54	8.97 ± 0.15*	16.19 ± 0.83*	11.30 ± 4.03	2.53 ± 0.13*	31.29 ± 1.68

SD: standard deviation, $^*p<0.001$ compared to the control group, $^{**}p<0.05$ compared to the control group

ovarian cancer [27-30]. Our study was conducted on prostate cancer to examine changes in LNCaP cells caused by high doses of AC in the long term and provided significant results concerning the level of APAF-1, which is located in the intrinsic pathway of apoptosis.

Other proteins that control apoptosis in cells are Bcl-2 and Bcl-XL. In a normal cell, these proteins are dominant and are anti-apoptotic markers [31,32]. However, as a result of mutations in the gene encoding these proteins in cancer cells, they are produced in high amounts [33]. Therefore, reducing the levels of these anti-apoptotic markers is important for cancer treatment. An in vitro study examining the effects of herbals on prostate cancer reported that *Melissa officinalis* plant extract inhibited Bcl-2 expression [34]. In another in vitro study conducted with sage plant extracts on a different type of prostate cancer cell (DU-145), there was a significant decrease in Bcl-2 and a significant increase in Bax and
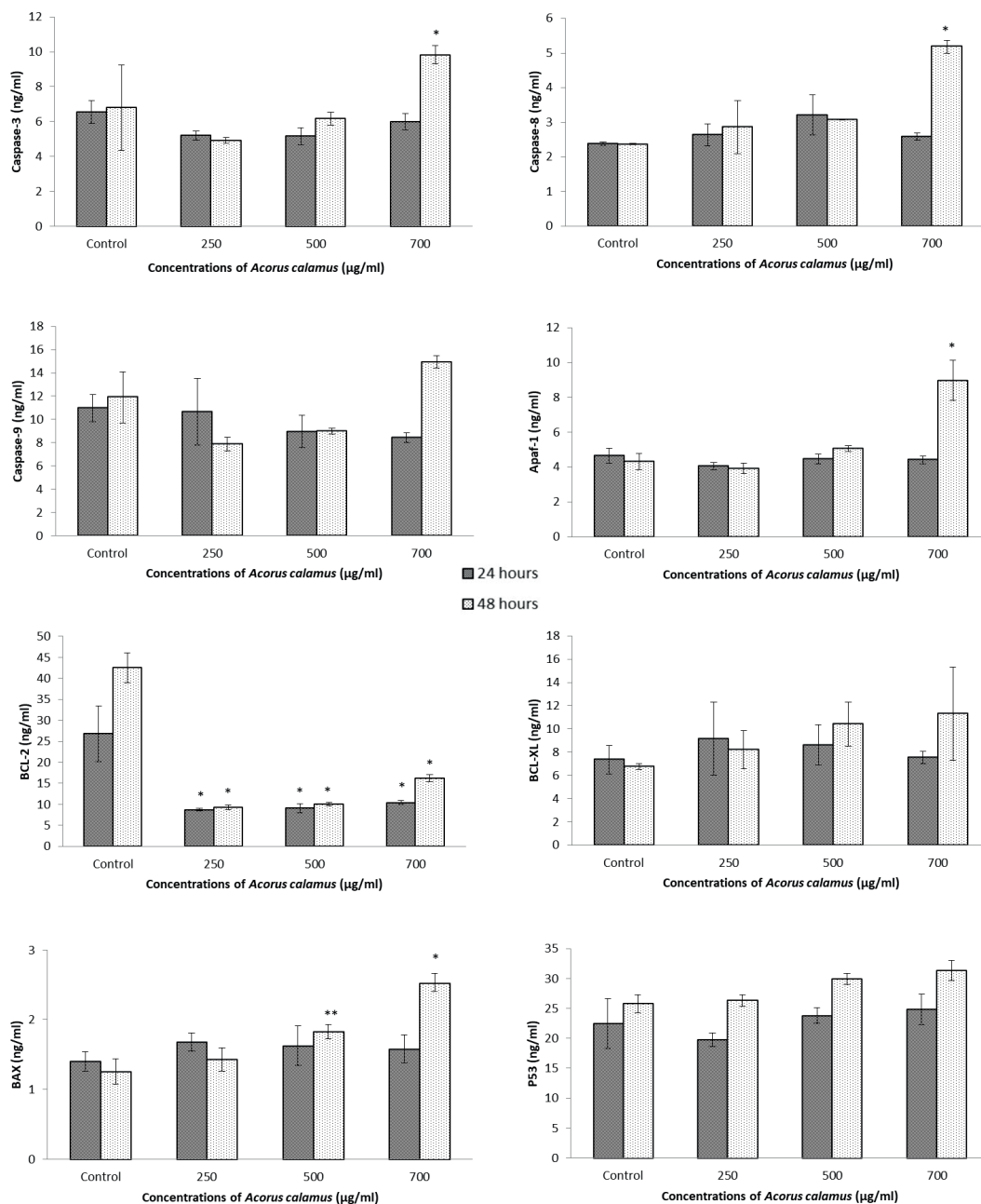

Fig 1. Effects of *Acorus calamus* concentrations on apoptotic markers. *$p<0.001$ compared to the control group, **$p<0.05$ compared to the control group.

caspase-9 [35]. Although the AC plant extract we used in our study did not cause a significant difference between the experimental groups in terms of the Bcl-XL ratio in prostate cancer cells, the significant decrease in Bcl-2 suggests that this plant may be a promising anticancer agent.

Bax, a protein belonging to the Bcl-2 family located in the mitochondrial membrane, is involved in the intrinsic pathway of apoptosis and plays a role in mitochondrial fragmentation [36]. As a pro-apoptotic marker, Bax is one of the important regulators of apoptosis [37]. The Bax/Bcl-2 ratio represents a corresponding change in mitochondrial permeability at the beginning of the induction of apoptosis. Therefore, this ratio is frequently used in cancer research. In clinical and pre-clinical studies, the Bax/Bcl-2 ratio has been investigated in different types of treatments for leukemia, prostate, colorectal, and breast cancers, and it has been suggested that this ratio can be used as a predictive marker in the follow-up of apoptosis potential and response to treatment [38-43]. It has also been used to show the presence of apoptosis in studies on different types of plant extracts and prostate cancer cells. One of these studies reported that the Bax/Bcl-2 ratio increased in prostate cancer cells (PC3) to which *Scutellaria altissima*, which is widely used in China, was applied, and this was accompanied by an increase in sensitivity to cisplatin, an anti-cancer treatment agent [44]. In a study

investigating the effects of a Mediterranean herb, rosemary (*Rosmarinus officinalis*), on prostate cancer cells (PC3), it was found that this plant caused an increase in the ratio of Bax to Bcl-2. [45]. Chen et al. examined the effects of dioscin, an herbal saponin, on LNcaP cancer cells and observed down-regulation of the expression of Bcl-2 and up-regulation of the expression of Bax, reporting similar results to our study in relation to the ratio of these two apoptotic proteins [46]. In our study, Bcl-2 and Bax significantly differed between the experimental and control groups, both when examined alone and in combination as a ratio, which is evidence that AC is also effective in the mitochondrial step of apoptosis in prostate cancer cells.

P53, also under the name of "guardian of the genome", is a transcription factor that is frequently mutated in malignancies [47]. It matters in the regulation of important cellular activities, such as apoptosis, cell cycle, and senescence. It can provide information on the prognosis of cancer [48]. It has also been proven to be one of the major prognostic factors in prostate cancer [49]. Previous publications have proven the presence of p53 mutations in various prostate cancer cells (PC-3, LNCaP, DU-145, and TsuPr-1) [50,51]. Studies using herbal antioxidant compounds, such as resveratrol and berberine, in prostate cancer cells have shown that these herbal compounds increase p53 and thus induce
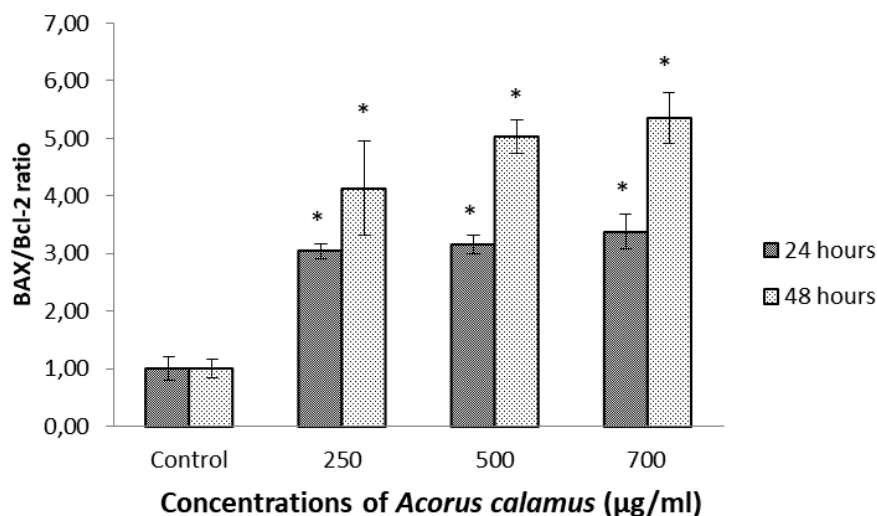

Fig 2. Effects of *Acorus calamus* concentrations on the Bax/Bcl-2 expression ratio. *$p<0.001$ compared to the control group.

apoptosis. In our study, although the p53 levels of the AC-administered groups were found to increase compared to the control group, not significantly different, which may be related to the duration of AC application being limited to 48 hours based on the literature. We consider that a statistically significant result could be achieved in the presence of a longer application time.

One of the most important components of the AC plant that plays a role in the apoptotic process is β-asarone. In a study in which in vivo and in vitro experiments were carried out together, it was found that β-asarone stimulated apoptosis and cell cycle arrest in G1 phase in glioma cells and increased the caspase-3 level [52]. In their in vitro study, Wu et al. found that β-asarone significantly activated caspase-3, -8, and -9 and Bax and suppressed Bcl-2 activity in gastric cancer cells [53]. In a study on colon cancer cells, the authors reported that β-asarone activated the steps of caspase-9 and caspase-3 and also induced apoptosis by decreasing theBcl-2/Bax ratio [54]. Wang et al. showed that β-asarone had effects on apoptotic processes, including caspase-3, caspase-9 activation, Bax up-regulation, and Bcl-2 down-regulation in lung cancer cells, as well as different molecular mechanisms, such as the inhibition of the Wnt/β-catenin signaling pathway [55]. In light of all these literature data and our findings, it is considered that the β-asarone component of AC may be effective in prostate cancer cells.

It has been proven that AC has antioxidant properties due to the presence of asarone in its structure. In a study conducted in rats administered cisplatin, the antioxidant effects of AC on the ovaries were examined, and it was found that AC caused a significant increase in superoxide dismutase levels [56]. AC has also been shown to have antimicrobial and anthelmintic properties [57].

In contrast to the information given above, in a study focusing on cognitive functions, Geng et al. found that β-asarone inhibited the activation of Bcl-2 and caspase-3 in the hippocampus of rats and therefore might be a potential treatment option for dysfunction related to Alzheimer's disease [58]. Similarly, Liu et al. reported that

β-asarone suppressed β-amyloid-induced neuronal apoptosis in the hippocampus of rats [59]. These different results can be attributed to study designs, the in vivo nature of experiments, dosing differences, and these assessments not being undertaken in cancer cells. While apoptosis is insufficient in cancer, excessive apoptosis is seen in neurodegenerative diseases [60]. The results of these studies also suggest that AC acts through different tissue-specific mechanisms and may act as a modulator.

AC has been used in traditional medicine for a long time and is generally considered safe. However, it should be noted that toxic effects can occur with high doses or long-term use. There are various indications in the literature that AC can cause hepatotoxicity, genotoxicity and cardiotoxicity in high doses. Alpha- and beta-asarone have been shown to cause cytotoxicity in human HepG2 liver cells [61]. Asarone has been reported to cause DNA damage and chromosomal abnormalities in vitro [62]. In another study, alpha-asarone was reported to cause cardiotoxicity by inducing cardiac defects and QT prolongation via mitochondrial apoptosis in zebrafish [63]. Most of these toxic effects have been observed at high doses or with long-term exposure. Therefore, caution should be exercised when using the plant and care should be taken not to exceed therapeutic doses. However, further research is needed to standardize, optimize doses and determine the long-term safety profile of AC.

In studies investigating the effect of AC on other cancers, the apoptosis-inducing effect of the alpha-asarone content of AC on lung cancer cells was found [64]. A review paper highlighted that in vitro studies have shown that AC inhibits the growth and proliferation of different cancers such as breast, prostate, colon, lung, liver and leukemia cancers and that these effects are due to the various bioactive components (α-asarone, β-asarone, calamusin, etc.) it contains. In addition, AC has been reported to be associated with mechanisms such as cell cycle arrest, induction of apoptosis and prevention of cell migration [65]. Although in vitro and in vivo studies on the potential role of AC in cancer treatment provide promising evidence, they

need to be substantiated by comprehensive scientific studies before the clinical application of AC can be addressed.

AC appears to have promising anti-cancer effects against prostate cancer and various types of cancer. However, the plant still needs to be supported by extensive scientific research before it can be put into clinical practice.

The studies investigating the effects of AC on normal cell lines have been referenced with the literature. In the study investigating the cytotoxic effect of AC on dermal fibroblast cells from experimental animals, AC did not significantly affect the viability of fibroblast cells even at high concentrations (up to 200 μg/mL) [66]. In the study investigating the effect of AC on human peripheral blood mononuclear cells in vivo, it was found that it did not significantly affect cell viability [67]. These results suggest that the plant may have a selective toxicity potential against cancer cells.

This study did not investigate the potential effects of AC on the tumor microenvironment, particularly MMP-9-mediated remodeling of the extracellular matrix. In future studies, investigating the effects of Achorus calamus on tumor-stroma interactions may help us to better understand the potential of this plant in the treatment of prostate cancer. Another limitation is herbal extracts usually contain several components and there may be synergistic, additive or antagonistic interactions between these components. A phytochemical analysis for AC could not be performed in the study. The composition of herbal extracts may vary depending on factors such as geographical origin, time of harvest and processing methods. A phytochemical analysis could have helped to confirm the composition of the extract and ensure comparability between different studies.

Conclusion

Cancer is a complex and multifactorial disease with a high mortality rate that constitutes a serious threat to human health. In this study, it was observed that AC had an effect on caspases and pro-apoptotic and anti-apoptotic proteins and played a role in both the intrinsic and extrinsic pathways of apoptosis. Significant results observed in the Bax/Bcl-2 expression ratio suggested that it also affected the mitochondrial step of apoptosis. These results were time- and dose-dependent. Our results were compared to those of many studies conducted with different types of herbal medicine on different types of prostate cancer cells. Although the β-asarone component of AC has been shown to have effects on different cancer cells, there are still limited data concerning its effects on prostate cancer. There is a need for further pre-clinical and clinical studies to fully elucidate the molecular mechanism of AC in different types of prostate cancer cells and demonstrate their utility alone or in combination with chemotherapeutic agents used in cancer.

Conflict of interest

The authors declare that they have no competing interests.

Ethics approval and consent to participate

Not applicable.

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ORIGINAL ARTICLE

Nursing profession during the pandemic: Perceptions, changes, and the role of media

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Abstract

The onset of the COVID-19 pandemic has dramatically highlighted the critical role of nursing in healthcare, transforming the traditional image of nurses from "sisters" or "white angels" to a more esteemed and academic profession. This study aims to evaluate nurses' perspectives on how the media influences the perception of the nursing profession during the COVID-19 crisis and its impact on their professional image. The study seeks to assess nurses' views on the media's role in shaping public perception of nursing during the COVID-19 pandemic and its implications for professional identity. A total of 208 nurses employed in a hospital setting between January 2022 – March 2022 participated in this research, with institutional approval. Data were collected using a researcher-designed questionnaire and the Nursing Profession Image Scale. Statistical analysis was performed using SPSS 22.0, employing descriptive statistics, Pearson correlation, and Student's t-tests, with a significance level set at $p<0.05$. The average age of the nurses was 35.02±7.80 years, with 81.7% being female. Only 7.3% were affiliated with a professional association. A notable 26.0% believed there were positive shifts in the nursing profession's image during the pandemic, while 80.3% observed increased discourse around nursing due to the pandemic. Moreover, 41.8% felt the media adequately portrayed nurses and their professional challenges. The mean score on the Nurses' Image Scale was 112.00±9.67. According to gender, affiliation with a professional organization, and perceptions of media coverage, the perception of the nursing profession's image has varied. However, no significant correlations were found between age, education, professional experience, and perception of the nursing profession. Nurses' perceptions of their professional image are moderately positive. Efforts to enhance the profession's public perception, particularly through media representation, are deemed imperative.

Keywords: COVID-19, nurse, professional image

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Introduction

The COVID-19 pandemic has profoundly impacted global health systems, presenting significant challenges, particularly for healthcare professionals such as nurses. Nurses have undertaken a critical role in combating the pandemic, facing intense workloads and coping with physical and emotional challenges. This challenging period has significantly influenced the public perception of the nursing profession, emphasizing once again the value of their profession [1-3]. Nurses have undertaken a critical role in combating the pandemic, facing intense workloads and coping with physical and emotional challenges. This challenging period has significantly influenced the public perception of the nursing profession, emphasizing once again the value of their profession [4,5]. Since the beginning of the pandemic, the rapidly increasing number of cases has exerted significant pressure on nurses working in the healthcare sector. Nurses, swiftly adapting to the treatment and care of COVID-19, have provided both physical and psychological support to patients. [6].

However, during this process, the overwhelming number of patients exceeding intensive care and hospital capacities compelled nurses to endure long and intense working hours. Nurses faced not only physical challenges but also psychological difficulties, such as the fear of transmitting the virus to their families [7,8]. Nurses who witnessed the pandemic experienced concerns such as burnout, fatigue, and the fear of transmitting the virus to their families due to increased workload and working hours. However, they continued their profession with great dedication to contribute to public health [9].

While highlighting the challenges caused by COVID-19, the current nursing workforce's unfavorable working conditions and the difficulties they face have also become evident [10]. Appreciation, gratitude, and love for nurses spread to a wide audience through social media. The missions of finding solutions to health problems, improving public health, and directly saving human lives were emphasized for nurses [7].

The majority of society perceives the nursing profession as an assistant to doctors, unquestionably implementing the doctor's instructions, and distributing medication. Representations in the media, especially in newspaper articles, advertisements, TV series, and films, significantly influence the image of nursing. However, these media representations often do not realistically reflect the image of nursing [11-15]. There is a direct relationship between public perception and the quality of nursing care. When nurses are subjected to the negative critical views of society, when they do not receive respect for their work, and when they face negative behaviors from patients, it has been reported that it affects their ability to focus on their job, lowers their motivation, leads to more mistakes, and results in less patient-oriented and respectful attitudes [14]. Therefore, understanding how the image of nursing and nurses' perceptions of professional image are shaped is a crucial factor that can impact nurses' professional performance and the quality of patient care.

Professional image perception is shaped by the combination of nurses' perceptions, views, and attitudes towards their profession, impacting job satisfaction, performance, and retention rates. Understanding the effects of the COVID-19 pandemic on the perception and image of the nursing profession is a fundamental factor in shaping nurses' perceptions of their roles and futures [4,5,7]. Enhancing these factors positively can contribute to increasing the benefits that nurses provide to society. However, the negative effects of professional image perceptions of nurses during the pandemic can lead to professional alienation, decreased job satisfaction, and a decline in the quality of care. In this context, the effects of the pandemic on the nursing profession should be thoroughly examined [4,7].

The role of the media stands out as a crucial factor in shaping the image perceptions of nurses [16-18]. During the pandemic, the media created societal awareness by sharing nurses' efforts and challenges with a broad audience [7]. Positive and supportive media content contributed to strengthening the professional

image of nurses and increased the respect society has for them [12-17]. In this context, understanding nurses' perceptions of their professional image during the pandemic, along with crucial factors such as societal respect, motivation, and professional satisfaction, is of great importance for the future development of the nursing profession and its impact on the healthcare system [19]. In this context, a detailed examination of nurses' perceptions of their professional image during the pandemic can provide valuable insights for developing coping strategies in the healthcare sector and enhancing nurses' levels of professional satisfaction. In our country, a limited number of studies have been found examining the impact of the COVID-19 pandemic on nurses' perceptions of professional image.There is no optimal number of studies in this field [12,14,20]. Therefore, the aim of this study is to assess nurses' thoughts on the role of the media in shaping the image of the nursing profession during the COVID-19 process and their perceptions of professional image.

Materials and Methods

Study Design

The research has a cross-sectional design and was planned as a correlational study. In order to enhance scientific reporting standards and accurately convey the methods during the research process, the STROBE checklist was utilized.

The primary objective of the study is to examine the impact of the COVID-19 pandemic on nurses' professional perceptions and professional image. In this context, the research focuses on the following questions:

1. What are the thoughts of nurses regarding the role of the media in the changing image of the nursing profession during the pandemic?

2. How does the COVID-19 pandemic affect the professional perception of nurses?

3. Do the descriptive characteristics of nurses affect the Nursing Profession Image Scale scores?

Study Place and Period

The study population consisted of nurses working at Osmaniye State Hospital. The data

were collected from the nurses who agreed to participate in the research between January 2022- March 2022.

Study Population

Voluntary participants who have actively worked as nurses for at least one year among the 224 nurses working at Osmaniye State Hospital were included in the research. Nurses who were on leave during the pandemic or had less than one year of work experience were excluded from the study. The data of 208 nurses were included in the research (92%).

Data Collection Tools

The data of the study were collected using a descriptive characteristics form (participants' certain characteristics, nurses' thoughts on the role of the media in changing the image of the nursing profession during the pandemic) and the Nursing Profession Image Scale. After obtaining ethical approval, the forms were distributed online through Google Forms to nurses who agreed to participate in the study. Additionally, a link and explanations about the study were distributed via email or WhatsApp.

To determine nurses' professional image perceptions, a scale developed by "Dost and Bahçecik" in 2015, consisting of a 5-point Likert scale with 6 factors and 42 items, was utilized. The scale consists of professional qualifications (11 items), working conditions (10 items), gender (8 items), education (5 items), professional status (5 items) and appearance (3 items). It consists of 6 subscales: item). 15 items in the scale (8, 14, 15, 17, 18, 20, 21, 22,23, 24, 25, 26, 28, 29, 31) are scored negatively. The lowest score that can be obtained from the scale is 42, the highest. The score is 210, 42-75 is very weak, 76-109 is weak, 110-143 indicates average, 144-177 indicates good and 178-210 indicates very good image perception. Taken from scale as the score increases, the image perception also increases positively. The Cronbach's Alpha coefficient of the scale is $\alpha=0.82$ [21] and in this study, it was found as $\alpha=0.80$.

Data Analysis

Statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS)

version 22 software. Descriptive statistical methods such as frequency, percentage, mean, and standard deviation, as well as the Kolmogorov-Smirnov distribution test were used to examine the normality of distribution. The data were found to be normally distributed. Student's *t*-test was applied to determine relationships between nurses' scale scores and sociodemographic variables. The Pearson correlation analysis was conducted to evaluate the relationship between age and the scores of the nursing profession image scale. The obtained *p*-values were considered significant at the <0.05 level.

Ethical Considerations

The present study received approval from the Ethics Committee of Korkut Ata University (Date: 22/12/2021, Decision no: 2021/8/7). Written approval was obtained from the institution where the study was conducted. They continued with the survey after indicating their consent and retention of online data collection records participants were asked to indicate their

willingness to volunteer in the online survey. Prior to the study, an informed consent form was obtained from participants who agreed to take part in the research. The research process adhered to the principles of the Helsinki Declaration.

Results

The participants in the study consist of nurses, of whom 81.7% are female. In terms of educational background, 67.3% have a university degree, and 14% are members of professional associations. The average age of the participants is 35.02±7.80 years, with an age range from 21 to 53 years. The average professional experience among the nurses is 12.18±8.06 years, ranging from 1 to 34 years (**Table 1**).

Approximately 74% of the nurses believe that there have been positive changes in the image of the nursing profession during the pandemic, attributing this change significantly to the influence of the media. Furthermore, 80% noted an increased representation of the nursing profession in various media outlets during the pandemic. However, 58% expressed the opinion

Table 1. Descriptive characteristics of nurses (N:208).

Features	n	%
Gender		
Female	170	81.7
Male	38	18.3
Education		
High School	17	8.2
Associate Degree	33	15.8
Bachelor's Degree	140	67.3
Master's Degree	18	8.7
Association Membership		
Yes	14	7.3
No	194	92.7
The association joined		
Turkish Nurses Association	8	57.0
Student Nurses Association	3	21.0
Turkish Midwives Association	2	15.0
Turkish Neonatology Association	1	7.0

Age: Mean±SD* 35.02±7.80 (minimum: 21, maximum: 53) years

Professional experience: Mean±SD* 12.18±8.06 (minimum: 1, maximum: 34) years

* Mean ± Standard Deviation

that, despite these increased representations, the media has not adequately highlighted the positive aspects of nurses or addressed the professional challenges they faced during the pandemic (**Table 2**).

The average score of nurses on the Nursing Profession Image Scale was found to be 112±9.67, indicating a moderate level of image perception. Sub-dimension scores were as follows: professional qualifications 20.03±1.96, working conditions 19.01±3.9, gender 22.67±4.8, education 13.97±1.67, professional status 1.18±3.09, and appearance 6.07±2.8. (**Table 3**).

Those who believe that men and those not affiliated with professional organizations or associations have not been adequately discussed in relation to the nursing profession during the pandemic, in comparison to those who believe they have been sufficiently discussed, found the

image perception of the nursing profession to be high and statistically significant ($p<0.05$) (**Table 4**).

The correlation between age and professional qualifications is weak and negative (r = -0.036). This indicates a slight tendency for a decrease in professional qualifications with increasing age. However, this relationship is very weak and statistically insignificant ($p>0.05$). The weak negative correlation between age and professional qualifications suggests a limited impact of age on professional qualifications. However, this effect is not statistically significant (**Table 5**). No significant relationship was found between educational status and the subscale scores (**Table 5**) ($p>0.05$).

There is a moderate negative correlation between professional qualifications and working conditions (r=-0.534). It is observed

Table 2. Nurses' views on the role of the media in the change of the nursing profession's image during the pandemic (N=208).

Features		n	%
"I believe that there have been positive changes in the image of the nursing profession during the pandemic, and I consider the media's influence to be significant in this transformation."	Yes	54	26.0
	No	154	74.0
"With the advent of the pandemic, nursing profession has started to receive more attention in the media."	Yes	167	80.3
	No	41	19.7
"During the pandemic, nurses were not sufficiently praised in the media, and the professional challenges they face were brought to the forefront."	Yes	87	41.8
	No	121	58.2

Table 3. The scores of nurses on the 'Nursing Profession Image Scale' and its sub-dimensions (N=208).

Scale and Subdimensions	Minimum	Maximum	Mean	Standard Deviation
Professional Qualifications	9	24	20.03	1.96
Working Conditions	14	46	19.01	3.9
Gender	12	32	22.67	4.8
Education	10	20	13.97	1.67
Professional Status	1	5	1.18	3.09
Appearance	3	15	6.07	2.8
Total	87	146	112	9.67

Table 4. Comparison of the total scores of the Nursing Profession Image Scale and its sub-dimensions according to the demographic characteristics of nurses (N=208).

Characteristics		Professional Qualifications M±SD	Working Conditions M±SD	Gender M±SD	Education M±SD	Professional Status M±SD	Appearance M±SD	Total Scale Score M±SD
Gender	**Female**	20.21±1.71	18.78±3.29	22.11±4.68	13.96±1.61	15.35±5.84	5.88±2.67	111.05±9.19
	Male	19.23±2.71	20.07±5.86	25.11±4.56	14.02±1.93	15.89±6.33	6.82±3.25	116.09±10.45
Statistical Significance		**t=2.824** **p=0.004**	t=-1.862 p=0.064	**t=-3.704** **p=0.000**	t=-0.204 p=0.838	t=-0.509 p=0.612	**t=-2.078** **p=0.039**	**t=-3.392** **p=0.001**
	High School	20.09±5.88	15.33±5.88	15.08±1.83	22.04±7.54	23.24±7.78	15.42±7.75	115.09±10.42
	Associate Degree	20.11±1.88	18.33±5.68	12.08±1.33	14.05±1.13	15.49±4.33	13.42±6.75	110.07±10.12
Education	**Bacheor's Degree**	20.11±1.62	11.82±4.71	16.08±1.43	15.28 ±6.55	15.50±5.13	15.02±5.85	112.06±9.12
	Master's Degree	20.01±1.52	11.12±3.71	14.06±1.33	16.12 ±4.55	12.38±4.22	11.21±4.52	111.05±7.11
Statistical Significance**		F=1.235 p=0.249	F=0.279 p=0.785	F=0.271 p=0.879	F=0.191 p=0.879	F=0.673 p=0.667	F=0.484 p=0.759	F=0.321 p=0.864
To Become a Member of a Professional Organization or Association	**Yes**	20.41±1.72	18.62±2.48	21.82±4.75	13.27±0.95	14.31±6.04	5.58±2.39	108.72±9.32
	No	19.97±2.00	19.08±4.08	22.81±4.80	14.08±1.73	15.63±5.90	6.15±2.86	112.64±9.64
Statistical Significance*		t=1.108 p=0.269	t=-0.592 p=0.555	t=-1.028 p=0.305	**t=-2.455** **p=0.015**	t=-1.119 p=0.264	t=-1.005 p=0.316	**t=-2.040** **p=0.043**
"I believe that there have been positive changes in the image of the nursing profession during the pandemic, and I consider the influence of the media to be significant."	**Yes**	19.82±2.30	19.09±4.91	23.41±4.78	13.98±1.55	13.74±5.73	5.91±2.77	109.22±9.99
	No	20.18±1.68	18.96±3.01	22.15±4.76	13.96±1.76	16.65±5.78	6.18±2.83	113.10±9.38

Table 4. (continued) Comparison of the total scores of the Nursing Profession Image Scale and its sub-dimensions according to the demographic characteristics of nurses (N=208).

Statistical *Significance		t=-1.312 *p*=0.191	t=0.228 *p*=0.820	t=1.879 *p*=0.062	t=0.089 *p*=0.929	**t=-3.588** ***p*=0.000**	t=-0.661 *p*=0.509	t=-2.572 *p*=0.110
"With the onset of the pandemic, there has been an increased focus on the nursing profession."	**Yes**	20.14±2.56	19.08±3.50	22.72±4.71	13.95±1.65	16.18±5.85	6.23±2.79	109.51±9.48
	No	19.72±1.70	18.83±4.89	22.53±5.03	14.03±1.75	13.35±5.65	5.61±2.81	113.96±9.40
Statistical *Significance		t=-1.375 *p*=0.171	t=0.482 *p*=0.685	t=-0.250 *p*=0.803	t=0.311 *p*=0.756	**t=-3.088** ***p*=0.002**	t=1.405 *p*=0.161	**t=-3.356** ***p*=0.001**
"During the pandemic, nurses were not sufficiently praised in the media, and the professional challenges they face were brought to the forefront."	**Yes**	20.01±1.94	18.98±4.32	22.55±4.96	14.25±1.70	20.01±1.94	5.58±2.40	110.95±10.52
	No	20.05±1.99	19.04±3.58	22.76±4.70	13.77±1.63	20.5±1.99	6.42±3.02	112.90±8.98
Statistical Significance*		t=-0.167 *p*=0.867	t=-0.096 *p*=0.924	t=-0.321 *p*=0.749	**t=0.601** ***p*=0.043**	t=-0.167 *p*=0.867	**t=-2.134** ***p*=0.034**	t=-1.434 *p*=0.153

*Independent samples test (T-test), ** Oneway ANOVA test, M: Mean, SD: Standart Deviation

that an increase in professional qualifications is associated with a slight decrease in working conditions. This relationship is significant ($p<0.01$). The significant negative correlation between professional qualifications and working conditions indicates that nurses with higher professional qualifications generally face more challenging working conditions (**Table 5**).

The correlation between working conditions and gender is very weak and positive (r = 0.066). This indicates a slight increase in gender with an increase in working conditions. This relationship is significant ($p<0.01$). The significant positive correlation between working conditions and gender suggests that certain gender groups are exposed to more challenging working conditions (**Table 5**).

The correlation between gender and education is very weak and positive (r = 0.066). This indicates a slight increase in gender with an increase in education level. This relationship is significant ($p<0.01$). The negative correlation between education and professional status suggests that nurses with higher education levels tend to have lower professional status (**Table 5**).

The correlation between education and professional status is high (r = 0.897), indicating a strong positive relationship. Nurses with higher education levels generally have higher professional status.

The correlation between professional status and total score is strong and positive (r = 0.697). This indicates that an increase in professional status is associated with an increase in the total score. This relationship is highly significant ($p<0.01$). The high positive correlation between professional status and total score suggests that nurses with higher professional status generally have higher total scores. This situation indicates that professional status influences overall professional perception (**Table 5**).

Discussion

This study aims to assess nurses' perceptions of their professional image during the COVID-19 process and their thoughts on the role of the media in shaping these perceptions. The findings include nurses' opinions on whether the media has shown enough interest in the nursing profession during the pandemic.

The majority of nurses participating in this research don't believe that there have been positive changes in the image of the nursing profession during the pandemic, In the pandemic an increased focus on the nursing profession in the media [4-7]. However, this study nurses feel that these news stories do not adequately express

Table 5. Comparison of sub-dimensions and total scores of the Nursing Profession Image Scale according to age (N=208).

Scale sub-dimensions translations	Age	Professional Qualifications	Working Conditions	Gender	Education	Professional Status	Total
Professional Qualifications	-0.036**		-0.534**	0.066**	-0.137**	0.032**	-0.192**
Working Conditions	0.550**	-0.534**		-0.117**	0.219**	0.172**	0.550*
Gender	0.020**	0.066**	-0.117**		-0.129**	-0.275**	0.219**
Education	-0.019**	-0.137**	0.219**	-0.129**		0.070**	0.897*
Professional Status	0.010**	0.032**	0.172**	-0.275**	0.070**		0.697**

*Correlation significance level is 0.05

**Correlation significance level is 0.01 [Correlation is significant at the 0.01 level (two-tailed)]

the importance of the profession. Studies indicate that the image of the nursing profession during the pandemic is shaped by the influence of the media, the efforts of nurses, and the perception of the community. Outbreaks, especially COVID-19, have highlighted nurses as heroes and angels [4-7]. Studies have concluded that the media positively affected the public image of nurses by highlighting their sacrifices and efforts during the pandemic [8-11,22]. The fact that the participants in this study thought otherwise may be due to the participants' belief that nurses are not given enough space in the Turkish media.

In this study, it was determined that the image of nursing is at a moderate level. Similarly, in the study conducted by Topuz et al. (2023), it was determined that the image perception of nurses is at a moderate level [12]. On the contrary, according to the study by Yurtsever et al., it was found that the professional image perception of nurses is at a good level [13]. In the study conducted by Karadeniz et al. (2022), it was determined that the professional image perception of nurses is at a moderate level [14]. The challenges brought by the pandemic may have negatively impacted the professional image of nurses. Additionally, studies conducted in different regions of the same country may vary due to the different working conditions in various cities and hospitals. This is because it has been emphasized that the working environment plays a significant role in the negative professional perceptions among nurses [15]. Practicing the nursing profession in Türkiye brings about some challenges. These include a hectic work pace and overtime, insufficient number of nurses, low salaries, communication difficulties, lack of equipment and resources, limited career development, and low societal prestige. These factors can impact the professional satisfaction of nurses and their perception of the professional image [16,20,23]. Understanding nurses' perceptions of the nursing profession and developing various strategies to increase their professional satisfaction are recommended. Improvements in areas such as professional education, working conditions, gender equality, and professional status can positively influence nurses' professional perceptions and, in general,

strengthen the image of the nursing profession.

An important finding of the study is that during the pandemic, male nurses and those not affiliated with professional organizations perceive that there is not enough discussion about the nursing profession. This result indicates that gender and organizational membership status can influence perceptions related to the nursing profession.Smilarl, in the study by Aydın Sayılan and Kulakaç, it was reported that female nurses have higher levels of positive image perception regarding the nursing profession compared to males [20]. In this context, the results of the research emphasize the necessity of a broad perspective to understand and improve the perception of the nursing profession. These data open the door to a more comprehensive discussion on how perceptions may change depending on factors such as membership status in a professional organization or gender. Considering the social image of the nursing profession and the role of the media in this regard, the public image has mainly led to misunderstandings and stereotypes, contributing to the perception of nursing as a female-dominated profession [23,24]. Male nurses were perceived as feminine or homosexual and were made invisible to society [17,18,25]. Perhaps in this study, male nurses may feel that they were overlooked both before and during the pandemic when discussing the nursing profession.

In the study, it was determined that the average scores of nurses in the "professional status" and "appearance" sub-dimensions were lower compared to other sub-dimension scores. Among nurses, 37% completely agreed with the statement "Nursing is a respected profession in society" in the "Professional status" sub-dimension. This situation might suggest that even during the pandemic, the media coverage did not sufficiently improve the professional perception in society. Unlike this study in the study by Blau et al. (2023), participants mentioned that the prestige of nursing increased in society with the pandemic, and awareness regarding the nursing profession and the role of nurses also increased [26]. Nurses, nurse managers, nurse educators, professional associations, and nursing educators

should make efforts to maintain and reinforce this positive image. These efforts should aim to increase public awareness on aspects such as the qualities, education, and working conditions of nursing, and it is believed that the media should support the nursing profession in these efforts.

In this study, participants received very low scores from the 'appearance' scale sub-dimension. When evaluating this situation, it is important to consider the challenges brought by the pandemic. The pandemic has imposed intense stress and pressure on healthcare workers, which may have led to limitations in personal care and appearance. Nurses may not have had enough time for their own care as a result of the demanding working conditions during this period. Additionally, it is essential to consider the impact of this situation on the overall health and well-being of nurses. Long and intense working hours, coupled with the uncertainty and stress brought by the pandemic, may make it challenging for nurses to focus adequately on their own health and care needs [6-9]. These findings may emphasize the need for health institutions and managers to take measures to support the health and well-being of nurses. Providing support for healthcare workers both physically and emotionally during the pandemic can be crucial for long-term health outcomes.

Conclusion

In conclusion, the image perceptions of nurses towards the nursing profession are at a moderate level in this study. This finding indicates the need for a more positive perception of the nursing profession in society. Various initiatives are required to enhance nurses' perceptions of their own profession and create a positive professional image. These initiatives should aim to increase respect for the nursing profession, correct misinformation or gaps in the public's knowledge about nursing, and emphasize the importance of the profession.

Stakeholders such as the media, educational institutions, and professional associations, especially, can shape public perception by highlighting the positive aspects of the nursing profession. The challenges, sacrifices, and expertise of nurses can be the focal point of these efforts. Additionally, effective strategies should be developed to increase public awareness of various aspects of the nursing profession, highlight the value that nurses bring, and enhance respect for their profession.

Such initiatives will not only strengthen the image of nurses' professions but will also contribute to building a healthier society by increasing public trust in healthcare services. Improving respect for and perception of the nursing profession can boost nurses' motivation and help them perform their duties more effectively.

Limitations of the Study

The methodology and sample of the study are its limitations.

Conflict of interest

The authors have no conflict of interest to disclose.

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ORIGINAL ARTICLE

Association of maladaptive daydreaming with behavioral addictions

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Abstract

Maladaptive daydreaming (MD) is a proposed syndrome characterized by extensive, immersive daydreaming. Case reports have suggested an association of MD with behavioral addictions such as excessive internet use, online gaming, and compulsive sexual behaviors. The aim of this study was to investigate maladaptive daydreaming and behavioral addiction symptoms among young adults. An online questionnaire was administered to 293 volunteer medical students. Participants were assessed by applying the 16-item Maladaptive Daydreaming Scale (MDS-16), Addictive Behavior Burden Form, and Adult Attention Deficit Hyperactivity Disorder Self-Report Scale (ASRS). Participants were divided into two groups: probable MD (MDers) and non-MDers, according to MDS-16. Age and gender did not significantly differ between the MD and non-MD groups. MDers had more severe symptoms associated with gaming addiction ($p = 0.03$; U = 4941), problematic social media use ($p = 0.01$; U = 4680), food addiction ($p = 0.01$; U = 4965), sex/pornography addiction ($p = 0.01$; U = 4787), and compulsive buying ($p = 0.03$; U = 4874) than non-MDers. Among the behavioral addiction dimensions assessed in the study, only gaming addiction was a significant statistical predictor of MD. These findings highlight a significant association between MD and behavioral addictions, particularly gaming addiction, in young adults. Furthermore, the findings of this study suggest a unique relationship between gaming addiction and MD.

Keywords: Maladaptive daydreaming, daydreaming, fantasy, addictive behavior, gaming disorder

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Introduction

Daydreaming is a common and normal mental activity [1]. Recent literature has explored the phenomenon of excessive, uncontrolled daydreaming that disrupts daily functioning [2]. Maladaptive daydreaming (MD), describes recurrent daydreaming activities characterized by a shift of attention to a rich inner world, to the neglect of social, occupational, and academic activities [3]. MD, currently under research, is not yet recognized as a formal disorder in the International Classification of Diseases (ICD) or Diagnostic and Statistical Manual of Mental Disorders (DSM) [4]. Limited research exists on the prevalence of MD, with a reported point prevalence of 2.5% in the Israeli population [5]. In addition to being a common condition, MD is thought to be associated with various mental disorders. MD has been reported to have a high comorbidity with attention deficit hyperactivity disorder (ADHD), anxiety disorder, depression, obsessive-compulsive and related disorders [6]. Moreover, recent studies have shown that MD doubles the risk of suicide [7]. This supports the need for MD to be addressed as an important public health problem and needs to be examined in more detail.

Despite its prevalence and potential negative consequences, MD may serve as a coping strategy for some individuals [8]. MD is experienced not only as a defense mechanism to distract attention from current stressors but also as a rewarding activity [2]. People talk about daydreaming as a relaxing, fun activity, that they prefer this behavior because their lives are boring, and that it would be sad for them if they did not have this activity [2]. Experiencing MD as pleasurable (positive reinforcement) or using it as a coping mechanism to avoid negative situations (negative reinforcement) can lead to the reinforcement of this behavior. Case reports suggest that individuals with MD may exhibit tolerance (increasing behavior to achieve desired effects) and withdrawal symptoms (restlessness when unable to daydream) [9]. It has also been reported that although MD may have desirable effects for individuals in the short term, it may negatively affect their lives in the long term [10]. Moreover, case reports have been reported

showing the association of MD with behavioral addictions such as excessive internet use, online gaming, and compulsive sexual behaviors [9,11]. Although all these findings support the idea that MD can be considered a behavioral addiction, there are not yet enough studies to provide conclusive evidence.

There is a strong association between ADHD and behavioral addiction [12]. It has been shown that up to 83.3% of those with behavioral addiction and 76.9% of those with MD may have ADHD comorbidity [13,14]. This high comorbidity suggests that different behavioral addictions, MD, and ADHD may be related to similar biopsychosocial etiologies.

The main aim of this study was to investigate the relationship between MD and behavioral addictions. In addition, it was aimed at evaluating the predictive effect of ADHD symptoms and different behavioral addiction symptoms on MD.

The study proposed the following hypotheses:

- Maladaptive daydreamers (MDers) demonstrate a greater burden of behavioral addiction as evaluated through self-report measures compared to non-MDers.

- Maladaptive daydreamers (MDers) exhibit higher levels of ADHD symptoms compared to non-MDers.

- Behavioral addiction and ADHD symptom severity predict maladaptive daydreaming severity.

Materials and Methods

Uşak University Medical Ethics Committee approved the study for Clinical Studies (280-12). The research was planned as a cross-sectional survey with analytical components to assess associations between variables. Students from Uşak University Faculty of Medicine who gave informed consent to participate in the study and completed the online questionnaire were included in the study. Online surveys using Google Forms were delivered to students via phone messages. Of the students invited to the study, 41% completed it. All participants who completed the online questionnaire were included in the study and no exclusion criteria were used. The study was conducted between

15th January and 26th February 2024.

Instruments

Sociodemographic and Clinical Characteristics Information Form

Clinical information such as age, gender, marital status, academic year, history of mental disorders and physical illness, information about the treatments they were using, and history of mental disorders in their family history were recorded. It was used to record the sociodemographic and clinical characteristics of the patients participating in the study in a detailed and systematic manner.

The Maladaptive Daydreaming Scale (MDS-16)

It is a Likert-type self-report scale used to measure the severity of MD [15]. It consists of 16 items, and each item is scored between 0 and 100 points. Higher scores on the scale are associated with more severe MD. The Turkish validity and reliability study was conducted [16]. The study found strong internal consistency for the total score, with a *Cronbach's alpha* of 0.89. It has been reported that scores of 50 and above on the scale can distinguish individuals with MD from normal controls [6]. MDS-16 was used in this study to measure the severity of maladaptive daydreaming and a cut-off value of 50 was used to divide the participants into two groups: probable MDers (MDS-16 total score ≥ 50) and non-MDers (MDS-16 total score < 50).

Burden of Behavioral Addiction Form

The Burden of Behavioral Addiction Form is an 11-point Likert-type scale that was developed to screen for symptoms that people may exhibit in compulsive buying, problematic social media use, and food, gambling, gaming, or sex/pornography addiction [17]. It consists of questions that evaluate six basic biopsychosocial areas for each behavioral addiction: "excessive focus on the problematic behavior or craving, changes in mood or feelings of relief, tolerance, withdrawal effects, social or occupational impairment, and relapse" [18]. Each question is scored between 0 and 10 points, and the total scores that can be received for each behavioral addiction dimension range from 0 to 60. Higher scores on the scale are connected with an increased probability of behavioral addiction and a greater impact of symptoms on the person's life within the addiction criteria. The scale score reveals strong internal consistency for the total score, with a *Cronbach's alpha* of 0.942. *Cronbach's alpha* coefficients for the whole group were between 0.89 and 0.93 (gambling: 0.933; problematic social media use: 0.921; gaming addiction: 0.931; sex/pornography addiction: 0.910; food addiction: 0.894; compulsive buying: 0.915).

Adult ADHD Self-Report Scale (ASRS)

ASRS is a five-point Likert-type self-report scale developed to screen for ADHD in adults [19]. It has two subscales: 'attention deficit' and 'hyperactivity/impulsivity'. Each item is scored between 0 and 4, with total scores ranging from 0 to 64. Higher scores on the scale are associated with more severe ADHD symptoms. The Turkish validity and reliability of the scale were assessed, revealing strong internal consistency for the total score with a *Cronbach's alpha* of 0.88 [20].

Statistical Analysis

The data was analyzed using the Jamovi software version 2.3.28. The normal distribution was assessed with the *Shapiro -Wilk* test, the *Kolmogorov -Smirnov* test, and histograms. Descriptive statistics were expressed as median, interquartile range, mean, and standard deviation values for continuous variables and percentages for categorical variables. The *Mann-Whitney U* test was used for comparing the medians of non-normally distributed continuous variables and the Student's *t*-test for comparing the means of normally distributed continuous variables between the two groups. The χ^2 test was used to compare the observed frequencies in each category between the groups. The correlation coefficients for the relationships between the variables were examined using the Spearman test. The statistical significance level was set at 5% ($p<0.05$) in this study.

When the groups (MDers and non-MDers) were supposed to be distributed at a rate of 1/5 based on the findings of previous studies [21], it was found that 250 participants should be included to achieve a modest effect size (d = 0.5) with 90% power and 5% type-1 error.

Results

Of the 721 students who received the online survey, 293 (41%) completed it. Participants were divided into two groups according to their score on the MDS-16 scale: MDers (n = 50; 17%) and non-MDers (n = 243; 83%). The sociodemographic characteristics of the participants are shown in Table 1. The frequency of mental disorders and methylphenidate use were found to be higher in the MDers. No difference was found between the groups in terms of age, gender, marital status, and educational status (**Table 1**).

The behavioral addiction and ADHD scale scores in the study groups are shown in **Table 2**. The MDers had significantly higher scores in terms of compulsive buying, problematic social media use, food addiction, gaming addiction, and sex/ pornography addiction severity than the non-MDers. There was no difference between the groups in terms of gambling addiction severity. The MDers had significantly higher ASRS scale scores than the non-MDers ($p<0.001$).

Positive correlations were found between the scores obtained from the MDS-16 total scores and compulsive buying, problematic social media use, food addiction, gaming addiction, and sex/ pornography addiction severity and ASRS total scores. Correlations are shown in **Table 3**.

Multiple regression analysis was applied to predict the scores obtained from the MDS-16. In the model, age, gender, compulsive buying, problematic social media use, food addiction, gaming addiction, sex/pornography addiction, gambling addiction, and ASRS total score were

Table 1. Sociodemographic and clinical characteristics of the study groups.

	Total (n= 293)	non-MDers (n= 243)	MDers (n= 50)	Statistics	p
Age					
Mean (SD)	21.7 (2.25)	21.7 (2.32)	21.4 (1.93)	0.858	0.391[b]
Median (IQR)	22.0 (3.0)	22.0 (3.0)	22.0 (3.0)	5640	0.421[c]
Sex				0.315	0.575[a]
Female	186 (63.48%)	156 (64.2%)	30 (60%)		
Male	107 (36.51%)	87 (35.8%)	20 (40%)		
Marital status				0.834	0.659[a]
Single	289 (98.63%)	239 (98.4%)	50 (100%)		
Married	2 (0.7%)	2 (0.8%)	0 (0%)		
Divorced	2 (0.7%)	2 (0.8%)	0 (0%)		
Academic year				8.78	0.118[a]
1st year	58 (19.80%)	48 (19.8%)	10 (20%)		
2nd year	29 (9.90%)	25 (10.3%)	4 (8%)		
3rd year	63 (21.50%)	47 (19.3%)	16 (32%)		
4th year	35 (11.95%)	26 (10.7%)	9 (18%)		
5th year	58 (19.80%)	52 (21.4%)	6 (12%)		
6th year	50 (17.06%)	45 (18.5%)	5 (10%)		
Mental disorders				8.93	**0.003[a]**
	51 (17.41%)	35 (% 14.4%)	16 (32%)		
Psychiatric treatment					
Antidepressant	42 (14.33%)	32 (13.2%)	10 (20%)	1.58	0.209[a]
Antipsychotic	1 (0.00%)	1 (0.4%)	0 (0%)	0.206	0.650[a]
Anxiolytic	0 (0.00%)	0 (0%)	0 (0%)		
Mood stabilizer	0 (0.00%)	0 (0%)	0 (0%)		
Stimulants	7 (0.02%)	3 (1%)	4 (8%)	8.14	**0.004[a]**
Physical disease	34 (11.60%)	29 (11.9%)	5 (10%)	0.151	0.697[a]
Cigarette use	87 (29.69%)	68 (28.0%)	19 (38%)	1.99	0.158[a]
Alcohol use	126 (43.00%)	103 (42.4%)	23 (46%)	0.221	0.638[a]
Family history of mental disorders	34 (11.60%)	25 (10.3%)	9 (18%)	2.40	0.121[a]

MDers: Maladaptive Daydreamers, SD: Standard Deviation, IQR: Interquartile Range. Significant p values are marked in bold.
[a] χ2 test for comparisons between the study groups.
[b] Student's t-test for comparisons between the study groups.
[c] *Mann-Whitney U* for the comparisons between the study groups.

Table 2. Comparison of Behavioral Addiction and Attention Deficit Hyperactivity Disorder Symptom Severity between the study groups.

	Total (*n= 293*)	non-MDers (*n= 243*)	MDers (*n= 50*)	Statistics	*p*	Effect Size
MDS-16						
Mean (SD)	31.4 (18.4)	25.4 (13.2)	60.8 (10.1)	-17.9[a]	**<0.001**	-2.78
Median (IQR)	29.4 (25.0)	24.4 (20.6)	57.5 (11.3)	0.00[b]	**<0.001**	1.00
ASRS						
Mean (SD)	30.5 (13.5)	28.037 (12.30)	42.48 (12.87)	-7.50[a]	**<0.001**	-1.165
Median (IQR)	30 (18.0)	28.00 (17.0)	41.00 (13.8)	24.92[b]	**<0.001**	0.5899
BBAF						
Gambling addiction						
Mean (SD)	0.997 (4.84)	0.786 (4.31)	2.02 (6,81)	-1.65[a]	0.101	
Median (IQR)	0.00 (0.00)	0.00 (0.00)	0.00 (0,00)	5845[b]	0.449	
Social media use						
Mean (SD)	17.3 (15.7)	15.96 (14.81)	23.60 (18.15)	-3.19[a]	**0.002**	-0.496
Median (IQR)	12 (23.0)	11.00 (20.05)	19.50 (33.5)	4680[b]	**0.011**	0.2297
Gaming addiction						
Mean (SD)	7.45 (12.0)	6.543 (11.07)	11.86 (15.02)	-2.90[a]	**0.004**	-0.450
Median (IQR)	2 (9.0)	1.00 (8.00)	5.00 (21.0)	4941[b]	**0.030**	0.1867
Compulsive buying						
Mean (SD)	9.34 (11.6)	8.506 (10.87)	13.38 (14.30)	-2.72[a]	**0.007**	-0.423
Median (IQR)	5 (13.0)	4.00 (11.5)	9.50 (19.5)	4874[b]	**0.026**	0.1978
Food addiction						
Mean (SD)	14.3 (13.4)	13.198 (12.63)	19.38 (15.63)	-3.02[a]	**0.003**	-0.469
Median (IQR)	11 (19.0)	10,00 (17.0)	17.00 (22.5)	4965[b]	**0.011**	0.2272
Sex/pornography						
Mean (SD)	6.34 (10.5)	5.305 (9.00)	11.36 (15.06)	-3.80[a]	**<0.001**	-0.589
Median (IQR)	1 (9.0)	1.00 (6.00)	5.50 (16.3)	4787[b]	**0.014**	0.2121

MDS-16: The Maladaptive Daydreaming Scale, ASRS: Adult Attention Deficit Hyperactivity Disorder Self-Report Scale, BBAF: Burden of Behavioral Addiction Form, SD: Standard Deviation, IQR: Interquartile Range. Significant p values are marked in bold.
[a] Student's *t*-test for comparisons between the study groups.
[b] Mann-Whitney U for the comparisons between the study groups.

Table 3. Correlation analysis of the Scores of Maladaptive Daydreaming, Attention Deficit Hyperactivity Disorder, and Behavioral Addiction Scales.

		1	2	3	4	5	6	7
1.MDS-16	*r*							
	p							
2. BBAF, gambling addiction	*r*	0.063						
	p	0.285						
3. BBAF, social media use	*r*	0.260	0.096					
	p	**<0.001**	0.101					
4. BBAF, gaming addiction	*r*	0.242	0.219	0.367				
	p	**<0.001**	**<0.001**	**<0.001**				
5. BBAF, compulsive buying	*r*	0.264	-0.049	0.578	0.189			
	p	**<0.001**	0.404	**<0.001**	**0.001**			
6. BBAF, food addiction	*r*	0.270	0.021	0.609	0.334	0.570		
	p	**<0.001**	0.716	**<0.001**	**<0.001**	**<0.001**		
7. BBAF, sex/ pornography	*r*	0.258	0.217	0.378	0.446	0.128	0.364	
	p	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**0.029**	**<0.001**	
8.ASRS	*r*	0.554	0.080	0.346	0.208	0.284	0.366	0.300
	p	**<0.001**	0.172	**<0.001**	**<0.001**	**<0.001**	**<0.001**	**<0.001**

MDS-16: The Maladaptive Daydreaming Scale, ASRS: Adult Attention Deficit Hyperactivity Disorder Self-Report Scale, BBAF: Burden of Behavioral Addiction Form. Significant *p* values are marked in bold.
r: Spearman correlation coefficient

determined as independent variables, and the total score obtained from the MDS-16 was determined as the dependent variable. The regression model was found to be significant ($p<0.001$). The independent variables explained 34% of the variance ($R^2 = 0.347$). The effect of gaming addiction ($p=0.035$) and the ASRS total score ($p<0.001$) on the MDS-16 total score was statistically significant, and both were positive predictors of the MDS-16 total score. A summary of the model is shown in **Table 4.**

Discussion

In this study, MDers were found in 17% of the students. In studies conducted with university students, MDers rates have been reported to vary between 5.5 and 34% [5,22]. Although these findings indicate that symptoms associated with maladaptive daydreaming are common among university students, the prevalence varies across different studies. These different rates may have been caused by reasons such as the fact that the studies were conducted in different cultures and different languages and non-response bias. In order to calculate the prevalence of MDers in Türkiye, field studies with clinical interviews are needed. Nevertheless, the findings related to MD symptom severity and prevalence rates found in this survey study will guide future comprehensive epidemiologic studies.

MD has been reported to have many common features with behavioral addictions [9]. Case reports have been reported showing the association of MD with behavioral addictions such as excessive internet use, online gaming, and compulsive sexual behaviors.[9,11]

However, not enough studies have been conducted on this topic in the literature. This study showed that MDers scored higher on the compulsive buying, problematic social media use, food addiction, gaming addiction, and sex/pornography addiction scales than non-MDers. Among the behavioral addictions assessed in this study, only the difference between gambling addiction and MD severity was not statistically significant. The fact that the severity of gambling addiction among the participants included in the study was lower than other behavioral addictions may have led to a lack of statistically significant change between the groups. In order to evaluate the relationship between gambling addiction and MD, further studies with samples including individuals at higher risk for gambling addiction are needed.

In this study, it was found that the rate of mental disorders was higher in MDers. Similarly, a strong relationship between MDers and mental symptoms has been reported in the literature [6]. In the literature, it has been reported that the most common mental disorder associated with MD is ADHD [6]. While ADHD was found in 76.9% of those with MD, it was reported that 20.5% of those diagnosed with ADHD had MD [6,13]. Although these are two conditions with high comorbidity, MD has been reported to have unique clinical features different from ADHD [13]. In this study, consistent with the literature, it was shown that ADHD and MD scores were positively correlated, and the MDers scored higher on ADHD scale scores than the non-MDers.

Table 4. Multiple regression analyses of variables predicting maladaptive daydreaming.

Variables	β	SE	t	p
Intercept	16.2617	9.0933	1.7883	0.075
Age	-0.4017	0.4043	-0.9935	0.321
Sex	-0.1442	2.2970	-0.0628	0.950
BBAF, gaming addiction	-0.3337	0.2031	-1.6431	0.101
BBAF, social media use	-0.0172	0.0839	-0.2053	0.838
BBAF, gaming addiction	0.2051	0.0969	2.1161	**0.035**
BBAF, compulsive buying	0.1529	0.1022	1.4953	0.136
BBAF, food addiction	-0.0478	0.0919	-0.,5203	0.603
BBAF, sex/ pornography	0.0919	0.1135	0.8094	0.419
ASRS	0.7118	0.0744	9.5628	**<0.001**

$R^2 = 0.347$; f = 16.7; $p<0.001$

ASRS: Adult Attention Deficit Hyperactivity Disorder, BBAF: Burden of Behavioral Addiction Form, β: Standardized coefficient, SE: Standard Error. Significant p values are marked in bold.

In this study, the findings obtained from multiple regression analysis, in which age, gender, ADHD symptom severity, and different behavioral addictions were included as independent variables, indicate that there is a unique relationship between MD and game addiction. Unlike other behavioral addictions, daydreaming plays a major role in computer game addiction. Players spend time in a fantasy world during the game, distancing themselves from real life. Moreover, people with gaming addiction are known to continue fantasizing about the game when they are not playing or when they should be focusing on another activity [23,24]. The relationship between gaming and daydreaming has been known for a long time. It has been noted that gaming and fantasy are two highly interrelated psychological processes and that, in some cases, one can stand as an analog for the other [25]. It has been argued that gaming and fantasy are intertwined in the first years of life, that in early childhood gaming becomes a social activity with rules and differentiates itself from fantasy, and that with adolescence gaming decreases and fantasy activities begin to replace gaming [25]. The conceptual similarity of gaming and daydreaming suggests that there may be common factors that also lead to the excesses of these two behaviors. Further studies on the etiopathogenesis of gaming addiction and MD are needed.

This study has several limitations. This study was a single-center study conducted with young adults. Multicenter studies with samples other than the young age group will enable generalization of the results found in this study. Moreover, the cross-sectional nature of the study prevents establishing a cause-and-effect relationship. Longitudinal studies evaluating the relationship between behavioral addictions, MD, and ADHD with larger samples are needed. Approximately half of the students invited to participate in the study responded to the survey, which may have contributed to the non-response bias. However, the response rates were similar to the average response rate found in online survey studies (36–44%) [26,27]. Finally, symptoms were screened with self-report scales. Further studies using clinical interviews will allow the exclusion of additional diagnoses and a more reliable comparison of mental symptoms.

Conclusion

The main aim of this study is to examine the relationship between MD and other behavioral addictions. In this study, MD was shown to be associated with behavioral addictions such as compulsive buying, problematic social media use, food addiction, gaming addiction, and sex/pornography addiction. Furthermore, the findings of this study suggest that MD and gaming addiction may have a unique relationship that is different from other behavioral addictions. This relationship between behavioral addictions and MD may help to provide a better understanding of the MD's underlying mechanisms. Furthermore, addiction treatments may be a potential intervention avenue for individuals struggling with MD.

There was a positive relationship between ADHD and MD symptom severity. However, it is important to highlight that the study's findings do not imply causation. Further research is needed to determine the causal link between ADHD and MD.

Moving forward, further research is warranted to investigate the underlying mechanisms of the association between MD and gaming addiction, explore the effectiveness of interventions targeting multiple behavioral addictions in individuals with MD, and examine the temporal relationship between these conditions.

Conflict of interest

The author reports no conflict of interest in this work.

Data availability statement

The data that support the findings of this study are available on request from the corresponding author.

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STUDY PROTOCOL

The effect of the "Femininity Identity Improvement Program" based on cognitive behavioral and expressive techniques applied to gynecological cancer patients on prolonged grief reactions: Study protocol for randomized controlled trial

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Abstract

The research was designed to determine the impact of the Femininity Identity Improvement Program based on cognitive-behavioral and expressive techniques applied to gynecological cancer patients on their prolonged grief responses. The research is the study protocol created for a randomized controlled experimental study. The research protocol prepared in accordance with SPIRIT 2013 was registered in the Clinical Trials System (NCT05529303). The research was conducted with 80 patients (40 patients in the intervention group and 40 patients in the control group) who underwent surgery in the gynecology and obstetrics clinic of a university hospital within the last year. Among these patients, those who had a surgery at least three months ago were included in the study. The intervention group participated in the Femininity Identity Improvement Program for 10 weeks, with sessions once a week, each lasting 90-120 minutes. The control group received no intervention. Measurements were taken before the program (pretest), at the end of the program (posttest at the 10th week), and three months after the program (follow-up measurement) to determine the effect of the program. Data were collected using the Prolonged Grief Disorder Scale-Patient Form. The Analysis of Covariance (ANCOVA) was used to analyze the data obtained at different times. The study integrates two psychotherapeutic interventions (cognitive-behavioral theory and art therapy) in an innovative way to structure the Femininity Identity Improvement Program for psychiatric nursing practice. Psychiatric nurses in oncology and other relevant clinical settings are recommended to apply this program to provide comprehensive care using current approaches.

Keywords: Femininity, cognitive behavioral therapy, art therapy, grief, cancer

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Introduction

Cancer is one of the most complex diseases of our era and its incidence is rapidly increasing, making it one of the most significant threats to human health [1]. According to the latest data published by the World Health Organization (WHO) in 2022, it ranks among the top causes of death worldwide, with 10 million deaths reported in the year 2020 [2]. Gynecological cancers refer to malignancies affecting the female genital organs and are a major cause of mortality and morbidity in women, following breast and lung cancer [3]. It ranks among the top 10 cancers diagnosed among women in Türkiye [4].

The symptoms arising from gynecological cancers and the application of radiotherapy, chemotherapy, and surgical treatments can lead to issues related to a woman's reproductive capacity. Consequently, this may result in feelings of infertility, inadequacy, and imperfection, as well as a decrease in body image and self-esteem, a lack of self-confidence, hesitation in engaging in intimate relationships with the opposite sex, and a sense of incompleteness within the family [5-7]. Particularly, the loss of body parts associated with femininity significantly influences a woman's body image. This is because fertility, being a spouse, and the role of motherhood play a significant role in society's perception of 'feminine identity' [5,8-10]. For a woman who feels unable to fulfill this significant role, cancer diagnosis may signify a sense of inadequacy and a genuine mourning experience [5,7,11-15].

It is acknowledged that the experience of suffering, grief, and mourning, which arises from life-threatening illnesses and entails physical, emotional, and social losses, is a universal and natural response [16]. Prolonged grief is characterized by pronounced symptoms and significant functional impairment, and the individual believes that their life has ended with the loss and that their pain will never cease. In the context of cancer, it is unclear at which point the perception of loss initially occurs in the individual (whether it is during the pre-diagnosis stage, the diagnosis process, or upon learning of the terminal stage, etc.). Distinguishing where normal grief ends and prolonged grief begins is a challenging task [17]. In longitudinal studies involving gynecological cancer patients in the sample, it has been reported that the levels of grief in patients tend to increase over time [18]. Studies aiming to examine the grief process in cancer patients have indicated that patients experience high levels of grief, with variations in grief averages depending on certain variables [19,20].

Interest in psychological interventions has grown over time alongside research aimed at understanding the various challenges cancer patients face and improving their quality of life. When the individual and group-based psychological interventions are examined, it is observed that the effects of techniques such as Cognitive-Behavioral Therapy (CBT), mindfulness-based interventions, acceptance and commitment therapies, and expressive techniques including mindfulness-based art therapy on cancer patients have been investigated [21-25]. The CBT techniques are compatible with the use of expressive art techniques. Both CBT and expressive arts share the common goal of facilitating behavior change [26]. In group settings, individuals find relief by sharing their experiences of loss, realizing that intense emotions like grief and pain are also experienced by others [27]. A study conducted with gynecological cancer patients undergoing chemotherapy to determine the impact of art therapy on anxiety and hope levels reported significant improvements in both anxiety and hope levels following art therapy sessions [28]. In a study involving cervical cancer patients, a cognitive-behavioral stress management intervention was found to reduce anxiety and depression levels and was deemed an effective intervention for improving the quality of life [29].

Creative art therapies such as visual arts and music are categorized as nursing interventions. Nurses play a significant role in integrating these interventions with medical treatments and activating the patient's creative potential. Nursing care interventions of this nature have been increasingly employed by nurses both globally and in Türkiye [30].

When the literature was reviewed, no study addressing prolonged grief responses related to the losses experienced by gynecological cancer

patients concerning their femininity identity was found. This study aims to use cognitive-behavioral theory-based expressive interventions to address the psychosocial issues arising from the losses experienced by gynecological cancer patients related to their femininity identity. The concept of 'femininity identity improvement' in gynecological cancer patients is addressed for the first time in this study. In addition, this program is intended to contribute to the literature as a psychiatric nursing practice by integrating two separate psychotherapeutic interventions (cognitive-behavioral theory and art therapy) in an innovative approach to enhance femininity identity. Enabling psychiatric nurses in oncology and related clinics to implement this program is expected to significantly enhance the quality of life for patients by offering them comprehensive care through contemporary approaches.

Aim of the Research

The study was designed to determine the impact of the cognitive-behavioral and expressive techniques-based "Femininity Identity Improvement Program" applied to gynecological cancer patients on their prolonged grief responses. The hypotheses of the study are as follows:

H1$_0$: "The Femininity Identity Improvement Program" based on cognitive-behavioral and expressive techniques applied to gynecological cancer patients has no effect on prolonged grief responses.

H2$_0$: There is no change in the mean scores of the Prolonged Grief Disorder Scale for gynecological cancer patients in the intervention group across measurement times.

H3$_0$: There is no difference in the mean scores of the Prolonged Grief Disorder Scale-Patient Form between the intervention group and the control group in the post-test measurements.

H4$_0$: There is no difference in the mean scores of the Prolonged Grief Disorder Scale-Patient Form between the intervention group and the control group in the follow-up measurements.

H1$_1$: "The Femininity Identity Improvement Program" based on cognitive-behavioral and expressive techniques applied to gynecological cancer patients has an effect on prolonged grief responses.

H2$_1$: There is a change in the mean scores of the Prolonged Grief Disorder Scale for gynecological cancer patients in the intervention group according to the measurement time.

H3$_1$: There is a difference in the mean scores of the Prolonged Grief Disorder Scale-Patient Form between the intervention group and the control group in the post-test measurements.

H4$_1$: There is a difference in the mean scores of the Prolonged Grief Disorder Scale-Patient Form between the intervention group and the control group in the follow-up measurements.

Materials and Methods

Ethical Considerations

Ethical approval for the research was obtained from the Clinical Research Ethics Committee of the Afyonkarahisar Health Sciences University Faculty of Medicine on August 5, 2022, with decision number 423. Written permission was also obtained from the Chief Physician's Office of the University's Health, Practice, and Research Center on May 11, 2022, with document number E.82432, to conduct the research within the institution. In the implementation phase of the research, necessary permission was obtained from the university rectorate for the use of a room specifically designed for group interventions. All participants in both the intervention and control groups who agreed to participate in the research were informed by the researchers about all the details of the research process. They were provided with voluntary informed consent forms, and written permissions were obtained after reading the informed consent form. The confidentiality of all data obtained from the participants was ensured by the researchers. The research protocol was registered in the Clinical Trials registry with the number NCT05529303.

Research Type

This research has been designed as a randomized controlled experimental study with pre-test, post-test, and follow-up assessments for the intervention and control groups. The randomized controlled trial protocol for this study has been prepared in accordance with the SPIRIT 2013 guidelines [31] (**Table 1**).

Table 1. Standard protocol items: Recommendations for design and outcome evaluations and experimental trials (SPIRIT).

Time	Selection/ At the start — Patients who have undergone surgery within the last year and who had their surgery at least three months ago (t_{-1})	Baseline — Before the intervention (t_0)	Intervention (Femininity Identity Improvement Program)										Follow-up	
			Week 1 (t_1)	Week 2 (t_2)	Week 3 (t_3)	Week 4 (t_4)	Week 5 (t_5)	Week 6 (t_6)	Week 7 (t_7)	Week 8 (t_8)	Week 9 (t_9)	Week 10 (t_{10})	End of the intervention program	Three months after the final intervention
Registration														
Eligibility screening	x													
Informed consent	x													
Assignment		x												
Interventions														
Intervention group			x	x	x	x	x	x	x	x	x	x		
Control group														
Evaluations														
Sociodemographic information	x													
Prolonged Grief Disorder score	x												x	x

Randomization

All eligible patients meeting the inclusion criteria were administered the 'Prolonged Grief Disorder Scale-Patient Form' as a pre-test by the researcher [17]. After collecting the pre-test data, a statistician independent from the research performed the random assignment of groups. Group assignments were made as Group A and Group B, and the researcher was informed about these assignments with two separate opaque envelopes after the homogeneity of the data was checked. An external faculty member, independent of the study, determined Groups A and B through a random drawing. After the intervention program was implemented, the post-test and follow-up test data were also collected by an external faculty member for statistical analysis. The data were transferred to

a computer by an independent statistician, who was blinded until all analyses were completed. The randomization process was performed according to the CONSORT 2017 (Consolidated Standards of Reporting Trials) guidelines [32]. The Consort flowchart for the intervention and control groups in the study is presented in **Figure-1**. In the study, random allocation and concealment of the randomization process ensured the control of selection bias.

Blinding

In this randomized controlled study, the Femininity Identity Improvement Program to be applied to the intervention group must be administered by a researcher with specialized training. For this reason, blinding of the researcher was not be possible since the researcher was also
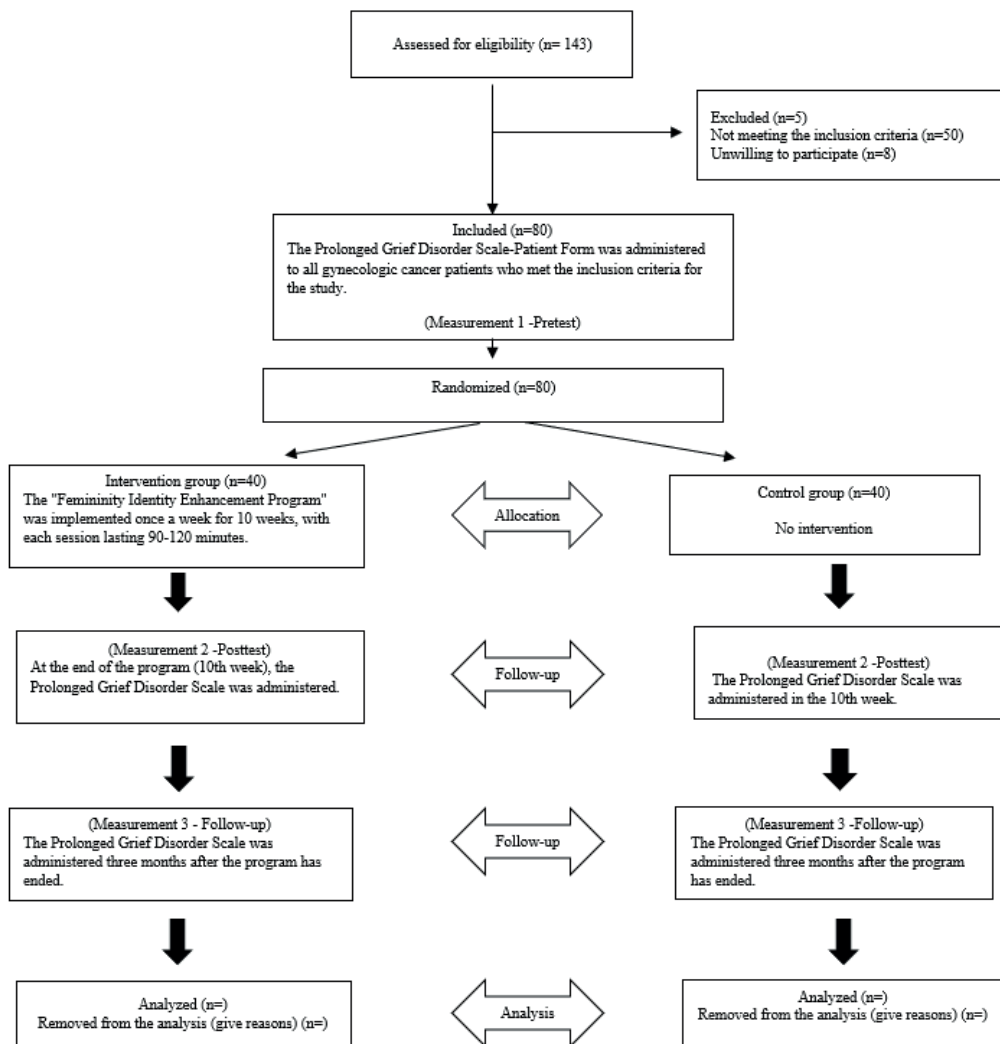

Figure 1. CONSORT 2017 flow diagram.

responsible for implementing the intervention program. As a 10-session 'Femininity Identity Improvement Program' was administered to the participants in the intervention group, participant blinding was also not feasible. Post-test and follow-up assessments were conducted by another researcher who did not know the participants' group assignment. The data, coded as Groups A and B, were transferred to a computer by the same individual. The analysis of data for Groups A and B was performed by an independent statistician. Following the completion of the statistical analysis and report writing, the coding for the intervention and control groups was revealed by the researcher responsible for data transfer to the computer. The process of data collection for the post-test and follow-up assessments, data coding, statistical analysis, and reporting were conducted while ensuring blinding to mitigate detection bias, statistical bias, and reporting bias.

Inclusion Criteria

• Being between the ages of 18-65,

• Being diagnosed with gynecological cancer,

• Knowing that you are diagnosed with cancer,

• At least three months have passed since the completion of surgical treatment,

• Being able to read and write,

• Volunteering to participate in the study.

Exclusion Criteria

• Having a diagnosis of any mental disorder (such as depression, anxiety, schizophrenia, bipolar disorder)

• Having an obstacle to establishing and maintaining effective communication

• Being in terminal stage

Criteria for Exclusion during the Study

• The participant states that she wants to leave the research,

• The participant does not attend/cannot attend at least two sessions for any reason,

• The participant has a deterioration in mental well-being and/or the development of a medical condition that will prevent the participant from participating in the sessions.

Research Population and Sample

The sample size calculation was conducted using power analysis (G*Power 3.1.9.7 software). The study is based on the mean scores of the Prolonged Grief Disorder Scale-Patient Form, which is used as a measurement for prolonged grief in cancer patients. The reason for considering the mean scores of the Prolonged Grief Disorder Scale-Patient Form as the variable is the content of the "Femininity Identity Improvement Program" applied to the intervention group, which is designed to address prolonged grief and femininity perception. Upon reviewing the literature, it was found that the scale itself or a parallel form of it was not utilized in previous studies. In cases where there is no literature available, conducting a pilot study and performing power analysis based on the values obtained from the pilot study is a reliable method. Therefore, a pilot study was conducted with a total of 30 cases (15 cases in the intervention group and 15 cases in the control group) to run the power analysis process [33]. Based on the measurement results of the pilot study and the two-tailed Mann-Whitney U test, with a 95% confidence level (1-α), a 95% test power (1-β), and an effect size of d=0.932, it was determined that each group should include 33 cases, resulting in a total sample size of 66 cases for the study (**Figure 2**). However, considering potential losses during the study, the number was increased by 20% to reach a total of 80 women [34].

Research Setting and Characteristics

The research was conducted with patients who met the inclusion criteria of having undergone surgery within the last year at the Gynecology and Obstetrics Clinic of a university hospital and having at least 3 (three) months passed since the surgery. Patients who were eligible according to the inclusion criteria and came for outpatient check-ups or were receiving treatment in the chemotherapy or radiotherapy units at the time of the study were asked to give verbal and written consent to participate in the study.

Data Collection Instruments

Personal Information Form

The form, created by researchers based on a literature review [8,28,35], contains a total of 15 questions, including information about gynecological cancer patients' age, marital status, place of residence, educational background, status of having a child, family structure, employment status, and type of cancer and treatment.

Prolonged Grief Disorder Scale-Patient Form (PG-12-Patient Form)

This scale is used to assess the emotional experiences and grief responses of cancer patients related to the losses caused by the disease. It was initially developed to measure grief symptoms by a group of researchers led by Prigerson and was originally called the Complex Grief Inventory. With the inclusion of Prolonged Grief Disorder in the proposed classification for ICD-11 (International Classification of Diseases), the scale was renamed as the Prolonged Grief Disorder Scale. The Turkish version of the scale's validity and reliability was assessed by Danışman et al. in 2017. It is a 5-point Likert scale consisting of 12 items. An increase in the total score from the scale indicates an increase in grief symptoms [17].

Implementation Phase of the Research

The intervention groups, consisting of 8-10 participants each, [36] determined through randomization, received the Femininity Identity

Improvement Program based on cognitive-behavioral and expressive techniques for 10 weeks (**Table 2**).

The program sessions were conducted once a week, each lasting 90-120 minutes. The program and the participant information booklet for the intervention group were created by the researchers after consulting with 10 experts in the field. The Lawshe technique was used to gather expert opinions [37]. The control group did not receive any interventions during the study. To assess the program's effectiveness, measurements were taken from both groups before the program (1st measurement - pretest), at the end of the program (2nd measurement - posttest - 10th week), and three (3) months after the program ended (3rd measurement - follow-up). Based on the results of the study, the control group participants also received the program.

Statistical Analysis

The SPSS software package was used for data analysis, which was blinded to the intervention and control groups and conducted by an independent researcher. The normality of data distribution was assessed. In the data analysis, the number, percentage, standard deviation, mean, chi-square, independent and dependent t-tests were performed, and the magnitude of the test statistic and degrees of freedom (t, F, r, etc.), effect size (ES), confidence interval (CI), and statistical power were calculated. The study involved comparing the values obtained at each time point for groups, posttest measurements
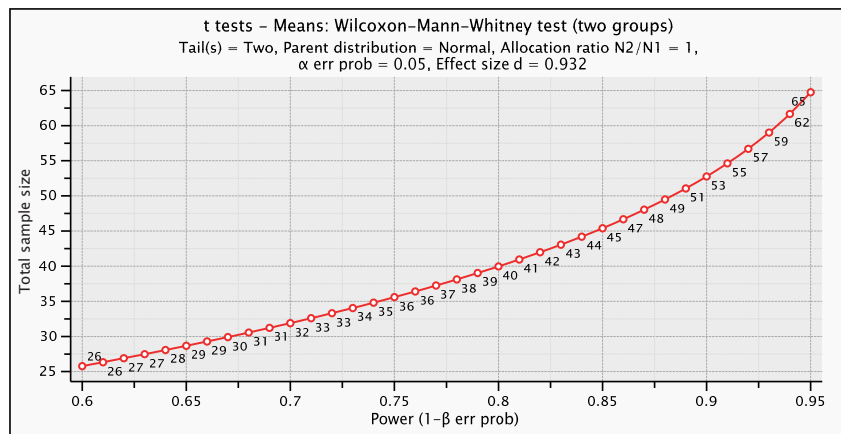

Figure 2: G Power report.

of groups, follow-up measurements of groups, and differences between groups after follow-up measurements (posttest). These comparisons were performed using the Analysis of Covariance (ANCOVA). Similarity analysis was conducted for the intervention and control groups. Cronbach's alpha was calculated to examine the scale's reliability.

Discussion and Conclusion

According to Kübler-Ross, when patients can no longer deny their illness, when they need more treatment, and when they become weaker while trying to cope with more symptoms, they may no longer be able to view this situation with a smile, and their emotional numbness or anger may be replaced by a deep sense of loss. This

Table 2. Femininity Identity Improvement Program.

Sessions	Aim	Content	Duration	Materials
Session 1: Introduction & Getting to know each other	• Getting to know the group members • Explanation of the group process • Expression of group members' expectations regarding group work • Sharing the group's purpose with group members • Setting the group rules • Sharing experiences related to the illness	• Introduction of the group leader • Providing detailed information to group members about the meeting place, frequency, duration, and session content of the group • Distributing the informative booklet prepared for participants • Ice-breaking game • Distributing name tags prepared for group members • Activity titled "Flowers in Our Garden" • Setting group rules through interactions after the activity • Relaxation exercise • Informing about homework assignments • Summarizing the session • Sharing feelings and thoughts about the session among group members • One-minute silence practice	90-120 minutes	A3-sized paper, flower pots made of colored cardboard, colored pencils, crayons, scissors, glue, various art supplies (different colored craft papers, background cards, cotton, string, buttons, glitter, etc.)
Session 2: Psychoeducation and Cognitive Model	• Raising awareness among group members about the biopsychosocial effects of cancer • Presentation of the cognitive model	• Emotion and mood regulation • Review of the first session • Homework assignments • Informing about the agenda • "Bring Emotions to Life" icebreaker game • Presentation of the biopsychosocial effects of cancer and their relationship with emotions, thoughts, and behaviors • "Drawing the Picture of the Illness" activity • "4-4-6-2" Breath Counting Exercise • Informing about homework • Summarizing the session • Group members sharing their emotions and thoughts about the session • One-minute silence practice	90-120 minutes	A4 paper, pencil, colored pencils
Session 3: Self-Exploration and Body Awareness	• Helping group members recognize the changes in their bodies due to cancer • Increasing body awareness	• Emotion-mood regulation • Briefly reviewing the previous session • Sharing homework assignments • Informing about the agenda • Body scan (in a sitting position)	90-120 minutes	3 sheets of A4 paper, a pencil, and colored pencils

Table 2. (continued) Femininity Identity Improvement Program.

	• Helping individuals perceive their bodies as a whole	• "Draw Yourself" activity • "Reach for Your Star" game • Providing information about homework • Summarizing the session • Allowing group members to share their feelings and thoughts about the session • One-minute silence practice		
Session 4: Self-Exploration - Emotions	• Being able to recognize suppressed emotions • Being able to identify unmet needs and express them • Expressing the overall body image	• Emotion-mood regulation • Briefly reviewing the previous session • Sharing homework assignments • Providing information about the agenda • "Silent Scream" exercise • Tree Meditation • Providing information about homework assignments • Summarizing the session • Allowing group members to share their feelings and thoughts about the session • One-minute silence practice	90-120 minutes	A4 paper, pencil, colored markers
Session 5: Femininity Perception	• Discussing the concept of "femininity (being a woman)" with group members. • Allowing group members to openly express their feelings regarding femininity • Encouraging group members to openly express their feelings about their bodies as women	• Emotion-mood regulation • Briefly reviewing the previous session • Sharing homework assignments • Informing about the agenda • Working on the concept of "femininity (being a woman)" by associating it with an object • Playing the "Waterfall" game • Providing information about homework • Summarizing the session • Allowing group members to share their feelings and thoughts about the session • One-minute silence practice	90-120 minutes	A4 paper, a pencil
Session 6: Inner Change	• Recognizing challenging emotions related to loss • Developing the ability to manage recognized challenging emotions • Perceiving oneself as a whole • Developing self-compassion	• Emotion-mood regulation • Briefly reviewing of the previous session • Sharing of homework assignments • Informing about the agenda • Psychoeducation • "Creating a Woman" Exercise • "Self-compassion break" meditation • Informing about homework assignments • Summarizing the session • Sharing feelings and thoughts about the session • One-minute silence practice	90-120 minutes	A3 size drawing paper, pencils, various coloring materials (dry, felt-tip, watercolor, etc.), scissors, glue, various art supplies (different colored craft papers, cardboard, cotton, string, buttons, glitter, etc.)
Session 7: Acceptance	• Loving one's body and accepting the losses	• Emotion-mood regulation • Briefly reviewing the previous session • Sharing homework assignments	90-120 minutes	A4 paper, a pencil, dry coloring markers
	• Accepting one's limitations • Overcoming difficulties in self-expression	• Informing about the agenda • "Draw Your Emotion" Exercise • Seated meditation with acceptance • Providing information about homework • Summarizing the session • Allowing group members to share their feelings and thoughts regarding the session • One-minute silence practice		

loss can manifest in different ways. For example, a woman with breast cancer may react to losing her feminine shape, while a woman with uterine cancer may feel that she is no longer a real woman [38]. In gynecological cancers, many women experience symbolic losses during the treatment process that distance them from their feelings of womanhood. Women may use negative descriptions of their bodies as asymmetrical, deficient, or shattered. Surgical and medical interventions can affect reproductive capacity, leading to issues such as feeling incomplete,

inadequate, and flawed, decreased body image and self-esteem, reluctance to enter new intimate relationships with the opposite sex, and a sense of being unable to complete their family [5,7,8,11-14,39].

There are several studies in the literature that have been conducted with gynecologic cancer patients, involving both individual and group interventions. Many of these studies have addressed issues such as sexual dysfunction, quality of life, treatment side effects,

Table 2. (continued) Femininity Identity Improvement Program.

Session 8: Self-Esteem	• Identifying negative beliefs and distortions about femininity • Highlighting strengths • Identifying areas open to empowerment • Enhancing the individual's adaptability	• Emotion-mood regulation • Briefly reviewing the previous session • Sharing homework assignments • Providing information about the agenda • A brief presentation on cognitive distortions • Sharing cognitive distortions related to femininity by group members • Role-play to expose cognitive distortions • Greeting practice • Informing about homework assignments • Summarizing the session • Allowing group members to share their thoughts and feelings about the session • One-minute silence practice	90-120 minutes	-
Session 9: Self-Esteem	• Building self-confidence • Enhancing self-esteem	• Emotion-mood regulation • Briefly reviewing the previous session • Sharing homework assignments • Informing about the agenda • "I Have My Basket with Me, I'm Going Shopping" activity • Providing information about homework • Summarizing the session • Allowing group members to share their feelings and thoughts about the session • One-minute silence practice	90-120 minutes	Cardboard-made baskets, colored cardstock, scissors, glue, pencil
Session 10: Hope and the Purpose of Life	• Instilling hope • Determining life purposes • Concluding the group process	• Emotion-mood regulation • Briefly reviewing the previous session • Sharing homework assignments • Informing about the agenda • "Our Garden" activity • Creating a "Goals Poster" using the "Sentence Completion Game" • Summarizing the session • Allowing group members to share their feelings and thoughts about the session • One-minute silence practice	90-120 minutes	Canvas suitable for painting (American cloth), brushes of various thicknesses, oil paint, acrylic paint, disposable protective apron, glue

menopause, body image, levels of anxiety and depression, coping skills, sleep quality, and marital satisfaction in women [7,39-45]. In our country, interventions have been conducted for individuals diagnosed with genital organ cancer to assess their reactions to cancer, body image, coping, and psychosocial adaptation. These interventions have included progressive muscle relaxation exercises and reflexology applications for patients undergoing chemotherapy, nursing care based on Watson's Human Caring Theory to assess the impact on anxiety, depression, and quality of life, reflexology applications based on Watson's Human Caring Model to evaluate the effect on chemotherapy symptoms, as well as studies aimed at determining the effects of logotherapy-based life meaning interviews on traumatic stress symptoms, post-traumatic growth, finding meaning in life, and spiritual well-being [46-50]. However, none of the previous studies have addressed the concept of prolonged grief disorder associated with perceived or realized losses related to femininity identity. Additionally, in these studies, no group interventions were found to be based on cognitive-behavioral and expressive techniques. The concept of "improving femininity identity" is being introduced for the first time in this study with gynecologic cancer patients. In the Femininity Identity Improvement Program prepared by the researchers in line with the literature, various expressive techniques such as art, writing, role-playing, guided imagery, along with cognitive-behavioral techniques, are used within the framework of psychiatric nursing roles. Two separate psychotherapeutic interventions are integrated in an innovative approach, contributing to psychiatric nursing practice. This research, as a single-blind randomized controlled trial, may provide evidence-based guidance to psychiatric nursing.

Limitations

The research results are only applicable to gynecological cancer patients between the ages of 18-65 who were included in the study. Limitations of the research include the recruitment of patients from only one university hospital, the exclusion of illiterate patients from the study, and the restriction of the intervention program to ten sessions in a group setting.

Conflict of interest

There is no conflict of interest between the authors.

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