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Our dear readers,

We are proud to publish the fifth issue of our journal for 2022 with 50 articles. Currently, the COVID-19 pandemic has lost its power, and we sure that all researcher would devote a significant part of their strength to valuable articles. As we mentioned before, we want to contribute to international literature at an increasing level and to increase the success bar of our journal by entering valuable international indexes such as SCI-Exp and Pubmed. We would like to thank all authors for submitting articles contributing to both domestic and international literature with their comprehensive scientific content for publication in our journal.

Sincerely yours

Assoc. Prof. Alpaslan TANOGLU, MD
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Comparison of tolterodine, trospium chloride, solifenacin treatments and its side effects on patients with pure urinary and mixed incontinence

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ABSTRACT

Objective: Urinary incontinence is defined as urinary incontinence that is a social or hygienic problem and can be objectively demonstrated. It is aimed to compare tolterodine, trospium chloride and solifenacin treatments, and its side effects on patients who have complaints of pure urinary and mixed incontinence.

Material and Method: Totally 98 patients, who applied to Ankara Etlik Zubeyde Hanım Gynecology Training and Research Hospital, Urogynecology Outpatient Clinic between November 2009 and October 2010 with compliants of urinary incontinence and met the criteria to participate in the research, have been included in this study.

Results: A significant improvement in each three of the drug group at third and six months was determined. Solifenacin is generally more effective than the other two treatments. When total values of UDI-6 (Urinary Distress Inventory) survey is analysed, it is seen that each of three antimuscarinic drug group ensured improvement on symptoms at the end of the third month as not to be different from the improvement at the six month. Each three antimuscarinic drug group has a significant therapeutic effect on the IIQ-7 (Incontinence Impact Questionnaire) survey which questions the life quality. Whereas complaints of constipation was seen more at patients that use tolterodine and trospium chloride, there was not a significant difference despite a slight increase in the solifenacin group ($p>0.05$). It is determined that solifenacin caused desert mouth less than the other two drug groups

Conclusion: Tolterodine, trospium chloride and solifenacin as anticholinergic drugs meaningfully reduced the activity of bladder and increased the quality of life. Drug therapy provided an effective and efficient improvement on incontinence.

Keywords: Pure urinary incontinence, tolterodine, trospium chloride, solifenacin, urogenital distress inventory, incontinence impact questionnaire

INTRODUCTION

Urinary incontinence is defined as urinary incontinence that is a social or hygienic problem and can be objectively demonstrated by International Continence Society (ICS) (1). Urinary incontinence impairs the quality of life of people, restricts the social life of the person and causes psychological problems. It is more common in females than males and can affect females at all age groups. This situation is caused by lower urinary tract pathologies, which affects 10-70% of women (2). Previous studies have shown that, especially overactive bladder symptoms increase with age (3,4). Urinary incontinence is a symptom, not a diagnosis. Although its frequency increases with age, it is not a normal part of aging and should not be considered as an insignificant complaint (5).

The differential diagnosis of urinary incontinence etiology is quite broad. Because of the different pathophysiologies of incontinence, treatment should be etiologically oriented, most effective and most accurate. Today, there are a wide range of treatment options ranging from drug therapy to behavioral therapies and, if necessary, surgical interventions. With a better understanding of the pathophysiology of incontinence, these treatment options will be longer-lasting and more

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successful in the coming years (6,7). The effectiveness of the treatment can be understood by questioning whether the changes after the treatment have a positive effect on the quality of life.

Patients benefit from anticholinergic agents, but they discontinue treatment in the long term. The most important reason for this is the long duration of treatment and possible side effects of anticholinergic agents (8-10). Today, anticholinergic agents that patients can tolerate better are also used. The M3 muscarinic receptor is specifically found in the detrusor muscle. However, anticholinergic drugs block not only the M3 receptor, but also the M1 receptor (brain, salivary gland) and M2 receptor (heart, intestine, etc.) found in other organs. This causes the drugs to show some side effects. Depending on the detrusor muscarinic receptor selectivity of the drug, these side effects vary in severity and type, but depending on the dose, blurred vision, constipation, dry mouth, anxiety, confusion, dizziness, insomnia, nausea and urticaria can be observed. These side effects affect the patient's use of the drug and satisfaction (11,12). For this reason, more selective M3 receptor blockers are being developed.

In 2004, the FDA (Food and Drug Administration) approved the use of darifenacin, solifenacin and trospium chloride in the treatment of overactive bladder (13). Although there are studies in which drug efficacy is compared with placebo, it is seen that some of them are compared within themselves (14-16). Quality of life scoring criteria were mostly established by placebo studies (17-19).

In this study, tolterodine, trospium chloride and solifenacin from the group of anticholinergic agents were compared with each other in terms of efficacy and potency, based on the scoring of the voiding diary, quality of life and drug side effects. It was aimed to evaluate the changes in the voiding diary, UDI-6 (Urinary Distress Inventory) questionnaire, IIQ-7 (Incontinence Impact Questionnaire) questionnaire, and anticholinergic drug side effects in overactive bladder and mixed type cases.

MATERIAL AND METHOD

The study was conducted with the permission of the Noninvasive Clinical Education Planning Board of 3rd step Training and Research Hospital in Ankara (Date: 25.11.2019, Decision No: 2009/119). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

A total of 98 patients who applied to the Urogynecology outpatient clinic of 3rd step Training and Research Hospital between November 2009 and October 2010 with the complaint of urinary incontinence and met the inclusion criteria of the study were included. Detailed informed consent was taken from all the patients. In this prospectively planned and conducted study, women who applied to the 3rd step Training and Research Hospital Urogynecology outpatient clinic with the complaint of urinary incontinence were evaluated at the first stage. Anamnesis of the cases was taken, physical examinations were performed and laboratory tests (full urinalysis, urine culture and fasting blood glucose) were requested.

In the anamnesis, in which condition and how urinary incontinence occurred was questioned. Whether there is involuntary leakage of urine during physical activity, movement (eg. playing sports, running, climbing stairs, sitting up, lifting heavy), sneezing, coughing or laughing. It was questioned whether there was a sudden urge to urinate due to sudden pain, pressure, pressure or discomfort and whether there was urinary incontinence due to this. Involuntary leakage of urine during physical activity, movement, sneezing, coughing or laughing "stress urinary incontinence (SUI)", a sudden and severe urination feeling that cannot be prevented and delayed with a sense of urgency caused by sudden pain, pressure, pressure or discomfort, and related incontinence was defined as "urge urinary continence (UUI)" and urinary incontinence in both ways was defined as "mixed urinary incontinence (MUI)".

Age, menopausal status, parity and mode of delivery, weight, height, overt diabetes, hypertension, urinary stone and chronic kidney disease history or history of surgery, neurological disease, antidiabetic, antihypertensive, diuretic, antidepressant or antipsychotic drug use In his anamnesis, it was questioned whether he had received medical treatment for incontinence, whether he had a previous operation involving the pelvic floor, whether due to incontinence or not.

External genital organs and urethral orifice were observed in the local genitourinary system examination of the cases while they were in the lithotomy position. Vaginal walls and cervix were observed with speculum examination. The presence of urinary incontinence was determined by applying a stress test with Valsalva maneuvers while the patient was in a semi-constricted state. In addition, pelvic relaxation status, which contributes to the development of urinary incontinence and may affect the treatment method, was evaluated with the Baden-Walker Halfway staging system. With Valsalva maneuver, uterine cervix descent, rectocele, cystocele, urethrocele, bladder neck mobility were noted. The Q-Tip Test evaluated the anatomical support of the paraurethral tissues, the degree of mobility of the bladder neck and proximal urethra.

In the design phase of the study, it was planned to perform urodynamic examination in order to objectively demonstrate the efficacy of the diagnosis in the initial evaluation of the cases and then in the 3rd and 6th months of the medical treatment. However, during the two years for which the data of our study were collected, urodynamic examination could not be performed on the patients due to technical inadequacy.

All patients were asked to keep a two-day voiding diary in which the amount of fluid consumed, the number of urination, the total amount of urine, the number of urgency to urinate, and the amount of incontinence were questioned. In addition, the Urogenital Distress Inventory-6 (UDI-6) questionnaire was applied to the patients, in which urinary incontinence complaints were questioned over six questions validated for the Turkish society and the Incontinence Impact Questionnaire-7 (IIQ-7) questionnaire form with seven questions evaluating the social life and quality of life of the patients. The first two questions in the UDI-6 form questioned the patients irritative symptoms, the next two questions asked their stress symptoms and the last two questions were asked about obstructive and voiding symptoms, and the questions were scored as none (0), mild (1), moderate (2) or a lot (3). In the IIQ-7 questionnaire, the physical activities of the patients were questioned with the first two questions, their travel quality with the next two questions, their social relations with the next question and their emotional health status with the last two questions.

The cases with pure SUI as a result of the examinations and tests were not included in the study group.

In addition, those whose blood glucose level is not regulated despite using antidiabetic drugs, those who use antihypertensive, diuretic, antidepressant or antipsychotics, those with 3rd and 4th degree pelvic organ prolapse, those who have received medical treatment for overactive bladder before, whether for incontinence or those who had undergone surgery involving the pelvic floor, those with urolithiasis and chronic kidney disease or a history of surgery and those with a history of neurological disease were also excluded from the study.

Patients who were found to have pure UUI or MUI after the initial diagnosis were given detailed information about the need to start anticholinergic drug therapy which was developed as an M3 muscarinic receptor blocker to reduce or eliminate urinary incontinence complaints.

Ophthalmology consultation was requested from the patients who agreed to participate in the study before starting the treatment to investigate the presence of narrow-angle glaucoma, which is a contraindicated condition for the use of anticholinergic drugs.

Complete urinalysis was repeated after the patients with urinary tract infection detected in complete urinalysis and urine culture were treated appropriately. Cases whose urinary tract infections were treated but still had complaints of UUI or MUI and cases whose blood glucose levels were regulated by the use of antidiabetic drugs were included in the study.

Ninety-eight cases met the inclusion criteria and agreed to participate in the study. Anticholinergic agents, which are M3 receptor blockers, were started as tolterodine SR 4 mg tablet (once daily), trospium chloride tablet (30 mg in the morning, 15 mg in the evening) and solifenacin 5 mg tablet (once daily) according to the order of admission to the hospital. It is recommended to use them for at least 6 months.

Treatment efficacy was observed with the UDI-6 and voiding diary form applied to the patients in the third and sixth months of the treatment and the changes in the effect of incontinence on life with the IIQ-7 form. Investigation of side effects after treatment with anticholinergic drugs was done with an anticholinergic side effect evaluation form. Blurred vision, constipation, dry mouth, anxiety (feeling nervous, scared or irritable), dizziness, insomnia (insomnia, inability to sleep or waking up earlier than usual), nausea-vomiting, urticaria (lesions that may be itchy on the skin) / allergic reaction symptom and its findings were questioned.

A total of five patients, one in the trospium chloride patient group and two each in the tolterodine and solifenacin patient group were excluded from the study because they dropped out of follow-up or did not comply with their drug use. The study was completed with the remaining 93 patients.

Statistical analysis of the data obtained at the end of the sixth month was performed using the SPSS 15.00 program. One Way Anova, Paired-Samples T Test, Mann Whitney. It was analyzed using U, Chi-Square, Wilcoxan tests. A p value of <0.05 was considered significant.

RESULTS

A total of 93 patients whose results were analyzed in the study were found to be equal in 3 treatment groups (trospium group n=31, tolterodine group n=31, solifenacin group n=31). The mean age, number of births and body mass index of the women in the 3 groups were similar ($p>0.05$) (**Table 1**).

Table 1. Demographic data of the cases

	Treatment group	Average±SD	Min–Max	p
Age	Trospiyum (n=31)	51.9±12.3	32–86	0.416
	Tolterodin (n=31)	55.0±11.9	31–80	
	Solifenasin (n=31)	55.7±11.4	40–80	
Parity	Trospiyum (n=31)	3.74±2.6	1–13	0.317
	Tolterodin (n=31)	4.74±2.4	0–10	
	Solifenasin (n=31)	4.42±2.9	0–13	
Body mass index (kg/m^2)	Trospiyum (n=31)	29.8±5.1	21.5–47.1	0.402
	Tolterodin (n=31)	31.5±5.2	21.9–44.4	
	Solifenasin (n=31)	30.1±5.3	22.7–39.5	

The mean number of urination per day, the number of urgency to urinate and the amount of urinary incontinence, which are the voiding diary parameters, were similar in all three drug groups before the start of treatment (p>0.05). When the efficacy of treatment was evaluated over time, a significant improvement was found in all parameters of the voiding diary in the third and sixth months compared to the initial values (p<0.05) (**Table 2**).

However, it was determined that the decrease in the number of urination was the highest in the solifenacin group and this decrease was significantly higher than the other groups (p<0.05).

While there was a statistically significant decrease in the number of urgent urination in all groups compared to the pre-treatment period in the 6-month period, it was observed that this decrease was the least in the tolterodine group. In cases using trospium and solifenacin, a similar decrease was found in the rate of urgency to urinate (**Table 2**).

The mean decrease in urinary incontinence was higher in the tolterodine and solifenacin groups than in those using trospium (**Table 2).**

Table 2. Comparison of voiding diary parameters by treatment groups

		Tolterodin	Trospiyum	Solifenasin	p
Number of urination (n)	Beginning	7.4±2.2	7.7±3.8	6.5±0.7	>0.05
	3 months	5.6±0.9	7.0±3.3	4.6±0.6	<0.05
	6 months	5.2±1.3	5.6±2.7	4.4±0.5	<0.05
p		<0.05	<0.05	<0.05	
Number of urgency to urinate (n)	Beginning	4.7±1.4	4.0±1.8	4.2±2.5	>0.05
	3 months	3.4±0.2	2.2±1.3	2.0±1.4	<0.05
	6 months	2.3±0.0	1.3±0.9	1.4±1.1	<0.05
p		<0.05	<0.05	<0.05	
Amount of incontinence (ml)	Beginning	197±43	207±95	188±61	>0.05
	3 months	118±14	143±79	117±49	<0.05
	6 months	62±33	87±55	65±35	<0.05
p		<0.05	<0.05	<0.05	

When the total scores in the UDI-6 questionnaire were examined, the initial UDI-6 scores of all three antimuscarinic drug groups were similar (p>0.05). However, there was a significant improvement in symptoms at the end of the third month in all three groups during the treatment process (p<0.05). Although this improvement continued significantly at the 6th month, the changes in the UDI-6 scores at the 3rd and 6th months were similar in all 3 drug groups (p>0.05) (**Table 3, Table 4**).

The IIQ-7 (Incontinence Effect Questionnaire-7) groups were similar to each other before treatment (p>0.05). In the IIQ-7 evaluation performed at the 3rd and 6th months of the treatment, it was observed that there was a significant decrease in IIQ-7 scores in all drug groups compared to the pre-treatment period, thus a significant improvement in the effect of incontinence (p<0.05). The IIQ-7 scores at 3 and 6 months were similar in all three groups (p>0.05) **(Table 5, Table 6**).

When the side effects of anticholinergic drugs used in the treatment (blurred vision, constipation, dry mouth, anxiety, dizziness, insomnia, nausea, urticaria) were examined, it was observed that complaints and symptoms were similar in all groups at the beginning (p>0.05).

While the rates of blurred vision at the 3rd and 6th months of the treatment did not differ from the initial rates in the trospium and solifenacin groups (p>0.05), it was found to increase significantly in the tolterodine group at the 6th month (p<0.05).

Constipation increased significantly in the groups using tolterodine and trospium both at the 3rd and 6th months compared to the baseline (p<0.05). While the constipation rates in the group using trospium were similar at the 3rd and 6th months (p>0.05), the constipation rate at the 6th month was found to be significantly higher in the group using tolterodine compared to the 3rd month (p<0.05). In the group using solifenacin, the rate of development of constipation did not differ significantly from the baseline (p>0.05).

Table 3. Urinary Distress Inventory, Short Form (UDI-6).

For each question, circle the number that best describes this problem for you over the past month. Do you experience and, if so, how much are you bothered by:

	Not at All	A Little Bit	Moderately	Greatly
Frequent urination?	0	1	2	3
Urine leakage related to urgency?	0	1	2	3
Urine leakage related to physical activity? (walking, running, laughing, sneezing, coughing)	0	1	2	3
Small amounts of urine leakage? (drops)	0	1	2	3
Difficulty emptying your bladder or difficulty urinating?	0	I	2	3
Pain or discomfort in your lower abdominal, pelvic, or genital area?	0	I	2	3

Table 4. Comparison of UDI-6 scores during treatment according to drug groups

Drug	Beginning	3 months	6 months	p
Trospiyum	1.06±0.28	0.48±0.19	0.54±0.23	<0.05
p		>0.05		
Tolterodin	1.10±0.23	0.54±0.23	0.65±0.34	<0.05
p		>0.05		
Solifenasin	1.18±0.37	0.47±0.37	0.55±0.41	<0.05
p		>0.05		

Table 5. Incontinence Impact Questionnaire, Short Form (I1Q-7).

Some people find that accidental urine loss may affect their activities, relationships, and feelings. For each question, circle the response that best describes how much your activities, relationships, and feelings arc being affected by urine leakage over the past month. Has urine leakage (incontinence) affected your:

	Not at All	Slightly	Moderately	Greatly
Ability to do household chores (cooking. housecleaning, laundry)?	0	1	2	3
Physical recreation such as walking, swimming, or other exercise?	0	1	2	3
Entertaining activities (movies. concerts, etc.)?	0	1	2	3
Ability to travel by car or bus more than 30 minutes from home?	0	1	2	3
Participation in social activities outside your home?	0	1	2	3
Emotional health (nervousness, depression, etc.)?	0	1	2	3
Feeling frustrated?	0	1	2	3

Table 6. Comparison of IIQ-7 values during the treatment process according to drug groups

Drug	Beginning	3 months	6 months	p
Trospiyum	1.65±0.79	0.44±0.40	0.36±0.42	<0.05
p		(p>0.05)		
Tolterodin	1.71±0.69	0.97±0.49	0.88±0.46	<0.05
p		(p>0.05)		
Solifenasin	1.70±0.84	0.56±0.52	0.49±0.49	<0.05
p		(p>0.05)		

Dry mouth increased strongly at the end of the 3rd and 6th months of treatment in all three drug groups (p<0.05). In the tolterodine group, this increase was higher than in the other two groups. Although statistically significant, the least increase was observed in the solifenacin group. It was determined that the rate and severity of dry mouth did not change between the 3rd and 6th months in the trospium and solifenacin groups (p>0.05). In the tolterodine group, the rate and severity of dry mouth continued to increase after the 3rd month (p<0.05).

When the complaints of anxiety were examined, a significant increase was observed in only the tolterodine group at the 3rd month compared to before the treatment (p<0.05), while there was a statistically significant increase in anxiety at the 6th month in all 3 drug groups compared to both the baseline and the 3rd month (p<0.05).

It was determined that the complaint of vertigo was significantly increased only in the tolterodine group at the 3rd month compared to the pre-treatment (p<0.05). At the end of the 6th month, there was a significant increase in the complaints of dizziness in both the tolterodine and solifenacin groups compared to the 3rd month, while there was no significant increase in the complaints of dizziness in the trospium group at any time (p>0.05).

When the complaint of insomnia was compared, no statistically significant increase was found in any treatment group at the end of the 3rd month compared to the pre-treatment (p>0.05) At the end of the 6th month, there was a significant increase in the complaint of insomnia in all treatment groups compared to the 3rd month (p<0.05).

When the complaints of nausea and vomiting were examined, a statistically significant increase was found in the 6th month only in the solifenacin group compared to the pre-treatment (p<0.05), while no difference was observed in the complaints of nausea and vomiting in the other treatment groups (p>0.05).

No significant urticaria development was observed during the treatment period in the trospium and solifenacin groups (p>0.05). A statistically significant increase was observed in the rate of development of urticaria only in the tolterodine group after the 3rd month compared to before treatment (p<0.05).

DISCUSSION

In the study conducted by Burgio et al. (21) in Baltimore, 58% of 541 healthy women aged 42-50 years reported urinary incontinence at any time and 30.7% reported urinary incontinence at least once a month. In this study, the mean age of all patient groups was found to be 54.18 years, and almost all of them were perimenopausal and postmenopausal patients.

In another study, prevalence rate of incontinence to be 37.6% in all women aged 50 years and older. Stress was found in 26.7% of these women, urge in 9.1% and mixed incontinence in 55.3% (22). In our study, the mean age of all patient groups was found 54.18 years. Moreover, mixed and urge incontinence was consistent with the expected group. Dwyer et al. (23) found that women with detrusor instability and stress incontinence were 20% more overweight compared to their age and height. Increased body weight causes an increase in abdominal wall weight and an increase in intra-abdominal pressure and intravesicular pressure. Bump et al. (24) showed a significant improvement in incontinence with weight loss. The mean BMI of the patients in our study was 30.4 for the whole patient

group, 29.8 for the trospium chloride patient group, 31.5 for the tolterodine patient group, and 30.1 for the solifenacin patient group. There was no statistically significant difference between the groups in terms of BMI (p=0.402). The mean BMI of the study group was consistent with the expected values for incontinence.

In a study conducted by Burgio et al. (25) the parity average was found to be 2.6 for continent women, 2.5 for those with rare urinary incontinence, and 2.7 for those with regular incontinence. In this study, the mean parity of the trospium chloride patient group was 3.74, the mean parity of the tolterodine patient group was 4.74, and the mean parity of the solifenacin patient group was 4.42, which supports Burgio et al. In addition, the p value for age was 0.317 in our study, and no significant difference was found between the groups. There were six cesarean sections in total, one in the trospium chloride patient group, three in the tolterodine patient group, and two in the solifenacin patient group, and this value was not statistically significant.

Trospium chloride, tolterodine and solifenacin are M3 receptor blockers which were compared with each other in this study. Comparisons of these three molecules were made not only in terms of their efficacy but also in terms of their side effects. In general, the usability and tolerability of all three drugs in both mixed and pure urge incontinence cases were determined based on the scores in the UDI-6, IIQ-7 and anticholinergic side effect evaluation questionnaires. Thus, it was evaluated to what extent the side effects that the patients were exposed to due to the drugs prevented the improvement in their complaints and quality of life.

In 2007, Cam et al. (26) investigated the applicability of IIQ-7 and UDI-6 on Turkish people in their study. They conducted these studies on 302 patients who applied to İstanbul Zeynep Kamil Hospital between March 2004 and October 2004 had urinary incontinence at least once in the past 12 months. As a result of the studies, they concluded that IIQ-7 and UDI-6 gave consistent results on Turkish people and were usable. In this study, an improvement was found in IIQ-7 quality of life scores with all three antimuscarinic drug groups on patient groups.

In the study performed by Abrams et al. (27) in 2005, they found that solifenacin achieved a good balance in treatment and tolerability and increased patient compliance and satisfaction in the long term. Solifenacin was found to be better in terms of side effects compared to the other two drug groups, in this study. In addition, the use of a single dose per day was considered as an important advantage in terms of patient compliance with the treatment. Chapple et al. (28) determined that solifenacin provided a statistically significant improvement in all incontinence symptoms compared to tolterodine. They also concluded that the complaint of blurred vision was more with tolterodine. Similarly, it was found that solifenacin was more effective than tolterodine in terms of incontinence symptoms, and blurred vision was more common in the tolterodine patient group. In another previous study, it was mentioned that solifenacin caused decrease in incontinence complaints faster than tolterodine (29). In this study, all three antimuscarinic drug groups had a significant effect on reducing incontinence complaints, but solifenacin provided a faster recovery on incontinence symptoms at the end of the third month, although not different from the sixth month.

In terms of irritative symptoms, there was a statistically significant difference between the tolterodine patient group and the solifenacin and trospium chloride patient group at baseline, and this difference persisted at the sixth month because of the higher baseline values of irritative symptoms in the tolterodine patient group or because solifenacin and trospium chloride had a better effect on irritative symptoms. The question of whether it is effective requires further research. The fact that the tolterodine patient group had fewer complaints in terms of obstructive and voiding difficulties at the beginning, and the closure of this difference between the tolterodine patient group and the solifenacin and trospium chloride patient groups at the end of the sixth month, suggests that tolterodine has a stronger effect on obstructive and voiding difficulty.

In many studies, the effects and side effects of existing drugs were compared with placebo, while comparisons of drugs with each other were less studied (30,31). Comparative studies of trospium chloride and tolterodine were insufficient. It was observed that their efficacy was not different from each other in only one study (32). In the UDI-6 questionnaire, in which we evaluated the drug efficacy together in our study, similar efficacy was found between the two drugs at the end of the third month, not different from the sixth month.

Trospium chloride, with its quaternary amine groups and positive charge, is low lipophilic and non-receptor specific. It passes to the central nervous system to a lesser extent than tolterodine, and although the receptor is not selective, it affects the muscarinic receptors in the central nervous system much less (33). In our study, while tolterodine causes dizziness in patients in the early period, the same effect occurs with solifenacin in the later period. Trospium chloride, on the other hand, did not have an effect of increasing dizziness on patients.

In a 2003-published literature (34) review of 6800 people and 32 separate studies, it was stated that anticholinergic drugs provided statistically significant improvement compared to placebo. However, side effects such as dry mouth and increased residual urine were emphasized in all studies. In our study, we also found a significant increase in dry mouth and residual urine in all three drug groups, with varying amounts. In the study of Metello J. et al. (35) solifenacin was found to be more tolerable than trospium. Constipation and dry mouth, which are the most common side effects of anticholinergic use, were less common in solifenacin than in trospium, in this study (**Table 6**).

In a meta-analysis by Thomas M. Kessler and his team (36) in February 2011, they reviewed 69 studies with a total of 26229 patients. According to the results of the meta-analysis, they concluded that all three anticholinergic drugs used in our study cause similar side effects and that overactive bladder treatment should be started with any of them, and if the drug is found to be ineffective on the patient, dose adjustment or switching to another drug is required. While similar side effects were observed with solifenacin and trospium chloride in this study, tolterodine was found to be more risky in terms of side effects compared to these two drugs.

CONCLUSION

Tolterodine, trospium chloride and solifenacin as anticholinergic drugs meaningfully reduced the activity of bladder and increased the quality of life. Drug therapy provided an effective and efficient improvement on incontinence.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was conducted with the permission of the Noninvasive Clinical Education Planning Board of 3rd step Training and Research Hospital in Ankara. (Date: 25.11.2019, Decision No: 2009/119).

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

Referee Evaluation Process: Externally peer-reviewed.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version

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Original Article

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Evaluation of treatment results of stereotactic body radiotherapy for spinal metastases: A single center experience

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ABSTRACT

Aim: To assess oncological outcomes and adverse events of patients receiving single or multi-fraction stereotactic body radiotherapy (SBRT) for spine metastases.

Material and Method: Patients with any pathologically proven solid tumor histology who had SBRT to the spine for recurrent or metastatic disease between the years 2010 and 2021 at our department were identified from institutional database. Patient, tumor and treatment characteristics, and follow-up medical records were retrospectively reviewed. Local control (LC) and overall survival (OS) rates were calculated, and adverse events were evaluated.

Results: A total of 47 patients were treated to 50 spine metastases. Median age was 53 years for all patients. Histologies included breast cancer (45%), non-small cell lung cancer (NSCLC; 21%), prostate cancer (15%) and other types (19%). Median follow-up was 16 months for all patients. Of 47 patients, six (13%) developed local failure and 15 (32%) died without local failure. One and two-year actuarial LC rates were 90.1% and 83.6%, respectively. One and two-year OS rates were 75.1% and 62.7%, respectively. Twenty-two (47%) patients had pain before SBRT. Fifteen (68%) of them had complete or partial pain response at 3 months after SBRT. Vertebral compression fracture, which was grade 1 in severity according to the Common Terminology Criteria for Adverse Events (CTCAE [v.4.03]), was observed in only one (2%) patient and it occurred 46 months after SBRT. No cases of treatment-related radiation myelopathy or any≥grade 3 RT induced acute or late toxicities occurred.

Conclusion: This study supports that SBRT to the spine results in high LC without any significant toxicity. The results of ongoing phase 3 trials will highlight whether this high LC benefit reflects to survival in oligometastatic disease.

Keywords: Stereotactic body radiotherapy, Spine, Metastasis

INTRODUCTION

Palliative radiotherapy (RT) is effective in achieving pain relief, preventing the morbidity of bone metastases and therefore, it has been used as one of the standarts of care in bone metastases (1,2). 8 Gy in a single fraction provides equivalent pain and narcotic relief at 3 months compared to 30 Gy in 10 fractions for patients with painful bone metastases from breast or prostate cancers however, the 8-Gy arm have a higher rate of re-treatment but have less acute toxicity than the 30-Gy arm (3).

Stereotactic body radiotherapy (SBRT), which is an innovative modality based on high precision planning and delivery, has the ability to dose escalate the tumor volume while sparing the adjacent organs-at-risk compared to conventional external beam RT (4, 5). Pain relief was similar between SBRT and conventional RT arms in NRG Oncology/RTOG 0631 phase 3 trial (6). However, Sahgal et al. (7) demonstrated that SBRT was associated with significantly higher complete response rates for pain compared to conventional external beam RT at 3 months and 6 months after treatment. Thus, there has been a paradigm shift in the management of spine metastases towards SBRT due to improving pain relief.

We, herein, reviewed and analyzed the data of our patients who received SBRT to the spine.

MATERIAL AND METHOD

The study was carried out with the permission of the Kartal Dr. Lütfi Kırdar City Hospital, Clinical Researches Ethics Committee (Date: 30.03.2022, Decision No: 2022/514/222/15). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

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Patients

Patients with any pathologically proven solid tumor histology who had SBRT to the spine for recurrent or metastatic disease between the years 2010 and 2021 at our department were identified from institutional database. Prior therapy including previous RT was not an exclusion criterion. Patient, tumor and treatment characteristics, and follow-up medical records were retrospectively reviewed.

Spinal Instability Neoplastic Score (SINS) of spine disease was assessed according to the system devised by the Spine Oncology Study Group (8). It evaluates and scores 6 variables: location of lesion, characterization of pain, type of bony lesion, radiographic spinal alignment, degree of vertebral body destruction, and involvement of posterolateral spinal elements. The SINS ranges from 0 to 18, with higher values indicating greater instability; a SINS score of 0–6 denotes stability, 7–12 denotes potentially unstability, and 13–18 denotes unstability.

Stereotactic Body Radiotherapy and Follow-up Evaluation

Axial T1-weighted post-gadolinium and axial T2-weighted non-contrast enhanced magnetic resonance imaging (MRI), including the target vertebral segment and at least one vertebral body above and below, those were acquired with a slice thickness of 1 mm were ordered before SBRT planning. Patients underwent immobilization with vac-loc bags and planning computed tomography (CT) scan was obtained in the treatment position. Pre-SBRT MRI was fused to planning CT scan for delineation of the gross tumor volume (GTV), clinical target volume (CTV), spinal cord and thecal sac. GTV, CTV, and organs at risk were deliniated. The planning target volume (PTV) was defined as CTV plus a 1 mm margin. Radiation dose and fractionation were determined for each patient on the basis of PTV volume, prior RT dose, and spinal cord and thecal sac tolerances. Treatment plans consisted of one, two, three, or five fractions for median doses of 17, 16, 21, and 22 Gy, respectively. Biological effective dose (BED) was calculated using the linear quadratic formula utilizing an α/β ratio of 10 for tumor. Two of two patients treated with two fractions, one of 29 patients treated with three fractions, and six of nine patients treated with five fractions had previously received RT.

Spinal cord D0.1cc and thecal sac Dmax were restricted to 10 and 12.4 Gy in one fraction, 18 and 20.3 Gy in three fractions, and 23 and 25.3 Gy in five fractions for de novo treatments, respectively (9, 10). Thecal sac and spinal cord dose constraints were individualized in the retreatment setting, and prior radiation spine dose and time interval since the prior RT were taken into account. Dose planning was carried out with the Multiplan Software (Accuray Inc., Sunyvale, CA, USA). CyberKnife treatment was performed in an outpatient setting. Treatment was delivered utilizing Xsight spine image tracking.

Follow-up care consisted of clinical examination and positron emission tomography - computed tomography (PET/CT), spine MRI with contrast, or spine CT according to the physician preference every three months unless clinically indicated at an earlier time point.

Outcomes

All times to event were measured from the date of SBRT. Event was defined for local control (LC) as a progressively enhancing lesion or soft tissue mass at the treated vertebral level that was depicted by MRI, CT or PET/CT scans, or pathology that demonstrated malignancy. Patients without an event were censored at the last contact date and patients were also censored when they died. OS was defined as the time from SBRT to death from any cause.

Toxicity was assessed according to the Common Terminology Criteria for Adverse Events (CTCAE [v.4.03]) (11).

Statistical Analysis

Rates of LC and OS were estimated using the Kaplan–Meier method. The log rank method was used for statistical comparisons of groups. Mann-whitney U test was used to compare the differences between two independent groups.

P values<0.05 were considered statistically significant. The data processing and statistical analysis were performed with statistical software package IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., Armonk, N.Y., USA).

RESULTS

Patient and Tumor Characteristics

A total of 47 patients were treated to 50 spine metastases. Baseline patient characteristics are showed in **Table 1.** Median age was 53 years for all patient cohort whereas 58 and 51 years for men and women, respectively. Of 47 patients, 39 (83%) patients had metastasis at one spinal segment, five (11%) patients had metastases at two consecutive spinal segments, and three (6%) patients had metastases at two non-consecutive spinal segments. Histologies included breast cancer (45%), non-small cell lung cancer (NSCLC; 21%), prostate cancer (15%) and other types (19%). Thirty-nine (81%) patients did not have RT history to target vertebrae whereas nine (19%) patients, presented with relapse of spine metastasis, had previously received 30 Gy palliative RT.

Table 1. Patients' characteristics

Patient characteristics	n (%)
Age, years	
Median (range)	53 (32-80)
Gender	
Male	21 (44.7)
Female	26 (55.3)
ECOG performance status	
0	19 (40.4)
1	26 (55.3)
2	2 (4.3)
No. of patients with primary tumors	
Breast	21 (44.7)
NSCLC	10 (21.3)
Prostate	7 (14.9)
Others	9 (19.1)
Previous RT to target vertebrae	
Yes	9 (18)
No	41 (82)
CT appearance of spine lesion	
Lytic	19 (38)
Sclerotic	20 (40)
Mixed (Lytic/Sclerotic)	11 (22)
SINS score	
0-6	28 (56)
7-12	19 (38)
13-18	3 (6)

Abbreviations: ECOG, Eastern Cooperative Oncology Group; NSCLC, Non-small cell lung cancer; RT, Radiotherapy; CT, Computed Tomography; SINS, Spinal Instability Neoplastic Score

Treatment characteristics are demonstrated in **Table 2.** SBRT was delivered in a median of three fractions (range one-five) with a median total dose of 21 Gy (range 13-28).

Table 2. Treatment characteristics

Treatment characteristics	n (%)
Site of target vertebrae	
Cervical	4 (8)
Thoracic	26 (52)
Lumbar	17 (34)
Sacral	3 (6)
Total SBRT dose	
Median (range)	21 Gy (13-28)
Number of SBRT fractions	
1	10 (20)
2	2 (4)
3	29 (58)
5	9 (18)
Volume of PTV	
Median (range), mm^3	33748 (4799-164310)
BED10 of the prescription dose	
Median (range), Gy	35.7 (28-65.1)

Abbreviations: SBRT, Stereotactic Body Radiotherapy; PTV, Planning target volume; BED, Biologically equivalent dose

Local Control, Overall Survival and Toxicity Outcomes

Median follow-up was 16 months for all patients and 23 months for alive patients. Of 47 patients, six (13%) developed local failure and 15 (32%) died without local failure during follow-up. Of six patients with local failure; four had breast cancer primary, two had other primaries. There was no local failure in patients with prostate cancer or NSCLC. The median BED10 of the prescription dose or the median PTV volume were not statistically different between the tumors with local failure and those without local failure (p=0.240 and p=0.302).

One and two-year actuarial LC rates were 90.1% and 83.6%, respectively (see **Figure a**). LC rates at one-year were as follows; 95.7% for patients with SINS 0-6 vs 82.5% for those with SINS 7-18 (p=0.253), 95.8% for patients with sclerotic or mix metastases vs 80.4% for those with lytic metastases (p=0.136), and 100% for patients with previous RT to target vertebrae vs 88.1% for those without previous RT to target vertebrae (p=0.769). One and two-year OS rates were 75.1% and 62.7%, respectively (see **Figure b**).

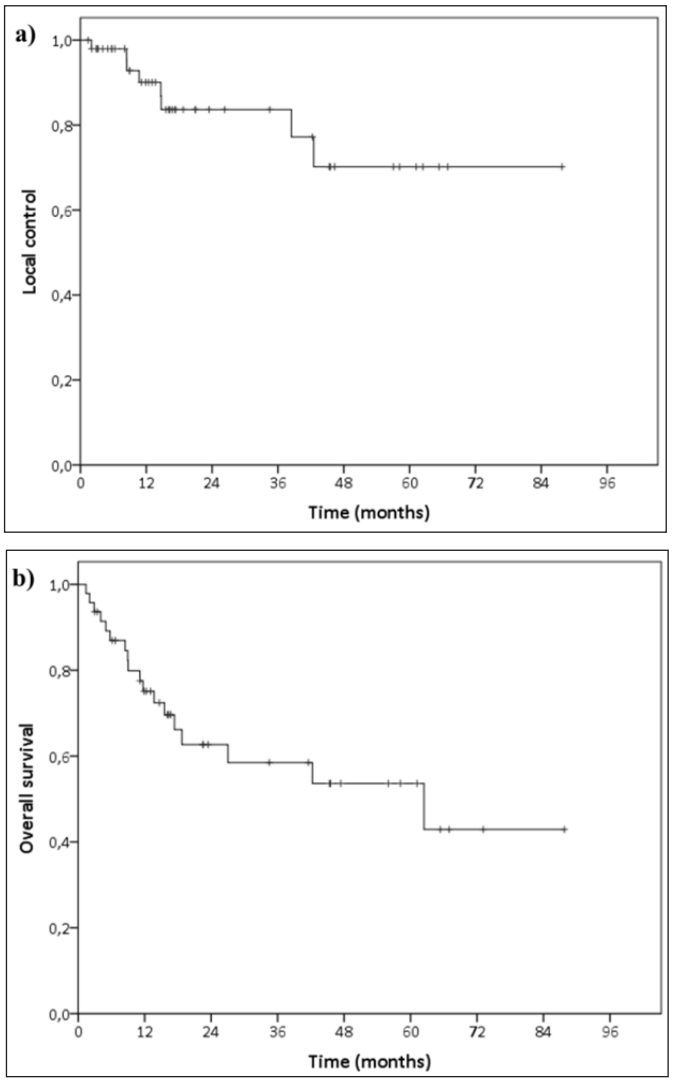

Figure 1. Operational duration according to the groups

Twenty-two (47%) patients had pain before SBRT. Fifteen (68%) of 22 patients had complete or partial pain response at 3 months after SBRT.

SBRT was altogether tolerated well. Vertebral compression fracture, which was grade 1 in severity according to the CTCAE (v.4.03), was observed in only one (2%) patient and it occurred 46 months after SBRT with the prescription dose of 17 Gy in one fraction. No cases of treatment-related radiation myelopathy or any≥grade 3 RT induced acute or late toxicities occurred.

DISCUSSION

There exists controversies in clinical trials about whether SBRT leads to improved pain control over conventional palliative RT (6,7,12). In the phase 2 trial conducted by Sprave et al. (12), 24 Gy single-fraction SBRT provided quicker and improved pain response compared to conformal RT with 30 Gy in 10 fractions. On the contrary, pain control at 3 months was not improved due to the lower pain control rate than expected in the SRS arm in NRG Oncology/RTOG 0631 phase 3 trial (6). In that trial, SBRT consisted of a total dose of 16 to 18 Gy delivered in one fraction, thus one may think that RT dose could be relatively low for producing greater pain relief. Nonetheless, Sahgal et al. (7) showed in their phase 2/3 trial that SBRT significantly improved the complete response rate for pain compared with conventional external beam RT. Patients received a total dose of 24 Gy in two consecutive daily fractions in SBRT arm in their trial which represents a high biologically equivalent SBRT dose than that used in NRG Oncology/RTOG 0631 trial. Of 22 patients who presented with pain prior to SBRT, 14 (64%) had complete or partial pain response at 3 months after SBRT in our study. This rate seems relatively higher than the pain response rate which was 53% at 3 months after SBRT in the randomized phase 3 trial conducted by Shagal et al (7). Pre-SBRT and post-SBRT pain evaluation was not done according to any pain scale in our patient population. Thus, this could be a limitation of our study. However, that type of pain response evaluation is beyond the scope of this study.

Apart from pain control, SBRT could be applied for improving survival for patients with limited burden of metastatic disease (13, 14). Although there exists several limitations (i.e. including multiple histologies and assigning the large majority of patients with prostate cancer to the SBRT arm), long term results of SABR-COMET phase 2 trial (15) demonstrated that SBRT was associated with a significant improvement in progression-free survival and OS in a group of patients with an oligometastatic disease (mostly with 1–3 metastatic lesions). Several phase-3 trials are accruing patients and are evaluating the impact of SBRT on survival in patients with oligometastases (16-18). Twenty-five of 47 (53%) patients did not have pain in our patient population but they had limited burden of disease and thereby being treated with SBRT.

Vertebral compression fracture is one of the common toxicities of spine SBRT (19-21). In the trial conducted by Sahgal et al. (19), the median time to vertebral fracture was 2.46 months (range, 0.03 to 43.01 months), and 65% developed in the first 4 months following SBRT. The two-year cumulative incidence of vertebral fracture was 13% and they observed that≥20 Gy in single-fraction posed a significant risk for fracture compared to lower SBRT dose. In the study conducted by Mehta et al. (20), patients treated with two to five fractions SBRT with a median total dose of 24 Gy (in a median of three fractions) and vertebral body fracture occurred in 5.3% of those without surgery or vertebroplasty prior to SBRT. Only one (2%) patient, who were treated with 17 Gy in single fraction, experienced vertebral fracture with grade 1 in severity in our study. It developed 46 months after SBRT. Up to us, the lower rate of vertebral fracture in our patient population is due to smaller sample size and our SBRT dose and fractionation schemes such as that 79% of patients were treated in multi-fraction with limited biologically equivalent dose.

SBRT for spine metastases was safely performed without causing any increase in adverse effects as compared to conventional EBRT in randomized controlled trials (6, 7). SBRT applied safely to our patient population, and no radiation myelopathy and any≥grade 3 toxicity was observed. This could be due to the our general clinical approach in which a threshold of less than 5% risk of serious adverse effects is chosen for organs at risk dose recommendations (9,10).

Several SBRT dose fractionation schedules were assessed compared to conventional RT as mentioned above. However, there are no dose finding randomized trials to evaluate the superiority of ideal dose fractionation in SBRT. One-year LC rate was 90% in our study population. This rate is consistent with the literature (22). However, heterogeneous dose-fractionation protocols were applied due to the organs at risk doses and prior RT history to the target vertebrae in our study. Thus, it is difficult to draw a conclusion for the effectiveness of a specific dose-fractionation protocol. This could be limitation of our study. In additon, small sample size and retrospective design are the other limitations of our study. Small sample size and high LC rate might have induced not to determine any significant factor assosicated with improved or decreased LC.

CONCLUSION

SBRT to the spine results in improved pain response and high LC without any significant toxicity. The results of ongoing phase 3 trials mentioned above will highlight whether this high LC benefit reflects to survival in oligometastatic disease.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of the Kartal Dr. Lütfi Kırdar City Hospital, Clinical Researches Ethics Committee (Date: 30.03.2022, Decision No: 2022/514/222/15).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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Original Article

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The relationship between polycystic ovary syndrome and irritable bowel syndrome

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ABSTRACT

Introduction: Polycystic ovary syndrome (PCOS) causes endocrine disorders that affect the functioning of the reproductive system and the body's metabolic system. Bowel movement disorders and abdominal pain are common complaints of PCOS patients. Few studies have been performed on the relationship between PCOS and irritable bowel syndrome (IBS), and the association between the two syndromes is unclear.

Material and Method: In the study, 72 patients were enrolled at gynecology and obstetric clinic in Turkey. The control group were (n=34) and women with PCOS (n=38). IBS diagnosis was made by using Roma IV criteria.

Results: The results showed that IBS prevalence was similar in PCOS (52%) and the control group (50%) ($p>0.05$). No statistically significant association was found between IBS-PCOS and non-IBS-PCOS in terms of gastrointestinal symptoms ($p=0.685$). These symptoms were associated with PCOS rather than IBS. Significant differences have been observed between IBS-PCOS and non-IBS-PCOS for fasting insulin (FI), luteinizing hormone (LH) and Homeostasis model assessment for insulin resistance (HOMA-IR) ($p<0.05$). Significant differences have been observed between IBS-control and non-IBS-control for FI, fasting glucose and HOMA-IR ($p<0.05$). Aging was significantly associated with co-occurrence with IBS and PCOS.

Conclusion: Although many PCOS patients were diagnosed with IBS based on Rome IV criteria, no significant relationship was found between PCOS and IBS prevalence in the study subjects. The prevalence of gastrointestinal symptoms is similar in both groups (IBS-PCOS and PCOS only). Age was an important risk factor for the co-occurrence of IBS and PCOS.

Keywords: Polycystic ovary syndrome, Irritable bowel syndrome, gastrointestinal symptoms

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a complex endocrine disorder associated with mild to moderate inflammation (1). This syndrome is a complex and heterogeneous disorder with fertility and metabolic complications (2-4). The irregular menstrual cycle is one of the most obvious symptoms of this disease, which indicates ovarian dysfunction (2). Oligomenorrhea, hyperandrogenism symptoms such as hirsutism and acne, hair loss, and infertility are common in patients with PCOS. PCOS prevalence is 3% to 26%, depending on the region studied (5). The syndrome is related to obesity, hyperinsulinemia, increased risk of insulin resistance (IR), type 2 diabetes mellitus (DM), dyslipidemia, and cardiovascular diseases (CVDs) (6). Gastrointestinal (GI) disorders such as abdominal pain, constipation, or bloating are more reported in women with PCOS than in healthy women. Abdominal pains and discomfort are more common in women with PCOS, although it may not be a symptom of this syndrome (7).

GI dysfunction is a chronic intestinal disease that affects with a prevalence rate of 10 to 20% (8)(8). Irritable bowel syndrome (IBS) is a disorder that manifests itself with abdominal pain and discomfort and changes in defecation habits. Patients also experience symptoms such as bloating and a feeling of incomplete excretion, which can last for years and an average of 10 years (9). These symptoms significantly affect patients' quality of life and consequently impose a heavy economic and social burden on society (10). Symptoms include changes in bowel habits and abdominal pain. Changes in

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bowel habits include conditions of an unspecified type, a combination of constipation and diarrhea (IBS-M), or predominant diarrhea (IBS-D) or constipation (IBS-C). Rome IV criteria for diagnosing IBS are frequent abdominal pain and changes in stool frequency or form that begin approximately six months before diagnosis and last for approximately three months (11).

IBS occurs in all ages and genders, but it is shown that the disease is more prevalent in younger people. Women experience this disease 1.5 to 3 times more than men (12, 13). IBS-C or IBS-M is more prevalent in women, while men report more symptoms of IBS-D (14). Thus, it can be concluded that young women are more at risk of IBS. This could further study the association between hormones and IBS in future research. PCOS and IBS affect women's quality of life as well as mental and physical health, and the study of the relationship between these diseases to find targeted therapies has been considered in limited research (15-17). This study examines the relationsip between PCOS and IBS in women.

MATERIAL AND METHOD

The retrospective study was conducted with the approval of the Clinical Research Ethics Committee of Beykoz University. (Date: 26.04.2021, Decision No: 1) All study processes were conducted under the principles of the Declaration of Helsinki and ethical rules.

Seventy-two participants in this study visited Medistate Hospital Gynecology and Obstetrics department from January 2020 to February 2021. Participants ranged in age from 18 to 45 years.

Subjects diagnosed with PCOS (n=38) and healthy subjects (n=34) were asked to partake in the investigation as the case group.

According to Rotterdam Criteria, the criteria for diagnosing PCOS is to have at least two of the following characteristics:

1) polycystic ovary on ultrasound; 2) clinical and/or biochemical hyperandrogenism signs; 3) oligoovulation or anovulation. On the other hand, in healthy women, menstrual cycles are regular, and no excess androgens were observed. IBS diagnosis was made by using Roma IV criteria (18,19). Gastrointestinal symptoms such as a postprandial saturation, functional dyspepsia, epigastric pain, early satiety, and epigastric burning were noted.

Inclusion and Exclusion Criteria

The inclusion criteria were the age of women between 18-45 years. The exclusion criteria were known chronic disease, pregnancy or lactating, premenopausal or menopausal, and active infection.

Statistical Analysis

Data were analyzed, tabulated, and subjected by SPSS (version 26). The continuous data were displayed as mean±SD. At the same time, categorical data were illustrated as percentages and numbers. The Kolmogorov-Smirnov test of normality was utilized to test the normality hypothesis. Based on the test results, proper parametric (Independent Sample t-test) and nonparametric tests (Chi-Squared test and Mann-Whitney U test) were used. A p-value of<0.05 was regarded as statistically significant.

RESULTS

72 women (mean age±SD: 36±4.06) were in the study. The participants' body mass indexes (BMIs) were 26.16±4.31. Although many participants with PCOS had symptoms of IBS based on Roma IV criteria, no significant relationship was reported between the prevalence of IBS and PCOS. **Table 1** indicates the general participants characteristics in different IBS groups.

In the IBS-PCOS group, fasting insulin (FI), luteinizing hormone (LH) and Homeostasis model assessment for insulin resistance (HOMA-IR) values were significantly lower than in the non-IBS-PCOS group (p<0.05). Age in the IBS-PCOS group was higher than non-IBS-PCOS group (P=0.009). Also, the BMI of people in the IBS-PCOS group was significantly lower than people in the non-IBS-PCOS group (P=<0.001). HOMA-IR values were significantly different between IBS-control and non-IBS-control groups, and also fasting glucose (FG) and FI in the IBS-control group were significantly lower than non-IBS-control group (p<0.05) There was significant difference in terms of age and BMI between IBS-PCOS and IBS-control patients (p<0.05). **Table 1** shows the examination of the significant relationship of variables between the PCOS and control groups and their subgroups.

As stated in **Table 2**, a chi-square test found no statistically significant association between IBS-PCOS and non-IBS-PCOS in terms of gastrointestinal symptoms (p=0.685). Whether or not they have IBS, PCOS patients have similar GI symptoms. There was a statistically significant association between IBS-control and non-IBS-control in terms of GI symptoms (p=<0.001). The majority of the IBS-control women had not symptoms. In contrast, the functional dyspepsia are most common in non-IBS-control. **Table 2** shows the symptoms present in PCOS and control groups.

As stated in **Table 3**, a chi-square test found a statistically significant association between IBS-PCOS and GI symptoms (p=<0.001). While the majority of the women

with IBS-PCOS had functional dyspepsia, most of the IBS-CON had no symptoms. The prevalence of GI symptoms in women with IBS-PCOS was functional dyspepsia (55%), postprandial saturation (10%), epigastric pain (20%), early satiety (5%), and epigastric burning (10%). Data in **Table 2 and Table 3** are presented as numbers (percentages).

Figure 1 shows that the prevalence of symptoms is similar in both groups.

Table 1. The examination of the significant relationship of variables between the PCOS and control groups and their subgroups

Variable		PCOS patients (n=38)			Controls(n=34)			IBS-PCOS vs. IBS-CON(P)
		IBS-PCOS (n=20) Mean(SD) n(%)	non-IBS-PCOS (n=18) Mean(SD) n(%)	P	IBS-CON (n=17) Mean(SD) n(%)	non-IBS-Control (n=17) Mean(SD) n(%)	P	
Age		33.05(1.87)	29.94(3.74)	0.009⁑	28.23(3.75)	26.82(3.86)	0.288*	<0.001⁑
BMI		25.85(2.03)	31.37(4.41)	<0.001*	23.42(2.46)	23.77(2.52)	0.693*	<0.001*
FG		91.1(8.19)	94.94(7.59)	0.142*	86.70(5.82)	97.41(7.96)	<0.001*	0.066*
FI		6.23(1.89)	15.56(6.35)	<0.001⁑	7.22(1.39)	12.72(3.15)	<0.001*	0.076*
FSH		6.15(1.88)	6.16(1.06)	0.974*	5.68(1.77)	6.30(1.79)	0.319*	0.444*
LH		4.78(2.02)	10.02(4.25)	<0.001*	4.84(1.46)	5.71(1.91)	0.306⁑	0.917*
Prolactin		15.83(5.83)	16.18(5.61)	0.854*	16.16(4.90)	16.87(6.76)	0.731*	0.851*
TSH		1.92(0.68)	2.02(1.24)	0.534⁑	1.83(0.94)	2.23(1.34)	0.413⁑	0.413⁑
E2		42.85(9.30)	41.05(16.44)	0.264⁑	45.29(20.81)	35.27(12.61)	0.182⁑	0.639*
HOMA-IR		1.38(0.36)	3.81(1.60)	<0.001⁑	1.54(0.27)	3.26(0.94)	<0.001⁑	0.159*
Infertility type	1	16(80)	15(83)	0.791†	12(70)	16(94)	0.072†	0.506†
	2	4(20)	3(17)		5(30)	1(6)		
Infertility duration	1	0(0)	0(0)	0.911†	0(0)	1(5)	0.511†	0.863†
	2	18(90)	16(89)		15(88)	13(76)		
	3	2(10)	2(11)		2(12)	3(17)		
Cigarette	1	12(60)	10(55)	0.782†	12(70)	15(88)	0.203†	0.501†
	2	8(40)	8(45)		5(30)	2(12)		

†Chi-Squared test ⁑Mann-Whitney U test *Independent Sample t-test, BMI , body mass index ; TSH, thyroid-stimulating hormone; LH, luteinizing hormone; FI, Fasting Insulin; E2, estradiol; HOMA-IR, homeostasis model assessment for insulin resistance; FSH, follicle-stimulating hormone; FG, Fasting Glucose; IBS-PCOS, polycystic ovary syndrome patients with irritable bowel syndrome; IBS-CON, controls with irritable bowel syndrome; non-IBS-PCOS, polycystic ovary syndrome patients without irritable bowel syndrome; non-IBS-CON, controls without irritable bowel syndrome.

Table 2. The symptoms present in PCOS and control groups

Variable	PCOS patients (n=38)			Controls(n=34)		
	IBS-PCOS (n=20)	non-IBS-PCOS (n=18)	P	IBS-Control (n=17)	non-IBS-Control (n=17)	P
Gastrointestinal Symptoms			0.685			<0.001*
No Symptoms	0(0)	0(0)		14(82.4)	3(17.6)	
Functional Dyspepsia	11(55)	12(66.7)		2(11.7)	14(82.4)	
Postprandial Saturation	2(10)	3(16.7)		1(5.8)	0(0)	
Epigastric Burning	2(10)	1(5.5)		0(0)	0(0)	
Epigastric Pain	4(20)	1(5.5)		0(0)	0(0)	
Early Satiety	1(5)	1(5.5)		0(0)	0(0)	

*A chi-square test

Table 3. The symptoms present in IBS-PCOS and IBS-CON

Symptoms	IBS-PCOS (n=20) n(%)	IBS-CON (n=17) n(%)	P-value
No Symptoms	0(0)	14(82.3%)	<0.001*
Functional Dyspepsia	11(55%)	2(11.7%)	
Postprandial Saturation	2(10%)	1(5.8%)	
Early Satiety	1(5%)	0(0)	
Epigastric Pain	4(20%)	0(0)	
Epigastric Burning	2(10%)	0(0)	

*A chi-square test

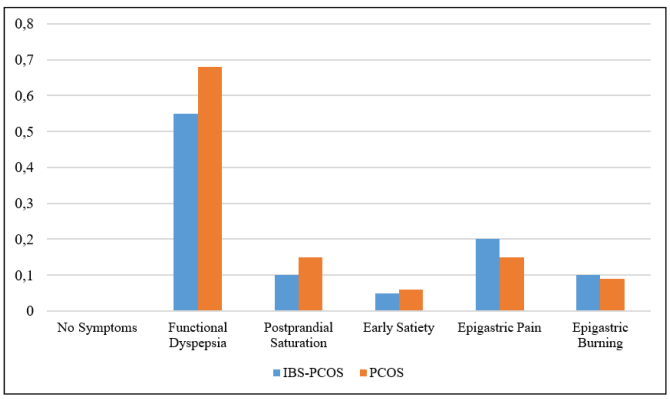

Figure 1. Prevalence of symptoms in IBS-PCOS and PCOS groups

DISCUSSION

Based on the present study's findings, IBS has a high prevalence, and in this regard, the group of PCOS patients is not significantly different from the control group. Among PCOS and control patients, IBS-C was the IBS most common type. Age was an important risk factor for co-occurrence of IBS and PCOS.

A very high IBS prevalence was observed in the case and control groups in this study. IBS prevalence is 52% in the PCOS group, 50% in the control group, and approximately 50% in the general group. Niemyjska et al. (20) reported a prevalence of IBS symptoms based on the diagnosis criterion of Roma III in Polish women at 40%. Akbayram et al. (21) reported a general prevalence of 16.2% among students in Turkey. In Turkey, as in other countries, the prevalence is higher among women than men (22-24). Oka et al. (8) has reviewed dozens of studies on the IBS prevalence and reported the prevalence of the disease based on the criterion of diagnosis of Rome III, 9.3%, and based on Roma IV, 3.8% prevalence was reported. All studies have emphasized the higher IBS prevalence in women than men (8). The general prevalence of IBS in the present study is higher than in other studies, which may be related to the number of samples or differences in the diagnostic criteria.

The IBS prevalence in the PCOS and control groups is approximately 50% in this study, and there is no statistically significant difference. Mathur et al. (17) reported IBS prevalence in PCOS patients of 41.7% versus 10.3% of healthy individuals. Bazarganipour et al. (25) reported a 29.7% prevalence in PCOS patients versus an 11% prevalence in healthy individuals. In these two studies, Roma I and III diagnostic criteria were used, respectively, and a significant difference was reported between the prevalence of IBS in the two groups. Kałużna et al. (26) reported the IBS prevalence based on the criterion of Roma IV diagnosis in 24% of the PCOS group and 21% in the control group and did not report a significant relationship between the IBS prevalence and PCOS disease. The present study results show no significant difference between the IBS prevalence in the PCOS and control groups, although further studies with the presence of young women with PCOS with a high number of samples are recommended.

Among PCOS and control patients, IBS-C was the most prevalent type of IBS. The IBS-C type had the highest prevalence in the present study, and IBS-M had the lowest prevalence in PCOS and control women. Half of the women in both groups had IBS-C, which is consistent with Kim et al. (12), reported a prevalence of 40% in subjects. Bazarganipour et al. (25) reported the highest prevalence of IBS-C type in PCOS group 70% and control 45%. Also, Galica et al. (27) reported the prevalence of IBS as follows IBS-C (58%), IBS-D (28%) and IBS-M (14%). According to research done by Palsson et al. (28) the distribution of IBS types in different studies significantly affects the identification method. The use of the Roma III or Roma IV method can significantly change the results related to the distribution of IBS types(28). To more accurately measure the IBS prevalence subtypes in PCOS patients, it is recommended that future studies be conducted with larger populations of different ethnicities.

In the present study, the mean BMI in the IBS-PCOS group was significantly lower than in the non-IBS-PCOS group. Thus, the hypothesis of an adverse effect of obesity on the development of IBS in PCOS women is rejected. However, in the IBS-PCOS group, the average BMI is higher than in the IBS-CON group because overweight PCOS patients is very common. Mathur et al. (17) reported the highest average BMI in the IBS-PCOS group. Sadik et al. (29) considers high BMI to be effective in aggravating IBS symptoms. In contrast, Kałużna et al. (26) and Bazarganipour et al. (25) did not show a significant relationship between BMI and IBS occurrence in PCOS patients. More studies are needed on the relationship between IBS occurrence in obese PCOS patients.

Patients with IBS-PCOS were older than non-IBS-PCOS. IBS-PCOS patients were also older than IBS-control. Accordingly, older women were more prone to getting PCOS and IBS simultaneously. FI, LH, and HOMA-IR levels were higher in IBS-PCOS than in non-IBS-PCOS. FG, FI, and HOMA-IR levels were also higher in IBS-control than in non-IBS-control.

CONCLUSION

Although many PCOS patients were diagnosed with IBS according to Roma IV criteria, there was no significant relationship between PCOS and the IBS prevalence in the study population. The prevalence of GI symptoms is similar in both groups (IBS-PCOS and PCOS only). Aging increases the risk of co-occurring with IBS and PCOS, but more research is required on the effect of obesity on this issue.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Beykoz University Clinical Research Ethics Committee (Date: 26.04.2021, Decision No: 1)

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

Financial Disclosure: The author declared that this study has received no financial support.

Author Contributions: The author declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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Minimally invasive plate osteosynthesis for segmental humerus fractures with a helical plate. Which distal fixation—the anterior or lateral—is superior?

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ABSTRACT

Aim: In order to achieve adequate stability in segmental humerus fractures, the PHILOS fixation with minimal invasive approach comes into use instead of conventional plating. However, according to the AO classification, 12C type segmental humerus fractures treated with minimally an invasive method are prone to complications. The purpose of this prospective study is to compare functional outcomes and complication rates following two different angled helical PHILOS plate fixation.

Material and Method: This multicenter study is a prospective review of cases with a final follow-up outcome. Twenty-two patients with AO 12-C humerus fractures underwent PHILOS fixation with contoured PHILOS plates between January 2016 and June 2019. Patients evaluated in two groups. Group 1 consisted 12 patients who were treated with a 30° helical plate and Group 2 consisted 10 patients who were treated with 70° helical plate. Clinical outcomes were noted according to the Constant-Murley scoring system.

Results: The mean age of patients treated in groups 1 and 2 were 49±15.8 and 50.7±17, respectively. Fractures healed in an average of 13.1±3.9 weeks in Group 1 and 13.8±3.1week in Group 2, respectively. The mean follow-up period of the patients was 18±6.1months in Group 1 and 22±4.2 months in Group 2. Mean Constant-Murley scores at final follow-up were 88±2.7 and 90±2.5 in Groups 1 and 2 respectively (p=.665). Radial nerve neuropraxia was seen in 2 cases in Group 1, and a sensorial injury of the musculocutaneous nerve was seen in 1 patient in Group 2 (p=.365).

Conclusion: Similar union rates and successful clinical results were obtained from both groups. However, this study suggests that the 70° angled helical PHILOS technique could be performed relatively easily in AO 12-C fractures with fewer complication rates. Musculocutaneous nerve affliction can be as functionally destructive as radial nerve affliction.

Keywords: Humerus shaft fracture, radial nerve palsy, minimally invasive plate osteosynthesis, safe zone, pre-contoured plate

INTRODUCTION

Although segmental fractures of the humeral diaphysis are generally seen in older age groups, they are occur in young individuals who sustain high-velocity injuries (1,2). Decision making for the treatment of segmental humerus fractures extending to proximal or distal 1/3 diaphysis varies depending on patient age and comorbidities (3). The choice of implant in patients requiring surgical treatment is an important factor that determines the success rate. Because of the anisotropic morphology of the humerus, intramedullary and extramedullary differences between the proximal and distal parts were decided as factors that complicate plate osteosynthesis (4). Plate osteosynthesis was found to require safe incisions and less invasive techniques because of the neurovascular structures of the arm that contains potential risks at every level (5).

Anatomical structures in the proximal, middle and distal zones should be known and preserved during surgery with the MIPO technique (6). The main neural structures located in the path of the implant are the axillary nerve, radial nerve, musculocutaneous and lateral cutaneous nerves of the forearm (6,7). While the radial nerve is likely to be damaged, especially in lateral and posterior approaches, the risk was found to be less in anterior plating (2). However, fixation of proximal and distal fractures is not possible with the anterior opening

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MIPO technique. For this purpose, it has been reported in a limited number of studies that extended segment fractures could be treated by using the anatomical PHILOS plate (2,8,9). Hence, it is possible to protect the deltoid insertion, to use the distal anterior surface for fixation of the distal screws, and to move away from the radial nerve zone by applying PHILOS plates that are helically shaped manually.

PHILOS plates provide excellent fixation for proximal fractures. The extension to the distal region provides a bridge plate formation in diaphyseal fractures (10,11). However, the angular difference in the sagittal plane between the humeral head and its distal is approximately 30 degrees. This particular point makes it difficult to manage treatment with conventional plates (12). On the other hand, the radial nerve in the middle third and distal diaphysis is frequently in danger (13).

In the present study, the treatment of Arbeitsgemeinschaft für Osteosynthesefragen (AO) 12-C type humeral fractures extending from the proximal humerus to distal diaphysis with 30° and 70° contoured PHILOS plates was compared. Additionally, clinical and radiological results of distal lateral and anterior fixation in segmental fractures detected by the bridging MIPO technique were compared.

Our hypothesis was that the 70° contoured PHILOS plate might be more advantageous as compared to the 30° contoured plate regarding the treatment of AO 12-C fractures extending from the proximal humerus to distal diaphysis. Contoured plate fixation can be performed as safely as a standard lateral fixation.

MATERIAL AND METHOD

The study was carried out with the permission of Süleyman Demirel University/Training and Research Hospital, Clinical Researches Ethics Committee (Date: 25.02.2022, Decision No: 5/65). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants who participated in this study.

Patients were operated on by 2 senior trauma surgeons in two trauma centers with the same method. Twenty-two patients with segmental humerus fractures, specifically AO 12-C type fractures according to AO/OTA classification, treated with two different types of helical plates between January 2016 and June 2019 were included. AO 12-C type fractures were included in the study according to the AO/OTA classification. Patients (>18 years) with closed segmental humeral fractures admitted to the emergency department were included in the study. The demographic data of the patients are summarized in **Table 1**. All cases were examined in the emergency department and immobilized with a temporary 'u' splint until surgery. All cases were treated with the long PHILOS plate in accordance with the MIPO technique in the beach chair position under general anesthesia. While distal lateral locking was performed in 12 of the cases with a 30° contoured plate (Group 1), distal anterior locking was performed in 10 cases with a 70° helical plate (Group 2).

Table 1. Preoperative and postoperative patient demographics

	30° Encountered Group 1		70° Encountered Group 2		P values
	N	Mean, SD	N	Mean, SD	P<0,05
Age	12	49±15.8	10	50.7±17	.811
Side Involvement	13	R:7 L: 6	10	R: 4 L: 6	-
Follow-up (mo.)	12	18±6.1	10	22±4.2	.335
AO/OTA Classification	12	AO12C2-C3	10	AO12C2-C3	-
Fluoroscopy time (sec)	13	21.9±6.7	10	21.5±7.2	.890
Surgery time (min)	12	46±7.5	10	52±9.1	.107
Polytrauma	4/12	11.6%	2/13	7.7%	-
Average hospitalization (days)	12	9.1±4.7	10	4.8±1.7	.261

Open fractures, pathologic fractures, pseudoarthrosis, periprosthetic fractures, neglected fractures, short oblique/ transverse fractures that can be fixed with intramedullary nail or conventional plates were excluded from this study. Patients who admitted with nerve involvement were excluded from the study. In all cases, the MIPO technique was applied by utilizing proximally deltopectoral and distally anterior or lateral approaches. All cases were followed up using anteroposterior and lateral radiographs at the 1st, 2nd, 4th, 6th and 12th months to evaluate bone healing. In all patients, adequate fracture healing was documented by both X-ray and satisfactory clinical evaluation at follow-up. Cases resulted from 12 motor vehicle accidents, 6 industrial accidents and 4 were reported as falls from a height and all were operated on with in the first week of the initial injury. All patients were mobilized after surgery with an arm sling and range of motion exercises were started at the 2nd week post-operatively. A dynamic dorsal wrist splint was applied to patients with radial neuropraxia (9.09%, n=2).

Patients were excluded from the close follow-up period after fractures healed and were asked for a final check after 1-year or more. There was no loss of follow-up. Trabecular continuity and an absence of pain at the fracture site was considered to be a union of the fracture. Functional outcomes were assessed according to Constant-Murley scores.

Surgical Technique

All patients were placed in the beach-chair position under general anesthesia. First, a proximal incision was made with a minimal deltopectoral approach, making a 5- to 6-cm proximal incision approximately 4 cm distal to the anterior portion of the acromion process and exposure between the deltoid and pectoral muscles. The anterior one third of deltoid insertion was subperiosteally elevated from the insertion to prepare lateral cleavage for the long PHILOS plate. For the distal incision, the length of the plate was measured and 3 distal screw holes were marked roughly before plate placement in both groups.

A sterile humerus sawbones model was used for an ideal plate twisting under surgery conditions. Approximate degree of 30 and 70 was determined with goniometer. Distal and proximal end points of the plate were marked and angle between these two planes were measured. In order to determine the correct implant length, an equal size comparison was made on the contralateral arm under fluoroscopy. In Group 1, a 5-cm incision was made in the skin on the lateral projection of the distal humerus. For the 30° contoured plate group, an intermuscular approach between brachialis and brachioradialis was preferred. Regardless of the length of the fractured segment, the radial nerve was identified and protected in all cases in Group 1 followed by placement of the distal. A long PHILOS plate was located submuscularly through the distal anterior or lateral humerus. Fractures were then reduced indirectly. The distal end of the plate was bent upwards and adapted to the lateral epicondyle anatomy for distal screw placement. In Group 1, a 30-degree inward contour was given to the plate manually in order to not cause rotation resulting in greater dominance of the supracondylar region, and thus, the double cortex was fixed distally. When the length of the humerus was approximately restored and both ends of the plate in the correct positions, guide pins were placed to the both ends of plate. The proximal and distal portions of the plate were fixed under the C-arm respectively (**Figure 1**).

For the 70° contoured helical PHILOS plate Group, a 5-cm skin incision was made on the anterior projection of the distal humerus. The brachial muscle was split into the medial and lateral portions, and the anterior aspect of the humerus exposed. The musculocutaneous nerve branches were not routinely identified and dissected. Distal and proximal screw fixation was made as described in Group 1. At least 3 bicortical screws were used for distal screw fixation in both groups. From the posterior tip of the acromion to the olecranon, the length/shortening of the limb was measured with a ruler and compared to the contralateral side (**Figure 2**).

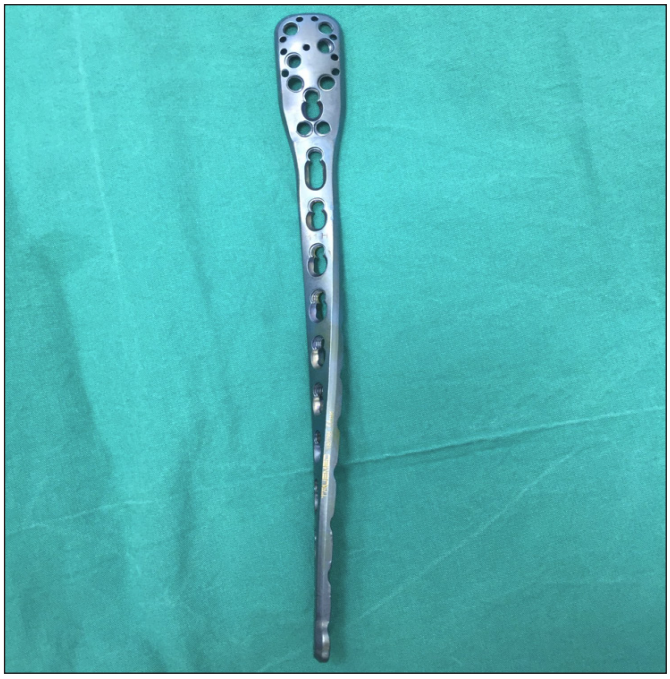

Figure 1. An image of 70-degree contoured PHILOS plate

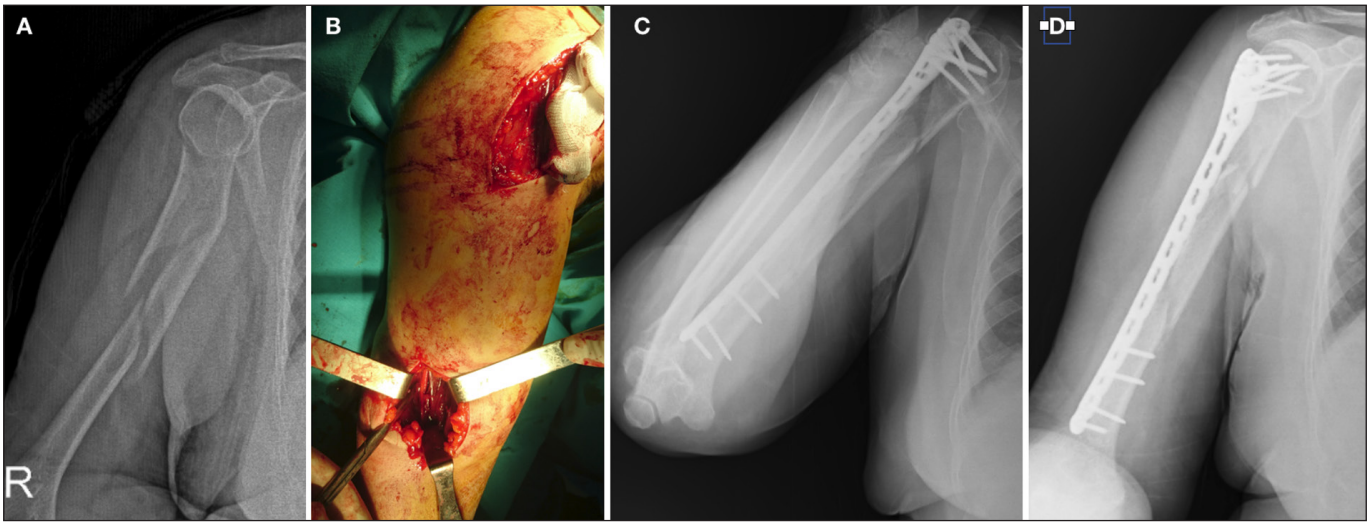

Figure 2. A 43-year-old male with AO 12-C type humerus fracture sustained in a traffic accident. (A) Anteroposterior plain radiograph of humerus fracture (B) Intraoperative image of the patient demonstrating MIPPO technique with incisions (C/D) Postoperative anteroposterior and lateral radiographs at 6th month follow-up.

Statistical Analysis

Statistical analyses were performed using SPSS version 23.0 software (IBM, Armonk, NY, USA). Comparisons of postoperative follow-up measurements and clinical outcomes were performed using a paired T-test and the analysis of variance and non-parametric Wilcoxon signed-rank tests. A post hoc power analysis, with an alpha error of 0.05, was conducted using SPSS (SPSS Inc, Chicago, IL). The observed power was .95% for all comparisons. A calculated effect size was found 0.76. The sample size planning showed an actual power of 0.951 with a total sample size of 20 patients. The nonparametric analysis of the two independent groups was compared using the Mann-Whitney U test. A value of $p<0.05$ was considered statistically significant.

RESULTS

The mean age of the patients included in the study was 49±15.8 (range 24 to 70) years in Group 1 and 50.7±17 (range 27 to 77) years in Group 2, respectively. One case in Group 1 was a bilateral humerus segmentary fracture, all other cases were unilateral humerus fractures. Mean surgery time was noted as 46±7.5 minutes in Group 1 and 52±9.2 minutes in Group 2 (p=.107). While the mean time to union was 13.2±3.9 weeks in Group 1, it was 13.8±3.1 weeks in Group 2. There was no statistical difference between the two methods in terms of union (p=.683). No intraoperative complications were encountered in any of the patients. The mean fluoroscopy time during surgery was 21.9±6.7 in Group 1 and 21.5±7.2 seconds in Group 2. The mean follow-up duration was

18±6.1 (range: 12-30) months in Group 1 and 22±4.2 (range 12-24) months in Group 2. A minimum of 3 and a maximum of 4 bicortical screws were used for distal fixation. Regardless of the length of the fractured segment, plate-screw insufficiency was not observed in any of the cases. The mean number of distal screws in both groups was 3.2 and 3.25 respectively. There was no significant difference between the 2 groups with respect to measured parameters (**Table 2**). Mean Constant-Murley scores were 88±2.7and 90±2.5 in Group 1 and Group 2 respectively at the final check-up. There was no statistically significant difference between the groups with regard to the Constant-Murley score.

Table 2. Clinical and radiological outcomes

Clinical and Radiological Outcomes	30° Encountered Group 1		70° Encountered Group 2		P values
	N	Mean, SD	N	Mean, SD	
Constant-Murley Scores	12	88.5±2.7	10	90±2.5	.665
Time to fracture union (w.)	12	13.1±3.9	10	13.8±3.1	.683
Follow-up	12	19.6±5.2	10	18±4.1	

SD: Standard deviation, w: week

Complications Implant related complications were seen in two cases. Plate related shoulder impingement was observed in 1 patient, and a 10° loss of extension at the elbow joint was observed in 1 patient from Group 1. In Group 1, temporary neuropraxia was observed in 2 cases. In these cases, symptoms related to nerve damage completely regressed within 6 months. The sensory bundle of the musculocutaneous nerve was affected in 1 case from Group 2 (**Figure 3**).

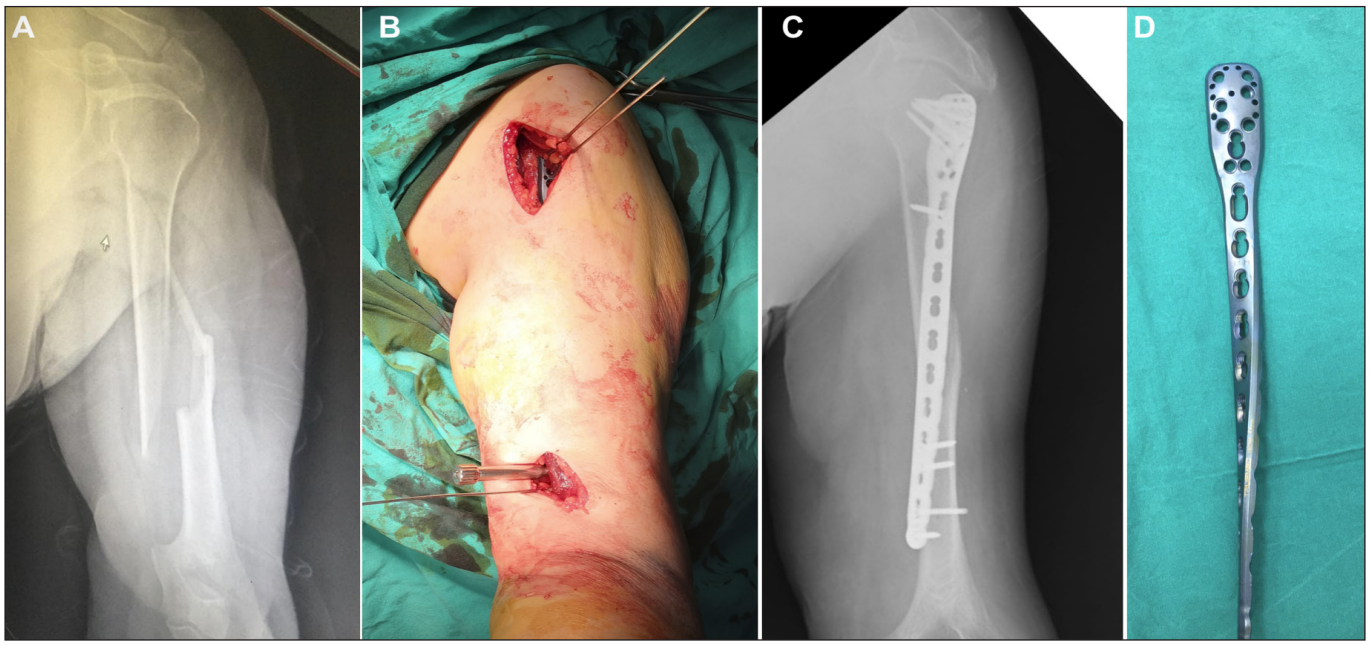

Figure 3. A 68-year-old female with AO type 12-C type humerus fracture. (A) Anteroposterior plain radiograph of humerus fracture (B) An intraoperative photo of the patient showing the both deltopectoral approach and distal- anterior incisions (C) Postoperative anteroposterior and lateral radiographs at 2nd month follow-up.

DISCUSSION

The present study reported the results of a series of segmental humerus fractures with proximal or distal extensions to the diaphysis treated with a minimally invasive long PHILOS plate. To minimize possible radial nerve damage and to adapt the PHILOS plate to the humeral anatomy, a helical plate model was created by contouring 30 and 70 degrees intra-operatively. To our knowledge, there is no study comparing anterior and lateral approaches for distal fixation of segmental humeral fractures treated using a helical PHILOS plate with different angles. Successful radiological and clinical results have been obtained with both methods. While nerve exposure is not required with helical plates angled at 70 degrees, it is essential to find and protect the radial nerve in 30° helical plates.

Due to high-energy trauma, multifocal fractures of the humerus were frequently seen in the elderly and young age groups, as well. The majority of these cases treated conservatively were reported to be associated with high rates of non-union, joint contracture or frozen shoulder (10). Surgical treatment was reported to produce good results for these types of fractures due to advantages such as allowing for early joint movement and a high rate of union (10). Although recent studies reported that plate fixation results are similar to the open or minimally invasive technique, minimally invasive techniques are becoming more popular (10).

Proximal humeral fractures have been treated with locking plates (PHILOS) since 2002. Rancan et al. (1) first reported the use of long PHILOS plates with a minimally invasive technique in metaphysiodiaphyseal humerus fractures. Opening could be achieved with a lateral split or deltopectoral approach in the proximal and a lateral mini-incision in the distal, but the radial nerve must be preserved. Although it was technically possible to find and protect the radial nerve, safe zones have been the most important subject of research due to the current risk. For this reason, many cadaveric studies have concluded that the anterior submuscular zone is safe (14,15). In the light of the available evidence, the idea of twisting and bending long PHILOS plates to conform to the humeral anatomy and to use them for fixation in metaphysiodiaphyseal fractures was first reported by Brunner et al. (16), in 2012. In the following years, a series of cases treated with the open or minimally invasive percutaneous osteosynthesis (MIPO) principle using long PHILOS or helical plates have been reported (2,6,8,12,13,17–22)

Conventional helical plate application was first introduced in 2005 by Yang et al. (23), however, the fixation was usually not possible in fractures extending to the proximal humerus with conventional helical plates (23). In these types of fractures, there may not be enough proximal fixation area for screw insertion. With the introduction of locking plates into clinical practice, it was predicted that angular stability could be achieved with a locking plate in proximal humerus fractures (24). Therefore, today long PHILOS plates are replacing conventional narrow plates. In 2014, 12 cases by Moon et al. (25), 46 cases by Wang et al. (15), in 2018, and 8 cases by Zamboni et al. (21), in 2019 a small number of cases were treated with the minimally invasive technique using the helical plate. The common aim of these cases was to create a safe submuscular tunnel between the distal and proximal humerus while bridging the fractured segment. The consensus of these studies was that less iatrogenic damage was noted with the MIPO technique using a helical plate (6,13,21).

Although there are case studies in which iatrogenic radial nerve damage was never seen in patients who underwent bridge plating with the MIPO technique, 3.4-4% radial nerve palsy and 3.4% nonunion complications were reported (6,10,14). On the other hand, the non-union rates with MIPO technique were reported to be lower than the functional brace (10,14). According to some meta-analyses, the MIPO technique did not have a clear advantage in terms of treatment time and union rates, but it resulted in lower complication rates and less iatrogenic radial damage when compared to ORIF (12,26). In our study, iatrogenic radial nerve injury (transient neuropraxia) was found in 2 cases in Group 1 (%16.6), and the sensory brunch of the musculocutaneous nerve was affected in 1 case in Group 2 (%10). These neural complications very likely occurred due to traction to the nerve during fixation of the plate distally. A deltopectoral approach was performed for proximal fixation and no axillary nerve damage was seen in any of cases.

There are some studies showing short-term results of humeral shaft fractures treated with the MIPO technique in the literature. In these studies, Constant-Murley scores have been reported between 76 to 88.6 (6,8,13,20,27). Clinical results of our study showed similar outcomes with the literature. In addition to the current research, there was no difference in clinical outcomes and union rates between distal anterior or lateral locking approaches (p=.665). The studies which addressed the MIPO technique for the treatment of humerus fractures reported between 13.2 to 17.9 weeks for the time of union (8,13,18). In our study, the mean time to radiological union was 13.2 in Group 1, and 13.8 weeks in Group 2. No superiority was observed between the distal anterior or lateral fixation methods over the duration of fracture union (p=.683).

Perioperative helical shaping of long plates may cause loss of strength and deterioration of locking screw holes

(8,12,23). No implant failure was found in our study, which confirms this prediction. Attention was paid especially to the twisting of the plate more proximal by preserving the last three screw holes in the distal.

The limitations of our study include the small number of patients (n=22) and short follow-up time, limiting advanced conclusions on rare complications and short-term outcomes. Even though segmentary humerus fractures are seen in individuals of all ages, including the elderly, examples tended to be from older individuals. Older specimens are more likely to be osteopenic, which this would more likely affect healing time and rehabilitation negatively.

CONCLUSION

Our prospective, randomized study suggests that the anterior or lateral distal fixation techniques in the treatment of AO 12-C type segmental humerus fracture provides good outcome. In order to minimize possible iatrogenic complications, contoured long PHILOS plate might be considered as a rational approach, but due to the anisometric structure of the humerus, a safe zone could not be precisely defined.

Depending on the direction in which the fracture line extends to the distal humerus, lateral or anterior fixation could be preferred. Considering the length of the fractured segment, fixation with at least three bicortical screws seems sufficient at the distal end of the plate for relative stability. This study concluded that intra-operative twisting of long PHILOS plates applied to AO 12-C type humerus fractures could be considered as a safe and effective surgical option with good radiographic and clinical outcomes and low complication rates.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Süleyman Demirel University/ Training and Research Hospital, Clinical Researches Ethics Committee (Date: 25.02.2022, Decision No: 5/65).

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

Referee Evaluation Process: Externally peer-reviewed.

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

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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Original Article

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Evaluation of the correlation of serum calcium, phosphorus levels and calcium phosphorus product with disease severity and ICU mortality in SARS-COV-2 pneumonia patients followed up in ICU

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ABSTRACT

Background: Calcium and phosphorus are important elements in the body that have been shown to decrease in critical inflammatory diseases. The aim of this study was to evaluate serum levels of calcium and phosphorus and the calcium phosphate product (CPP) in patients followed up in intensive care unit (ICU) due to hypoxemic respiratory failure caused by coronavirus disease 2019 (COVID-19) pneumonia. The secondary endpoint of the study were respiratory support therapies used in the evaluation of independent mortality and disease severity in ICU that were divided into four groups depending on the time of administration: (i) first 24 hours, (ii) 48-72 hours, (iii) 72 hours, and (iv) 72 hours-28 days.

Material and Method: The retrospective study included patients with critical and severe COVID-19 pneumonia followed up in ICU.

Results: The study included 369 patients with a mean age of 64.3±14.8 years. ICU mortality was observed in 142 (38.5%) patients, among whom 17 (4.6%) patients died within 24 hours, 28 (7.6%) died between 48-72 hours, 50 (12.7%) died within 72 hours, and 47 (12.7%) died between 72 hours and 28 days. Serum calcium level established a significant relationship with ICU mortality at 28 days and 72 hours ($p<0.05$). Serum phosphorus and calcium levels were not found as significant predictors of CPP ($p>0.05$).

Conclusion: Serial assessment of serum calcium may be a new criterion in the prediction of independent mortality in critical and severe COVID-19 pneumonia, which has been recently identified and has numerous unknown features.

Keywords: Mortality, calcium, phosphorus

INTRODUCTION

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was renamed as coronavirus disease 2019 (COVID-19) and was declared a pandemic by the World Health Organization (WHO) in 2020. This disease primarily affects the respiratory system and causes acute respiratory distress syndrome (ARDS). Clinical manifestations of COVID-19 comprise four categories: mild, moderate, severe and critical. In COVID-19 infection, laboratory abnormalities can be observed as a result of cytokine storm caused by exaggerated systemic inflammation, particularly in severe and critical cases (1-3).

Calcium and phosphorus are critical electrolytes found in the body. Calcium plays a role in neurotransmitter release, cardiac automaticity, skeletal, vascular, and smooth muscle function as well as blood coagulation, cell persistence, and functional activity of numerous enzymes (4). In a previous study conducted with COVID-19 patients, calcium alterations were associated with a poor prognosis in 70% of the patients (5). A study by Zhou et al. (6) evaluated 127 COVID-19 patients and found a significant relationship between calcium alterations and elevated inflammatory cytokines. The authors suggested that calcium alterations might be a novel and important indicator of mild, moderate, and severe COVID-19 (6). Phosphorusis an essential element playing an important role in intracellular oxygen delivery, immune system, acid-base balance, and coagulation cascade. Hypophosphatemia has been associated with respiratory failure as well as increased mortality and morbidity in

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critical diseases (7,8). In a previous study conducted with COVID-19, low calcium and phosphorus levels were found to be correlated with the disease severity (9).

Since COVID-19 is a recently identified disease, to our knowledge, there have been few studies on the relationship between serum calcium and phosphorus levels and CPP and the severity of COVID-19 and mortality. The aim of this study was to evaluate serum calcium and phosphorus levels and CPP in patients followed up in ICU due to hypoxemic respiratory failure caused by COVID-19 pneumonia. The secondary endpoint of the study were respiratory support therapies used in the evaluation of independent mortality and disease severity in ICU that were divided into four groups: (i) first 24 hours, (ii) 48-72 hours, (iii) 72 hours, and (iv) 72 hours-28 days.

MATERIAL AND METHOD

The study was carried out with the permission of Atatürk Sanatoryum Training and Research Hospital Clinical Researches Ethics Committee (Date: 12.04.2022, Decision No: 2012-KAEK-15/2502). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

The retrospective study reviewed medical records of patients that were hospitalized due to pneumonia and had a positive SARS-CoV-2 result on polymerase chain reaction (PCR) between March 2020 and May 2021. Patients that were hospitalized in ICU according to the Infectious Disease Society of America (IDSA) and American Thoracic Society (ATS) guidelines and had radiological infiltrations of >50%, tachypnea (respiratory rate >30/min), and a ratio of arterial oxygen partial pressure (PaO_2) to fractional inspired oxygen (FiO_2) (PaO_2/FiO_2) of <300 mmHg were included in the study (10). Laboratory parameters that were assessed within the first hour of ICU admission, including calcium, phosphorus, magnesium, vitamin D, albumin, ferritin, neutrophil count (NE), lymphocyte count (LYM), white blood cell count (WBC), D-dimer, C-reactive protein (CRP), and procalcitonin (PCT) were analyzed for each patient. The albumin-adjusted calcium level was calculated retrospectively, using the following formula: Albumin-adjusted calcium=total calcium + [0.9x(4-albumin)] (11). The albumin-adjusted calcium level was multiplied by the simultaneous phosphorus level (calcium x phosphorus). Respiratory support therapies administered within the first hour of ICU admission were classified as invasive mechanical ventilation (IMV), noninvasive mechanical ventilation (NIMV), high-flow nasal oxygenation (HFNO), and non-rebreather mask (NRB) therapy. Twenty-eight-day ICU mortality was divided into four groups depending on the time of death: (i) first 24 hours, (ii) 48-72 hours, (iii) 72 hours, and (iv) 72 hours-28 days. The Acute Physiology and Chronic Health Evaluation II (APACHE II) and the Sequential Organ Failure Assessment (SOFA) scores were used to predict mortality and prognosis within the first 24 hours of ICU admission (12). Pregnant women, individuals under 18 years of age, and patients with thyroid dysfunction, history of thyroid and parathyroid surgery, drug use within the last month that might affect serum levels of calcium, phosphorus, and vitamin D, history of collagen tissue and, an active infection other than SARS-CoV-2, and a history of dialysis were excluded from the study. Moreover, patients that had a creatinine value of >1.44 mg/dL due to a 0.3 mg/dL increase of creatinine within 48 hours prior to ICU admission due to SARS-CoV-2 pneumonia according to the Kidney Disease: Improving Global Outcomes (KDIGO) guidelines were also excluded from the study. Electrolyte replacement therapy was administered within the first hour of ICU admission accordance to laboratory parameters.

Normal reference ranges for calcium, phosphorus, albumin, magnesium, vitamin D, ferritin, NE, LYM, WBC, D-dimer, CRP, and creatinine were accepted as 8.8-10.6 mg/dL, 2.5-4.5 mg/dL, 3.5-5 mg/L, 1.9-2.5 mg/dL, 30-100 ng/ml, 22-275 ng/ml, 2-6.9×10^3 µL, 0.6-3.4×10^3 µL, 4.6-10.2×10^3 µL, 0-0.44 mg/L, 0-5 mg/L, and 0.81-1.44 mg/dL, respectively. A PCT level of <0.5 ng/ml was considered to indicate a low risk and a level of >2 ng/ml was considered to indicate a high risk. WBC, NE, and LYM were measured using a photometric analyzer (Mindary BC-6800 device), vitamin D level was measured using an ADVIA Chemistry XPT analyzer (Siemens, München, Germany), PCT immunoassay and the assessment of ferritin level were performed using an ADVIA Centaur XPT analyzer (Siemens, München, Germany), CRP, albumin, calcium, phosphorus, magnesium, and creatinine levels were measured using a Beckman Coulter hematology autoanalyzer (Beckman Coulter, USA), and D-dimer level was measured using a Sysmex autoanalyzer (Sysmex, Kobe, Japan) with the turbidimetric method.

Statistical Analysis

Data were analyzed using SPSS for Windows version 23.0 (Armonk, NY: IBM Corp.).Categorical variables were expressed as frequencies (n) and percentages (%) and continuous variables were expressed as mean, standard deviation (SD), and minimum-maximum. The conformity of variables to normal distribution was assessed using the Kolmogorov-Smirnov and Shapiro-Wilk tests. inary variables that did not conform to normal distribution were compared using Mann-Whitney U test. Correlations between continuous variables were assessed using Spearman's Correlation Coefficient. Logistic regression analysis was performed to determine the independent variables affecting mortality. A p value of <0.05 was considered significant.

RESULTS

Table 1 presents demographic and clinical characteristics of the patients. A total of 369 patients with a mean age of 64.3±14.8 years (223 men [60.4%] and 146 women [39.6%]) were included in the study. ICU mortality occurred in 142 (38.5%) patients, among whom 17 (4.6%) patients died within 24 hours, 28 (7.6%) died between 48-72 hours, 50 (12.7%) died within 72 hours, and 47 (12.7%) died between 72 hours and 28 days. In ICU, IMV was administered in 242 (65.6%), NIMV was administered in 82 (22.0%), HFNO was administered in 192 (52.0%), and NRB was administered in 86 (23.3%) patients. **Table 2** shows the mean values of laboratory parameters measured within the first hour of ICU admission. A positive correlation was found between calcium level and LYM, between phosphorus and magnesium, and between albumin and LYM (p<0.05 for all).

Table 1. Demographic and clinical characteristics

Variables	Frequency (N)	Percent (%)
Gender		
Male	223	60.4
Female	146	39.6
ICU mortality	142	38.5
24 hours ICU mortality	17	4.6
48-72 hours ICU mortality	28	7.6
72-hour ICU mortality	50	13.6
72 hours - 28 Days ICU mortality	47	12.7
PaO_2/FiO_2		
<100 mmHg	19	5.1
<150 mmHg	21	5.7
<200 mmHg	40	10.8
<250 mmHg	47	12.7
<300 mmHg	242	65.6
Comorbidities	213	57.7
HT	139	37.7
CAD	68	18.4
CHF	20	5.4
DM	82	22.2
Neurological	6	1.6
COPD	49	13.3
Bronchial asthma	10	2.7
Malignancy	16	4.3
CKF	2	0.5
Variables	**Mean±SD**	**Median (Min-Max)**
Age (years)	64.3±14.8	66(19-94)
APACHE II	21.2±6.2	20(11-38)
SOFA	6.2±3.45	5(2-30)

ICU: Intensive care unit, PaO_2/FiO_2: Ratio of the partial pressure of oxygen in arterial blood (PaO_2) to fractional inspired oxygen (FiO_2) HT: Hypertension, CAD: Coronary artery disease, CHF: Congestive heart failure, DM: Diabetes mellitus, CKF: Chronic kidney failure, APACHE II: Acute Physiology and Chronic Health Evaluation II score, SOFA: Sequential Organ Failure Assessment Score

Although there was a significant relationship between ICU mortality and calcium level(p=0.015),no significant relationship was found between serum phosphorus level and CPP (p=0.401 and p=0.144, respectively). Mortality within the first 24 hours, 48-72 hours, and 72 hours-28 days established no significant relationship with serum phosphorus and calcium levels and CPP (p=0.531, p=0.822, p=0.728, p=0.362, p=0.418, p=0.157, p=0.383, p=0.626, and p=0.321, respectively). In contrast, a significant relationship was found between 72-hour mortality and calcium level(p=0.012) and no significant relationship was found between phosphorus level and CPP (p=0.456 and p=0.841, respectively). Similarly, no significant relationship was found between serum calcium and phosphorus level and CPP and the administration of IMV, NIMV, HFNO, and NRB in ICU (p>0.05 for all) (**Table 3**). When factors affecting mortality were evaluated in multiple regression analysis, the risk of mortality was 1.23-fold greater in patients with a higher APACHE II score (OR:1.230;95% CI, 1.083-1.396, p=0.001) and was 1.001-fold greater in patients with a higher ferritin level (OR:1.001;95%CI,1.00-1.001, p=0.017).

Table 2. Laboratory parameters

Variables	Mean±SD	Med (Min-Max)
Phosphorus (mg/dL)	2.9±1.19	2.7 (0.8-11.6)
Magnesium (mg/dL)	2.15±0.53	2.1 (1.3-8.9)
Albumin (mg/L)	3.09±0.67	3 (1-4.9)
Calcium (mg/dL)	9.44±4.3	9.2 (6.49-90.8)
CPP	12.3±4.4	11.9 (9.4-92.4)
Vitamin D (ng/ml)	17.89±12.91	14.35 (1.73-79.36)
Ferritin (ng/ml)	655.9±551.6	482.7 (7.3-1650)
Neutrophil ($\times10^3\mu L$)	8.8±5.46	8.03 (1.13-34.87)
Lymphocyte ($\times10^3\mu L$)	1.05±1.14	0.81 (0.05-16.49)
C-Reactive protein(mg/L)	118.3±98.6	104.1 (0.37-562.9)
Procalcitonin (ng/ml)	1.43±4.53	0.12 (0.01-41.4)
D-Dimer (mg/L)	8.47±72.01	1.11 (0.19-1366)
White blood cell (WBC) count ($\times10^3$ µL)	10.3±5.6	9.43 (2.3-35.93)

CPP: Calcium phosphate product, SD: Standard deviation, The normal reference rangesof calcium, phosphorus, albumin, magnesium, vitamin D, ferritin, NE, LYM, WBC, D-dimer, CRP, and creatinine were accepted as 8.8-10.6 mg/dL, 2.5-4.5 mg/dL, 3.5-5 mg/L,1.9-2.5 mg/dL, 30-100 ng/ml, 22-275 ng/ml, 2-6.9×10^3 µL, 0.6-3.4×10^3 µL, 4.6-10.2×10^3 µL, 0-0.44 mg/L, 0-5 mg/L, and 0.81-1.44 mg/dL, respectively. PCT <0.5 ng/ml was considered to indicate a low risk, and >2 ng/ml was considered to indicatea high risk.

DISCUSSION

Clinical manifestations of COVID-19 comprise four categories: mild, moderate, severe and critical. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) enters the target cell by binding to the angiotensin-converting enzyme 2 (ACE2) molecule via the S protein. The ACE2 protein is released in large amounts in intestinal epithelial, renal tubular, alveolar, cardiac, and smooth muscle cells. It is also known that macrophage activation

Table 3. Relationship between calcium and phosphorus levels and CPPand ICU mortality

	Phosphorus		Calcium		CPP	
Variables	**Med (Min-Max)**	**p**	**Med (Min-Max)**	**p**	**Med (Min-Max)**	**p**
ICU Mortality						
No	2.7 (0.9-6.3)	0.401	9.24 (6.49-90.8)	0.015	11.96 (10.06-92.4)	0.144
Yes	2.6 (0.8-11.6)		9.12 (6.8-12.46)		11.71 (9.4-20.5)	
24-hr ICU Mortality						
No	2.7 (0.8-11.6)	0.531	9.2 (6.49-90.8)	0.822	11.9 (9.4-92.4)	0.728
Yes	2.5 (1.4-7.1)		9.1 (6.8-12.46)		11.7 (9.8-19.56)	
48-72 hour ICU Mortality						
No	2.7 (0.8-11.6)	0.362	9.2 (6.49-90.8)	0.418	11.92 (9.4-92.4)	0.157
Yes	2.3 (1.4-7.1)		9.15 (8.18-11.64)		11.27 (9.72-17.54)	
72-hour ICU Mortality						
No	2.7 (0.9-11.6)	0.456	9.23 (6.49-90.8)	0.012	11.9 (9.72-92.4)	0.841
Yes	2.8 (0.8-6.5)		9.1 (7.46-11.28)		11.96 (9.4-15.82)	
72 hours-28 days ICU Mortality						
No	2.7 (0.8-7.1)	0.383	9.2 (6.49-90.8)	0.626	11.92 (9.4-92.4)	0.321
Yes	2.7 (1.3-11.6)		9.18 (7.52-10.44)		11.74 (10.24-20.5)	

* p<0.05, Mann-Whitney U test, ICU: Intensive Care Unit, CPP: Calcium phosphate product

syndrome (MAS), which is characterized by cytokine storm due to exaggerated hyperinflammatory response, occurs particularly during severe COVID-19 infection. Additionally, MAS may also lead to cytokine storm due to systemic inflammation and involve multiple organs and systems. In such patients, laboratory abnormalities such as elevated D-dimer, CRP, ferritin and liver enzymes as well as lymphopenia, thrombocytopenia, and hypofibrinogenemia can be observed (2,13). In several studies, 28-day mortality rate in patients with COVID-19 pneumonia was reported as 40% (14). In our study, 28-day ICU mortality rate was 38.5%, which was consistent with the literature.

Cytokine storm in SARS-CoV-2 is known to decrease adenosine triphosphate (ATP) pools, thereby leading to an increased need for electrolytes, particularly including phosphate and magnesium, for ATP production. Studies have shown that low serum phosphorus and magnesium levels are associated with the severity of SARS-CoV-2. In turn, these low levels decrease the ATP production as well as the production of 2,3-bisphosphoglycerate that is required for oxygen release from hemoglobin as a result of hypophosphatemia, ultimately resulting in tissue hypoxemia. In addition, the clinical and laboratory findings in hypophosphatemia are characterized by thrombocytopenia, liver and kidney dysfunction, neurological impairment, rhabdomyolysis, respiratory and immune failure, and multiple organ failure, as in MAS (13). There are several studies recommending the monitoring of phosphorus, magnesium and vitamin D serum levels in the early stages of COVID-19 infection in the risky population and their replacement when necessary (13). Magnesium is known to improve vitamin D function in addition to its antihypertensive, antithrombotic, and bronchodilator effects (15). A previous study conducted with COVID-19 patients found that serum concentrations of sodium, potassium, and calcium decreased in the presence of severe infection. The exact mechanism of calcium dysregulation in COVID-19 infection remains unclear. Experimental studies have shown that the SARS-CoV-2 E gene encodes the protein for extracellular calcium ions to enter the cell and that serum calcium plays a vital role in a series of critical physiological functions, including calcium phosphate deposition, manipulation, neuron electrical signal transmission, hormonal regulation, and blood coagulation (16).

In our study, elevated CRP, PCT, and ferritin levels suggestive of MAS were detected in our patients, which could be due to the inclusion of severe and critical COVID-19 pneumonia patients in the study. However, the adjusted serum calcium, magnesium, phosphorus, and albumin levels measured on ICU admission were found to be within the normal range. In addition, in line with the literature, our patients were found to have vitamin deficiency (<20 ng/ml) (17). In our patients, serum samples were collected at the time of ICU admission, as performed in studies conducted with COVID-19 patients. Nevertheless, contrary to several studies in the literature, serum calcium, phosphorus, and magnesium values in our study were found to be within the normal range in our patients with pneumonia due to COVID-19 (9,17). On the other hand, simultaneous arterial blood gas levels, which could affect serum phosphorus and calcium levels, and ionized calcium and parathormone (PTH) levels, which are more determinant in the assessment of calcium level, could not be measured due to the retrospective nature of the study (18). Accordingly, we consider that the measurements of our calcium and phosphorus levels were affected.

To our knowledge, there are very few studies evaluating the relationship between serum calcium and phosphorus levels and the severity of the disease and mortality in patients with COVID-19, which is a recently identified disease with numerous unknown features. A study by Zhou et al. (6) evaluated 127 COVID-19 patients and found that low calcium levels were observed in the early stage of viral infection and that this decrease was more prominent in the early stage of the disease in severe/critical patients. The authors also found a significant relationship between interleukin 6 (IL-6), a proinflammatory cytokine, and calcium alterations in mild, moderate, and severe patients and suggested that the calcium level in the early stage of viral infection could be a biomarker of the severity of COVID-19 (16). In a retrospective study of 316 hospitalized COVID-19 patients by Torres et al. (19), hypocalcemia was associated with poor clinical outcomes such as NIMV and IMV requirement. In that study, the authors assessed serum calcium level within the first 72 hours. In another study, hypocalcemia was associated with higher rates of hospitalization, ICU admission, ventilation, and mortality (6). Similarly, a retrospective study conducted with patients hospitalized in ICU due to sepsis found that calcium replacement therapy reduced the 28- and 90-day mortality rates (20). In our study, serum calcium and phosphorus levels were evaluated together with CPP. It is known that an increase in CPP (70 mg/dL) causes an increase in the mortality rate in dialysis patients with chronic kidney failure (21). In our study, however, no significant relationship was found between phosphorus and CPP levels and mortality. Additionally, no comparison could be made with regard to the relationship between CPP and mortality since,to our knowledge, there is no study in the literature evaluating CPP in COVID-19 patients. On the other hand, the calcium levels in our study established a significant relationship with 28-day and 72-hour ICU mortality. These findings of our study were consistent with the findings of several studies in the literature, whereas our findings that were related to phosphorus level were inconsistent with the findings in the literature (6,19). This inconsistency could be attributed to the inclusion of patients who received medical treatment due to low calcium and phosphorus levels and to the non-administration of serial laboratory measurements in our study (20).

Our study was limited since it was a single-center and retrospective study. Moreover, only the data of severe and critical COVID-19 pneumonia patients followed up in ICU were evaluated and no data was available regarding the patients hospitalized in the general ward.

CONCLUSION

Our findings indicated that serial assessment of serum calcium levels could be a new criterion for the prediction of independent mortality in critical and severe patients with COVID-19 pneumonia, which is a recently identified disease with numerous unknown features. Multicenter, randomized and controlled studies are needed to substantiate our findings.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Atatürk Sanatoryum Training and Research Hospital Clinical Researches Ethics Committee (Date: 12.04.2022, Decision No: 2012-KAEK-15/2502).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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The length of distal skin incision of the postero-lateral approach affects the cup inclination during the total hip arthroplasty

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ABSTRACT

Aim: The primary aim of the study was to determine whether the length of the distal skin incision of the posterolateral approach affects the cup inclination during total hip arthroplasty (THA).

Material and Method: In this study, a cohort of 71 consecutive patients who performed between January 2017 and December 2021 with unilateral THA using a posterolateral approach was retrospectively assessed. Two groups were formed according to acetabular cup inclination with normal anteversion angle. There were 56 hips in the inside group and 17 in the outside group. A curvilinear skin incision of around 13 cm was performed. Component position evaluation was carried out through a radiographic assessment of the acetabular component on an anteroposterior pelvis radiograph. The rate of an outlier was compared between groups according to the safe zone defined as 30° to 50° of inclination and 5° to 25° of anteversion, which was described by Lewinnek et al.

Results: No significant difference in the average total incision length was found between the two groups (p=0.207). While the average distal incision length was 7.91±0.62 cm (range, 6.8-9 cm) in the inside group and 6.37±0.21 cm (range, 6-6.7 cm) in the outside group. According to ROC analysis, a patient with ≤6.7 cm of the distal length of incision (DLI) was 5.71 times more likely to be outside than a patient with >6.7 cm of DLI. Seventeen hips (23.3%) were found outside the safe range. Substantial differences were observed regarding radiographic cup inclination between the two groups (p=0.0001). In the inside group, the average cup inclination was 44.11°±3.44° (range, 37°-50°), whereas, in the outside group, it was 55.41°±2.5° (range, 52°-59°). However, there were no significant differences in the average radiographic cup anteversion between the two groups (p=0.960). Although 11 of 17 (64.5%) patients were classified as obese (BMI ≥30) in the outside group experienced higher rates of inaccurate cup orientation, logistic regression analysis showed that the individual effects of obesity on the occurrence of the inaccurate cup position were not observed (p=0.884). One posterior hip dislocation occurred after one month postoperative in the outside group.

Conclusions: Longer distal portion of the skin incision of the posterolateral approach should be performed to achieve optimal operative inclination angles of the acetabular cup during THA. The surgeon must have no hesitation in extending the distal skin incision when adopting the posterolateral approach.

Keywords: Total hip arthroplasty, posterior approach, distal length of incision, acetabular cup inclination, body mass index

INTRODUCTION

Primary total hip arthroplasty (THA) with a uncemented technique is considered one of the most successful orthopaedic surgeries, dependent on accurate acetabular cup orientation (1-3). There are many variables playing a role in THA failure, including patients' age, BMI, sex, comorbidities, soft tissue quality, surgical approach, surgeon experience, and malposition of the acetabular component (3,4). The single most significant variable is malposition of the acetabular cup during placing the acetabular component that has been associated with an early dislocation, reduced range of motion, edge loading, pelvic osteolysis, increased rates of polyethylene wear of the components, acetabular migration, impingement, leg length discrepancy, and patient dissatisfaction (5-8). To minimize these complications, a radiological "safe zone" for acetabular cup positioning after THA

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proposed by Lewinnek et al. (9) has been widely accepted as a safe range of the position of 40°±10°abduction and 15°±10° anteversion (10). Thus, acetabular component malposition, such as placing the acetabular component too vertically or too anteverted or retroverted, affects function and complications after THA, which is one of the most significant causes of revision hip surgery (6,11).

The accurate placement of the acetabular cup depends on the surgical approach (12). The most common surgical approach for THA is the posterolateral approach (13). Although the posterolateral approach is widely performed, some reports have revealed that the posterolateral approach may have a higher dislocation rate than the direct anterior approach (6,7). Kwon et al. (1) found a relationship between the increased risk of dislocation and the posterolateral approach since most dislocations are seen posteriorly. Furthermore, limited exposure to the area of the posterolateral approach at the time of surgery may lead to the risk of component malposition (6,14). Therefore, the optimal acetabular cup orientation is crucial to achieving good long-term results after THA, especially in the posterolateral approach (5,6).

The present study hypothesized that the length of the distal skin incision of the posterolateral approach affects the cup inclination during THA. To the best of our knowledge, no trial to date has investigated the possible effects of the length of the distal skin incision of the posterolateral approach on the cup inclination during THA. Hence, the primary purpose of this study was to investigate whether the length of the distal skin incision of the posterolateral approach affects the cup inclination during THA. Secondly, the secondary aim of the study was to determine whether BMI leads to inaccurate acetabular component position.

MATERIAL AND METHOD

The study was carried out with the permission of İstanbul Medipol University Clinical Researches Ethics Committee (Date: 04/02/2022, Decision No: E-10840098-772.02-753). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

This study prospectively collected data in an institutional database. A cohort of 71 consecutive patients who performed between January 2017 and December 2021 with unilateral THA using the postero-lateral approach for end-stage osteoarthritis was retrospectively assessed. Six patients diagnosed with Crowe type III or IV developmental dysplasia were excluded from this study. Two patients who did not want to participate in this study were also excluded. A total of 73 hips in 63 patients who met inclusion criteria were assessed whether the acetabular cup was placed within a safe zone of 40°±10° of inclination and 15°±10° of anteversion. According to literature, lower accuracy of acetabular cup positioning has been achieved using a posterior approach than an anterior approach (15). The correct angle of inclination or abduction plays a crucial role in dislocation, especially in the posterolateral approach (16,17). Thus, the current study focused on acetabular cup inclination. Two groups were formed according to acetabular cup inclination with normal anteversion angle. There were 56 hips in the inside group and 17 hips in the outside group. The rate of outlier was compared between groups according to the safe zone defined as 30° to 50° of inclination and 5° to 25° of anteversion according to the Lewinnek safe zone (9).

All surgeries were performed by a single orthopaedic surgeon who had approximately 19 years of experience in THA. Preoperative templating for cup size was done in all hips, using two digital-line methods described by Oddy et al. (18). All operations were performed with the same operative technique in which the patient was placed in the lateral decubitus position, using a posterolateral approach. A pubic and a lumbosacral positioning brace was used to keep the pelvis in optimal position. We checked that the operating table was parallel to the floor. A spirit level was utilized to confirm that the operating table was parallel to the ground. The anterior superior iliac spines (ASIS) were checked to be perpendicular to the operating table. A curvilinear skin incision of around 13 cm extending 4-6 cm proximal and about 6-9 cm distal to the tip of the greater trochanter was performed (**Figures 1, 2**). The femur was retracted anteriorly to expose and reame the acetabulum before placing the acetabular shell inserted press-fit after underreaming the acetabulum by 1 mm (**Figure 3**). The surgeon attempted to place the acetabular cup within safe zone of 40°±10° of inclination and 15°±10° of anteversion. Cup inclination and anteversion were visually evaluated by the surgeon intraoperatively. The straight cup inserter positioning relative to the floor and cup-positioning guides with the freehand technique were used to verify the acetabular inclination and anteversion. Intraoperative fluoroscopy exposes the patient and surgeon to additional radiation. Hence, no fluoroscopy was applied to assess cup orientation. All hips were treated with the same cementless prosthesis (Trilogy acetabular cup, Versys Fiber Metal Taper stem; Zimmer Biomet, Warsaw, Indiana, USA). Traditional manual methods with mechanical and anatomical guides were utilized to determine the optimal cup positioning in the current study.

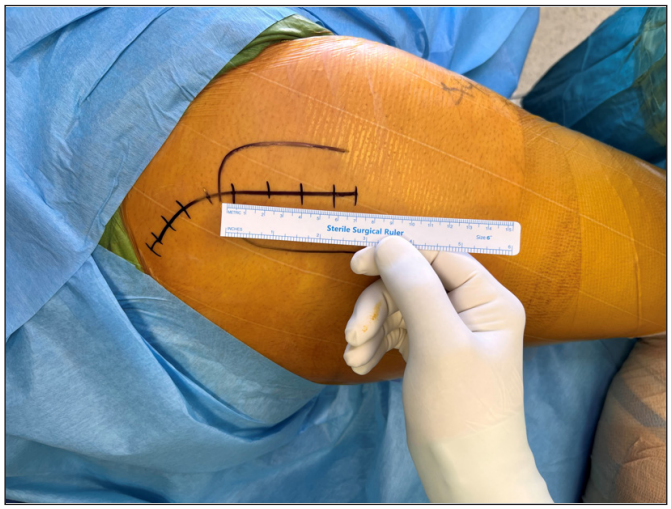

Figure 1. The image of the skin incision length of posterior approach before surgery.

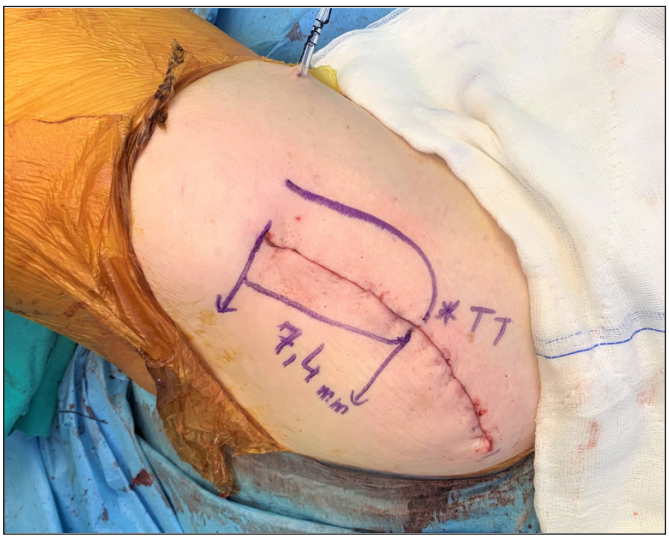

Figure 2. The image of the distal skin incision length of posterior approach after surgery.

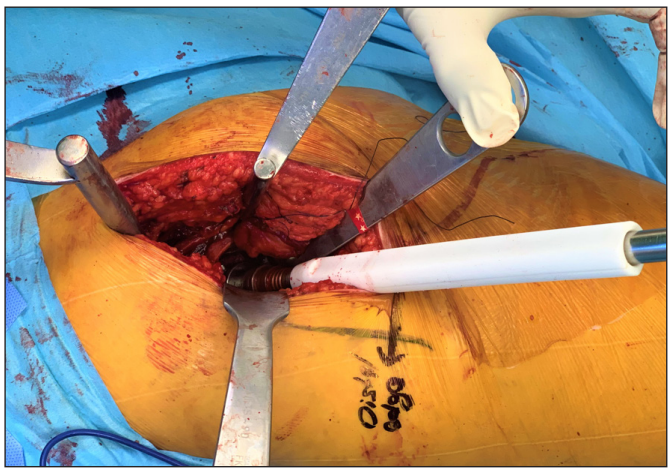

Figure 3. The image of the distal skin incision length of posterior approach during surgery.

Component position evaluation included cup inclination and anteversion was performed through a radiographic assessment of the acetabular component on an anteroposterior pelvis radiograph obtained using a standardized technique with the patients in the supine position. We utilized a picture archiving and communication system (PACS) to evaluate radiographic measurements (**Figures 4, 5**). The image of the distal skin incision length of posterior approach during surgery. The interteardrop line was used as the transverse axis of the pelvis to calculate cup inclination and anteversion measured from the post-operative radiographs. Cup inclination was measured using the angle between the plane of the cup opening and the interteardrop line. Anteversion was measured according to the method described by Liaw et al. (19).

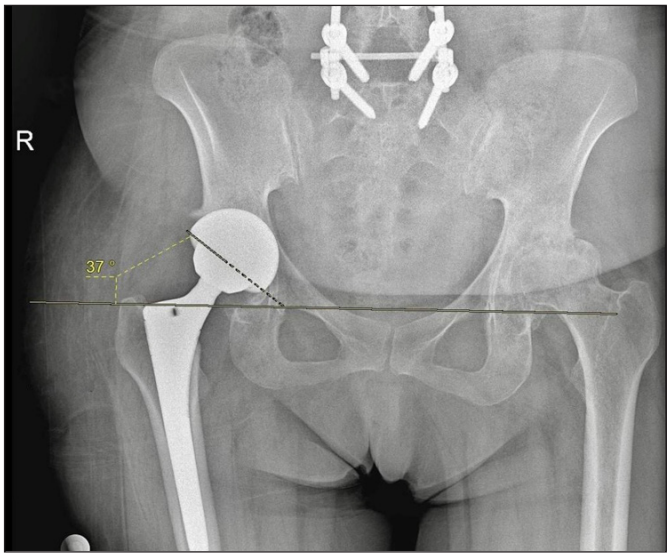

Figure 4. Evaluation of radiographic measurements of acetabular component inclination using PACS system. PACS: Picture Archiving and Communication System.

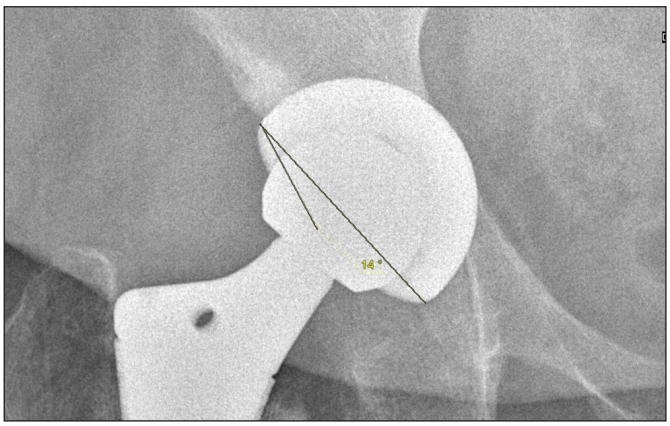

Figure 5. Evaluation of radiographic measurements of acetabular component anteversion using PACS system. PACS: Picture Archiving and Communication System.

The measurement of body mass index (BMI) was conducted in all patients who were classified as a obese according to BMI ≥30 that was described by the National Institutes of Health (20).

Statistical Analysis

NCSS (Number Cruncher Statistical System) 2007 Statistical Software (Utah, USA) program was used for statistical analysis. We expressed nominal data as

frequencies or percentages and quantitative data as mean±SD. The Shapiro-Wilk test was performed to test the normality of study data. Groups were compared using the independent t-test for normally distributed continuous variables. The chi-square test was used to analyse qualitative comparative parameters. ROC analysis of distal length of incision (DLI) was performed. Logistic regression analysis performed for individual effects of obesity and DLI on the occurrence of the inaccurate cup position. Sensitivity, specificity, positive predictive value, negative predictive value, and LR (+) (Likelihood Ratio) value were calculated to determine the calculated cut-off. P-value of ≤0.05 was considered statistically significant.

RESULTS

All subject demographics, including age, gender and BMI, were evaluated in the two groups (Table 1). The mean size of the acetabular component was 49.36±3.12 mm (range, 44-56 mm) for the inside group and 50.12±6.48 mm (range, 44-58 mm) for the outside group (p=0.745). The mean head size was 32.50±2.86 mm (range, 28-36 mm) for the inside group and 32.94±4.03 mm (range, 28-36 mm) for the outside group (p=0.119) (**Table 1**).

Table 1. Patient demographics data.

Variable	Inside group	Outside group	p value
Hips (n)	56	17	
Mean age (year)	61.7±13.32	60.35±12.2	0.712*
Gender (female/male)	47/9	15/2	0.664+
BMI (kg/m²)	27.61±4.27	30.91±4.79	0.008*
Side (right/left)	26/30	8/9	0.964+
Diagnosis			
Osteoarthritis	42 (75%)	12 (70.59%)	0.266+
Dysplasia			
Crowe I	6 (10.71%)	2 (11.76%)	
Crowe II	3 (5.36%)	3 (17.65%)	0.266+
Avascular necrosis	5 (8.93%)	0 (0.00%)	
Cup size (mm)	49.36±3.12	50.12±6.48	0.745*
Femoral head size (mm)	32.50±2.86	32.94±4.03	0.119*
TLI (cm)	13.10±0.61	13.31±0.59	0.207*
DLI (cm)	7.91±0.62	6.37±0.21	0.0001*

Values are given as mean (standard deviation) or n (%) as appropriate and p calculated by using the independent t-test (*), and the chi-squared test (+). BMI: Body mass index; TLI: Total length of incision; DLI; Distal length of incision.

No significant difference in the average total incision length was found between the two groups (p=0.207). The mean total length of incision was 13.10±0.61 in the inside group and 13.31±0.59 in the outside group. However, there were significant difference in the average distal incision length between the two groups (p=0.0001). While the average DLI was 7.91±0.62 cm (range, 6.8-9 cm) in the inside group and 6.37±0.21 cm (range, 6-6.7 cm) in the outside group. According to ROC analysis, a patient with ≤6.7 cm of DLI is 5.71 times more possible to be outside than a patient with >6.7 cm of DLI (**Figure 6**) (**Tables 2,3**).

Table 2. ROC analysis of DLI.

Variable	Area under the ROC curve (AUC)
DLI (cm)	0.961 (0.888-0.992)

Table 3. Sensitivity, Specificity, positive predictive value, negative predictive value, and LR (+) (Likelihood Ratio) value were calculated to determine the calculated cut off.

Cut Off	Sensitivity	Specificity	PPV	NPV	LR (+)
≤ 6.7	94.12	98.21	94.1	98.2	5.71

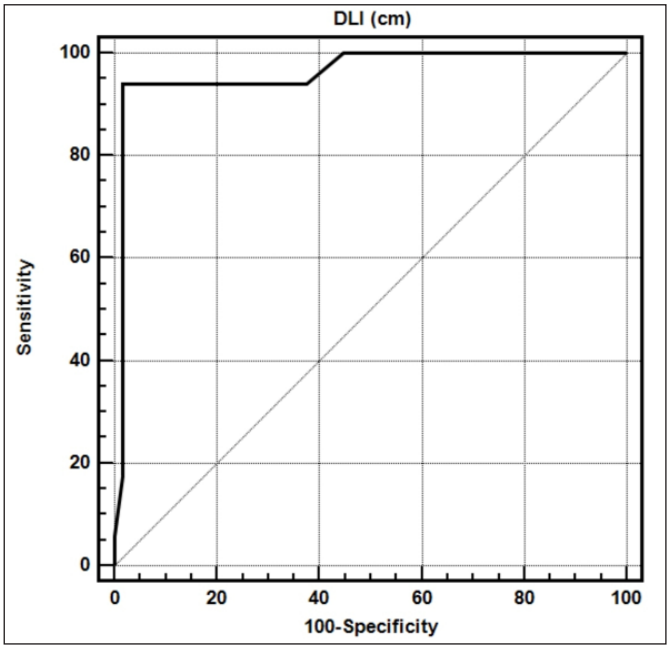

Figure 6. Sensitivity, Specificity, positive predictive value, negative predictive value, and LR (+) (Likelihood Ratio) value were calculated to determine the calculated cut off.

Seventeen hips (23.3%) were found outside the safe range according to Lewinnek et al. (9). Substantial differences were observed regarding radiographic cup inclination between the two groups (p= 0.0001). In the inside group, the average cup inclination was 44.11°±3.44° (range, 37°-50°), whereas, in the outside group, it was 55.41°±2.5° (range, 52°-59°). However, there were no significant differences in the average radiographic cup anteversion between the two groups (p= 0.960). In the inside group, the average cup anteversion was 15.52°±3.38° (range, 9°-22°), whereas, in the outside group, it was 15.47°±3.48° (range, 10°-23°) (**Table 4**).

Table 4. Postoperative radiographic data.

Variable	Inside group n=56	Outside group n=17	p value
Cup inclination angle	44.11°±3.44°	55.41°±2.5°	0,0001
Cup anteversion angle	15.52°±3.38°	15.47°±3.48°	0.960

Values are given as mean (standard deviation) or n (%) as appropriate and p calculated by using the independent t-test.

The BMI was 27.61±4.27 kg/m² in the inside group and 30.91±4.79 kg/m² in the outside group (p= 0.008). Although 11 of 17 (64.5%) patients classified as obese (BMI ≥30) in the outside group experienced higher rates of inaccurate cup orientation, logistic regression analysis showed that the individual effect of obesity on the occurrence of the inaccurate cup position was not observed (p= 0.884) (**Table 5**).

Table 5. Logistic regression analysis performed for individual effects of obesity and DLI on the occurrence of the inaccurate cup position

Variable	OR (95% CI)	p value
BMI	0.97 (0.61-1.54)	0.884
DLI (cm)	0.83 (0.20-1.60)	0.021

In the inside group, no hips encountered dislocation at the end of this study. However, one posterior hip dislocation occurred after one month postoperative in the outside group, which was treated in a closed manner under general anesthesia.

DISCUSSION

The present study showed that the distal portion of the skin incision of the posterolateral approach had a significant impact on acetabular cup inclination while placing the acetabular component in THA. Poor acetabular cup positioning was associated with the short distal portion of the skin incision of the posterolateral approach in the current study. To accomplish optimal cup position, especially acetabular inclination, surgeons should perform longer distal skin incision to place the cup more accurately in the posterior approach when additive soft tissue masses of the leg force the surgeon to insert the component at a higher inclination than aimed.

During acetabular cup implantation, the acetabular component position plays a crucial role in the success of THA (3,5). Optimal cup orientation within the safe zone relies on the surgeon's performance. However, accurate cup orientation is not always accomplished by experienced surgeons (2,12,21,22). In the present study, the surgeon had about nineteen years of experience in THA and encountered 17 of 73 outline cups. The ability of surgeons to accurately insert an acetabular cup depends on the surgical approach, especially when using the posterior approach (23). Furthermore, the exposure to the surgical field and the ability of the operating surgeon to place acetabular cup can be limited by a minimally invasive technique in THA, which increases a new dimension of difficulty in accurately positioning the cups (3,23). Callanan et al. (23) showed the inaccuracies of the minimally invasive surgical approach that may lead to a more constrained working space and decreased direct vision, resulting in the improper cup position. According to the present study results, given that all hips treated with a conventional posterolateral approach, some cases encountered poor inclination angles of acetabular cup owing to a short distal portion of the skin incision of the posterolateral approach during THA. Therefore, acetabular cup orientation is one of the most vital surgeon-controlled factors that must be considered during surgery (4,6). During the posterolateral approach, limited exposure to the acetabulum may lead to the risk of component malposition (6). In 2015, a study conducted by Garcia-Rey et al. (17) evaluated 1414 hips undergoing cementless THA, in which cups with a greater acetabular inclination angle had a higher risk for dislocation. Woerner et al. (12) believed that additive soft tissue masses of the leg could force the surgeon to insert the component in higher inclination than aimed. Grammatopoulos et al. (24) proposed that length of incision and depth of subcutaneous fat at the incision may result in impingement of the straight modular inserter handle on the skin and alter the cup orientation. A less distal skin incision through the posterolateral approach was performed in the outside group, which led to more inaccurate component orientation in the present study, which is consistent with Grammatopoulos et al. (24). In addition, the rates of the inaccurate cup position range from 30% to 75% in the literature (25,26). Danoff et al. (26) conducted a study in which 477 of 1289 components (37%) were outside the Lewinnek safe zone. In the other study by Bosker et al. (27), 29.5% cups were performed through the posterior approach located outside the Lewinnek safe zone. In the current study, 23.3% of components were inserted outside the Lewinnek safe zone, which is fewer than that in the literature. It seems reasonable to assume that the present study obtained this result because the surgeon who performed the THA had a high surgeon volume. Moreover, several reports observed lower anteversion angles in dislocating THAs placed through the posterolateral approach (16,28). Fujishiro et al. (28) analyzed 1,555 consecutive primary THAs using the posterolateral approach and revealed that the dislocation rate after THA was 3.22%. The dislocation risk was 1.9 times higher if cup anteversion was not between 10° and 30° in this report. They suggested that dislocated THA posteriorly had a significantly smaller acetabular anteversion compared to hips that did not dislocate. In the present study, the dislocation rate was 1.4%, which is lower than their results. The mean anteversion angle in the current study was 15.52°±3.38° in the outside group, which resulted in a lower dislocation rate, as Fujishiro et al. suggested.

The intraoperative view of the acetabulum in the posterolateral approach during THA is dissimilar to other approaches (6). Although some reports showed

no difference in cup inclination between the anterior approach and the posterior approach, some indicated that the anterior approach obtains more accurate acetabular component orientation (6,11,21). Callanan et al. (23) showed that the posterolateral approach could be one of the best to achieve an optimal cup orientation. In another trial, Goyal et al. (6) used a skin incision of approximately 15 cm in the posterior approach and compared the direct anterior approach and posterior approach regarding the component position. At the end of the present study, they did not find any difference between the two approaches. However, there was no information about the distal part of the posterior skin incision in this study. It seems reasonable to assume that the distal skin incision starts the greater trochanter bigger than the proximal part, which can allow the surgeon to place cup more accurately in the posterior approach. Conversely, a study by Hamilton et al. (11) found that the direct anterior approach provided more accurate component orientation than the posterolateral approach. In this study, placing the acetabular component vertically occurred in the posterolateral approach. Similarly, Ji et al. (21) showed that the surgical approach might play a crucial role in the accurate placement of the acetabular cup. In their report, a higher cup inclination angle occurred in the posterior approach. In the current study, the same conclusion can be drawn owing to the short distal portion of the skin incision of the posterolateral approach performed in the outside group that had a higher cup inclination angle. The present study suggested that optimal cup inclination angle can be obtained by performing longer distal incision of the posterolateral approach when the surgeon needs it during THA. Moreover, Lin et al. (29) performed a comparative study in which the direct anterior approach had higher rates of acceptable acetabular inclination compared with the posterolateral approach. In addition, they reported that a BMI of 30-34 was related to higher acetabular inclination compared with the normal weight group. Hence, longer incisions can be used in obese or highly muscular patients by surgeons who extend the incision of the posterolateral approach (30). However, the current study showed that BMI did not affect poor cup position. Some reports used a navigation system that might be applied to accomplish the accuracy of cup orientation in primary THA (3,22). However, the adoption of the navigation systems by orthopaedic surgeons has been slow due to multifactorial reasons, such as the increased cost (3). It is well-established that most surgeons evaluate the position of acetabular cup inclination and anteversion according to the alignment of the patient's pelvis by direct observation intraoperatively during hip arthroplasty (12). Hence, conventional methods with the freehand techniques were used in the present study.

Although surgeons attempt to accomplish accurate component position during the procedure, obtaining the targeted cup orientation remains a significant challenge for surgeons who have found high variability in the angle of acetabular cup radiographic inclination and anteversion (2). The inclination angle of the acetabular component may be related to implant failure due to suboptimal implant positioning and impingement, especially in the posterior approach that may lead to the increased risk of dislocation (31,32). A vertical acetabular cup with an inclination angle of more than 55° has been reported to be the most crucial factor related to higher rates of dislocations (23,33). Kennedy et al. (33) showed that three of 75 hips encountered recurrent dislocations necessitating revision of the acetabular component with a mean inclination angle of 61.9° (55°-69°), using the posterolateral approach. In the literature, dislocation rates in the posterior approach range from 1% to 5% (13). In the current study, one patient had an implant failure due to suboptimal acetabular cup positioning in which the acetabular component has an inclination angle of 57°. The dislocation rate in the current study was 1.4%, comparable to the literature. However, dislocation rate can be reduced using a novel way. Furthermore, optimal component positioning with good intraoperative evaluation is vital to avoid dislocation which is a major complication after THA (34). Patient-related, surgical factors or both can be responsible for dislocation (34). Some authors have found that BMI had a significant impact on the optimal cup position, whereas some have reported no significant impact (23,24,35). Woerner et al. (12) evaluated 65 patients who underwent THA through a minimally invasive technique and found a statistically significant correlation between the evaluating component inclination and BMI. Similarly, a study performed by Haffer et al. (35) confirmed that BMI impacted optimal acetabular component orientation. In another trial, Zhao et al. (36) encountered some difficulties in fully exposing the surgical field in the obese (BMI ≥30) or strong hips owing to the muscle or fat tissue gathered around the incision. Conversely, Grammatopoulos et al. (24) conducted a study in which no minimally invasive surgeries were performed. In this study, BMI had no important effect on the accurate cup orientation. The current study obtained the same conclusion, which confirmed that BMI had no important effect on the accurate cup orientation. Therefore, accurate placement of the acetabular cup is a crucial factor in preventing postoperative complications following THA, which is still difficult to accomplish the ideally intended component positioning owing to the difficulty in verifying the position during the procedure (2,21,22,33). These difficulties experienced during THA positioned using the posterolateral approach arose from the short distal portion of the skin incision in the current

study. Hence, the present study hypothesized that the length of the distal skin incision of the posterolateral approach affects the cup inclination during THA. To our knowledge, to date, no trial has investigated the possible effects of the length of the distal skin incision of the posterolateral approach on the cup inclination during THA. There is a lack of clinical reports that evaluate this problem that should be discussed in the literature. The present study tried to investigate whether the length of the distal skin incision of the posterolateral approach affects the cup inclination during THA. At the end of the current trial, some hips that underwent THA implanted with a posterior approach had higher cup inclination due to insufficient distal skin incision.

The retrospective design and small sample size were the main limitations of this present study. The current study did not have any comparing group owing to no matched control group. The other limitation was that we only monitored radiographs in the AP view, which cannot be optimal for the anteversion of the acetabular component. We are also aware that we could have performed a computerized tomography. Femoral component positioning using a posterior approach was not evaluated in this study. We focused on only acetabular component positioning because surgeons have little control of the femoral component position. However, acetabular cup position might be varied within anatomical limits. We have some strength. This is a single surgeon series, which might be considered the strength of this study. Moreover, we did not utilize different cups in this study.

CONCLUSION

A longer distal portion of the skin incision of the posterolateral approach should be performed to achieve optimal operative inclination angles of the acetabular cup during THA. The surgeon should have no hesitation in extending the distal skin incision when performing the posterolateral approach.

ETHICAL DECLARATIONS

Ethical Committee Approval: The study was carried out with the permission of İstanbul Medipol University Clinical Researches Ethics Committee (Date: 04/02/2022, Decision No: E-10840098-772.02-753).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Conflict of Interest Statement: The author declares no conflicts of interest.

Financial Disclosure: The author declared that this study had received no financial support.

Author Contributions: The author declares that they have all participated in the paper's design, execution, and analysis and that they have approved the final version.

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Original Article

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Six-year seroprevalence results for blood center mandatory donor screenings: single center experience

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ABSTRACT

Aims: Infections transmitted through blood and blood product transfusions are one of the most important health problems today. In Turkey; Microbiological screening for hepatitis B, hepatitis C, syphilis and HIV infections is mandatory for each unit of blood and blood components prepared for transfusion, regardless of the time between donations. In this study, it was aimed to determine the prevalence of HBV, HCV, HIV and syphilis in blood donors who applied to our Blood Center and to compare them with the prevalences in other regions of Turkey.

Material and Method: The study was conducted between January 2016 and June 2022 and included 68195 donors. Screenings for HBsAg, anti-HCV, anti-HIV and syphilis tests were performed with commercial kits using the Electro-Chemiluminescence Immunoassay (ECLIA) principle. Positive results were confirmed by validation studies.

Results: As a result of screening, 279 (0.4%) of 68195 donors tested positive for HBsAg, 56 (0.08%) for anti-HCV, 25 for anti-HIV (0.03%) and 197 (0.28%) for syphilis. Of the total donors, 2129 (3.1%) were volunteers and 66,066 (97%) were non-volunteer relatives. In total, 0.8% of the donors were positive for screening.

Conclusion: The significant (0.8%) positivity detected in the HBsAg, anti-HCV, anti-HIV and syphilis tests of the donors showed that blood transfusion screening tests were vital. In addition, considering the window period of infections and the pre-seroconversion period, it was concluded that there is a need for methods with high sensitivity and solutions such as avoiding unnecessary transfusions.

Keywords: Hepatitis B, HIV, HCV, syphilis, blood donors

INRODUCTION

Vitally important blood and blood products, which are used for treatment purposes in many diseases, are very costly and have a short period of use. These products cannot be produced synthetically yet under laboratory conditions and should be obtained from healthy individuals in the society (1).

Donor questioning and screening tests are applied for providing safe blood for the transfusion process. Donors are registered in blood transfusion centers, provided with a detailed 'Donor Inquiry Form' and physically examined. For safe blood supply; the World Health Organization (WHO) recommends supporting voluntary blood donors who donate regularly (2).

Immunological, and non-immunological infectious complications are among the various problems encountered in treatments performed with blood and blood products transfusion and conditions caused by microorganisms are common complications of blood transfusion. Although infections can be transmitted by bacteria, viruses, parasites, fungi and prions, in practice, especially human immunodeficiency virus (HIV 1-2), hepatitis C virus (HCV) and hepatitis B virus (HBV) have gained importance in terms of transfusion safety as the most important viral agents (3-5).

Furthermore, in addition to various viral agents such as Hepatitis A, Hepatitis D, Hepatitis E, Hepatitis G viruses, human parvovirus B19, human Herpes virus type 8, human Epstein Barr virus, cytomegalovirus, bacteria and parasites such as Treponema pallidum, Salmonella and Brucella can be transmitted by transfusion. Most of the infectious agents transferred from donors to recipients

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are able to maintain their viability in stored blood for a long time resulting in latent or asymptomatic infections (3,6).

In order to ensure transfusion safety, serological tests are requested from eligible donors (7). According to the regulation on the blood and blood products law in our country, HBsAg, anti-HCV, anti-HIV 1/2and syphilis standard tests are enforced as mandatory screenings. In Türkiye, the prevalences of HBV, HCV, HIV and VDRL in blood donors vary between 2.80-10.75%, 0.0-1.5%, 0-0.86%, and 0.02-0.2% respectively. Despite this, the risk of contamination with blood and blood products is estimated to be 1/100 000 for HCV, 1/63 000 for HBV and 1/680 000 for HIV (1).

In our study, it was aimed to determine the HBsAg, Anti-HCV, Anti-HIV and syphilis seropositivity rates by retrospectively examining the information of the donors who applied to our Blood Transfusion Center in the last 6.5 years, and to draw attention to viral or bacterial agents with increasing frequency.

MATERIAL AND METHOD

The study was carried out with the permission of the Health Sciences University, Şişli Hamidiye Etfal Training and Research Hospital, Health Practice and Research Center, Clinical Research Ethics Committee (Date: 24/05/2022, Decision No: 3567). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

The study was planned as a descriptive seroprevalence study. The results of blood donation screening tests of 68195 donors who applied to the Health Sciences University Şişli Hamidiye Etfal Training and Research Hospital's Blood Center between January 2016 and June 2022, filled the donor inquiry forms prepared and evaluated in accordance with the 'Blood and Blood Products Law No. 5624 and the provisions of the related legislation and donor selection criteria' and signed the 'Informed consent form' were included in our study. The demographic data of the donors registered in the automation system of the hospital were retrospectively analyzed (8).

Serological study

Individuals who were evaluated as safe and risk-free by questioning were accepted as donors. Anti-HCV, anti-HIV, Syphilis antibodies and HBsAg antigenes were investigated by Electro-Chemiluminescence Immunoassay (ECLIA) method from blood samples obtained from suitable donors. (Roche Cobas 6000 e601 Analyzer, Roche Diagnostics, USA). Positive tests were confirmed by repeating in the validation laboratory.

Statistics

The patients' data were evaluated descriptively and the percentages of the data were determined. The data were evaluated separately with classifications such as age and gender. SPSS v.26 was used for these descriptive data analysis.

RESULTS

Of 68195 donors, 279 (0.4%), 56 (0.08%), 25 (0.03%) and 197 (0.28%) were found to be respectively; HBsAg, anti-HCV, anti-HIV and syphilis positive. Of the total donors, 2129 (3.1%) were volunteer and 66066 (97%) were non-volunteer (patient relatives).

In our study, the general prevalence of transfusion-transmitted infections in a total of 68195 donors over 6.5 years was 0.54% (373 of 68195) and 279 (0.4%), 56 (0.08%), 25 (0.03%) and 197 (0.28%) donors were respectively; HBsAg, anti-HCV, anti-HIV and syphilis positive. The distribution and positivity rates of HBsAg, Anti-HCV, Anti-HIV and syphilis between 2016 and 2022 are given in **Table 1**.

Table 1. Distribution of HBsAg, Anti-HCV, Anti-HIV and syphilis in blood donors by years

Year	Number of donors	HBsAg (+)	Anti-HVC (+)	Anti-HIV (+)	Syphilis (+)
2016	14.619	83 (0.56%)	2 (0.01%)	10 (0.06%)	32 (0.21%)
2017	15.417	59 (0.38%)	3 (0.019%)	7 (0.04%)	46 (0.29%)
2018	12.611	68 (0.53%)	13 (0.10%)	1 (0.007%)	49 (0.38%)
2019	8.647	25 (0.28%)	11 (0.12%)	0 (0%)	21 (0.24%)
2020	6.389	23 (0.35%)	11 %0.17%)	1 (0.01%)	26 (0.40%)
2021	6793	13 (0.19%)	12 (0.17%)	4 (0.06%)	15 (0.22%)
2022	3719	8 (0.21%)	4 (0.10%)	2 (0.05%)	8 (0.21%)
Total	68195	279 (0.40%)	56 (0.08%)	25 (0.03%)	197 (0.28%)

* First 6 months of 2022

In the 6.5-year period, 5370 (8%) of the 18-65 aged 68195 blood donors were female and 62825 (92%) were male with a female/male ratio of 1/11.7. The majority of all donors, 43072 (63.1%) were aged between 25 and 44 (**Table 2**).

Table 2. Distribution of HBsAg, Anti-HCV, Anti-HIV and syphilis in blood donors by age and gender

Age range	Gender	Number of donors	HBsAg	Anti-HCV	Anti-HIV	Syphilis
18-24	Female	1323	4	1	0	2
	Male	11977	38	1	1	16
25-44	Female	2919	13	1	4	23
	Male	40153	133	33	14	87
45-65	Female	1125	9	5	1	17
	Male	10658	82	15	4	52
>65 age	Female	3	0	0	0	0
	Male	37	0	0	1	0
Total		68195	279	56	25	197

Of the total donors, 2129 (3.1%) were volunteer and 66066 (97%) were non-volunteer (patient relatives). By analyzing the number of donors by years, a gradual decrease in donation was observed. Additionally, the number of volunteer donors has been determined to be quite low compared to non-volunteer donors (**Table 3**).

Table 3. Distribution of volunteer and non-volunteer (patient relatives) donors by years.

Year	Volunteer donor	Non-volunteer donor
2016	627	13.992
2017	568	14.849
2018	392	12.219
2019	237	8410
2020	160	6229
2021	101	6692
2022	44	3675
Total	2129	66.066

HBsAg, anti-HIV, anti-HCV and several of the syphilis parameters were determined to be simultaneously positive in five of 68195 donors in our study (**Table 4**).

Table 4. Simultaneous positivity rates of HBsAg, Anti-HCV, Anti-HIV, syphilis in blood donors

year	Age	Gender	HBsAg (+)	Anti-HVC (+)	Anti-HIV (+)	Syphilis (+)
2016	39	Male	+	+		
2016	48	Male	+	+		+
2017	35	Male			+	+
2018	30	Male				
2018	27	Male			+	+

DISCUSSION

Providing safe blood transfusion is a primary goal of the blood centers. Recently, significant progress in terms of the safety of blood products has been made in developing and developed countries. Detailed examination of donors, questioning of their diseases and habits, routine screening of blood products for various pathogens are indispensable practices for the reliability of blood and blood products (9). Owing to the careful selection of blood donors, the advances in screening tests and the use of advanced methods; the risk of transfusion-transmitted infection, which is one of the most important complications of blood transfusion, is decreasing day by day. However, despite all the developments in modern medicine, the infection transmitted by transfusion has not been completely resolved yet. This situation continues to be an important health problem all over the world, especially in underdeveloped countries (7).

Despite all the precautions and technological developments, it is still not possible to identify individuals infected with infectious agents such as HBV, HCV and HIV by routine screening tests, as there is a possibility of transmission during the so-called "window period" between the infection and the attainment of measurable levels of antibody titers (9). The window period is approximately ten days for HCV, and the rate of transmission through fresh blood products is estimated to be 1 in 1.6 million units. Nowadays, the positive developments in the sensitivity of HIV antibody tests have shortened the window period from approximately 45 days to 22-25 days, reducing the rate of transfusion-associated HIV transmission to 1 in 1.8 million units (10).

In terms of safety of the blood supplied from the donors and transfused to the recipients; WHO (World Health Organization) has stated that blood must not pose any danger or disease and not contain infectious agents or harmful foreign substances. The safe human-to-human transfusion of blood and certain blood compounds is an extremely critical process. The “Safe Blood” WHO announcement at 2000 highlights the importance of this issue. In the world where millions of units of blood and blood components are transfused yearly, it is a serious public health problem that post-transfusion infections due to microorganisms, especially viruses, are still not prevented in some recipients (11). In order to solve this issue, it is worth noting that approaches such as regular donations from safe people should be popularized. For safe blood supply, screening studies for HBsAg, anti-HCV, anti-HIV and syphilis are routinely carried out in our country's blood banks.

In various regions of Türkiye; many studies have been conducted to determine the seroprevalence of HBsAg, Anti-HCV, Anti-HIV 1/2 and syphilis in blood donors (Table 5). In this study, which we conducted with a similar purpose, out of 68195 donors 279 (0.40%), 56 (0.08%), 25 (0.03%) and 197 (0.28%) were detected respectively as HBsAg, anti-HCV, anti-HIV and syphilis positive between 2016 and 2022.

In İstanbul; Ulutürket al. (9) detected HbsAg in 2.83% of 75747 donors between 1998 and 2008, Şanlı et al. (12) detected HbsAg in 2.03% of 51120 donors between 2003 and 2012 and Karagöz et al. (13) detected HbsAg in 1.4% of 10568 donors between 2009 and 2011 and reported that the rate of HBsAg positivity decreased within the process.

Considering the data of studies reported from various regions of our country, we see that HBsAg seropositivity rates vary between 0.5% and 3.17% (14-31) (**Table 5**). The rate of 0.40% we found in our study is below the reported rates. In addition, we see that HBsAg seropositivity rates are gradually decreasing every year in the period of 2016-2022 covered by our study. We think that the awareness of the society about HBV infection, vaccine applications and the effective implementation of the measures taken within the scope of safe blood supply are among the reasons responsible for this decrease.

Anti-HCV positive donors have been detected in the rates of 0.4% in Ulutürk et al. (9), 0.44% in Şanlı et al. (12) and 0.2% in Karagöz et al. (13) studies. As a result of the studies conducted on blood donors from various regions of our country, we see that the anti-HCV seropositivity rates vary between 0.05% and 0.92% (14-31) (**Table 5**). It is seen that the rate of 0.08% anti-HCV seropositivity detected in our study is low and consistent with the data of our country; especially the rates reported from our city.

HIV seropositivity rates in blood donors reported by Ulutürket al. (9), Şanlı et al. (12) and Karagöz et al. (13) studies were respectively; 0.001%, 0.06% and 0.03%. According to the reports of studies performed in various regions of Türkiye; the seropositivity rates of HIV in blood donors are between 0.0% and 1.06% (14-31) (**Table 5**). The rate of 0.03%, which we found in our study, is among these seropositivity rates and compatible with the data of Türkiye.

The incidence of syphilis, which ranks third after chlamydia and gonorrhea among sexually transmitted diseases in European Union countries, was reported as 4.5/100 000 in 2009 (32). The source of epidemiological data on syphilis in our country is largely based on donor screenings made by blood banks or data obtained from sex workers (33). 0.16% of Ulutürket al. (9), 0.33% of Şanlı et al. (12) and 0.7% of Karagöz et al. (13) donors were reported as syphilis antibody positive and according to studies from various regions of our country, syphilis seropositivity rates in blood donors range from 0% to 2.33% (14-31) (**Table 5**). The rate of 0.28%, which we found in our study, was among these seropositivity rates being compatible with the data of our country. Our study's rate also was found to be close to the incidence of the European Union sexually transmitted diseases in 2009.

A gradual decrease in HBsAg and syphilis screening test positivity has been observed in our hospital over the years. However, it is still not reset. The presence of anti-HCV and anti-HIV positivity points the importance of screening tests before blood transfusion. Although there is a decrease in the frequency of infectious diseases transmitted by blood transfusion in parallel with the advances in technology and science, a transfusion application with zero risk of infection does not seem to be possible in the near future. In addition, since the best transfusion is non-transfusion; limiting transfusion to absolute indications only seems to be the most effective protective method from the transfusion-transmitted infections.

Table 5. Seropositive rates of HbsAg, anti-HCV, anti-HIV and Syphilis detected in blood donors in different regions of our country

Province	Year	Researchers	Number of donors	HbsAg (%)	Anti-HCV (%)	Anti-HIV (%)	Syphilis (%)
İstanbul	1998-2008	Ulutürk et al.	75747	2.83	0.4	0.001	0.16
İstanbul	2003-2012	Şanlı et al.	51120	2.03	0.44	0.06	0.33
İstanbul	2009-2011	Karagöz et al.	10568	1.4	0.2	0.03	0.7
Zonguldak-Düzce-Sakarya-Kocaeli	2009-2014	Altındiş et al.	150787	0.8	0.38	0.0025	0.004
İzmir	2002-2006	Ağuş et al.	61409	2	0.54	0.028	-
İzmir	2004-2010	Uzun et al.	80454	1.31	0.38	0.002	0.04
Denizli	1999-2007	Akalın et al.	50521	0.97	0.44	0	0
Denizli	2007-2008	Balcı et al.	13334	1.3	0.5	0.023	0.13
Isparta	2000-2007	Kaya et al.	51361	1.1	0.44	0.09	0.08
Diyarbakır	2000-2010	Dayan et al.	266035	3.17	0.64	0.0004	0.07
Van	1995-2003	Dilek et al.	39002	2.55	0.17	0.036	0.057
Erzurum	2000-2011	Çelebi et al.	204000	3.14	0.92	1.06	2.33
Erzurum	2002-2003	Uyanık et al.	5028	2.6	0.4	0	-
Tokat	2003-2010	Bulut et al.	15696	1.29	0.16	0	0.02
Afyon	2001-2010	Altındiş et al.	37343	1.38	0.35	0.02	0.04
Çorum	2008-2013	Güreser et al.	13780	0.99	0.34	0.08	0.09
Kırıkkale	2003-2004	Deveci et al.	784	1.4	0.2	0	0
Adana	2007-2009	Yıldız et al.	62461	1.66	0.05	0.003	0.1
Malatya	2000-2007	Köroğlu et al.	13564	3.1	0.47	0.07	-
Kahramanmaraş	2012-2018	Kirişçi et al.	1326	0.5	0.15	0	0.07
Mersin	2006-2008	Öner et al.	30716	2.2	0.4	0.2	0.1
Hatay	2003-2004	Ocak et al.	12313	2.02	0.52	0.02	0.03
İstanbul *	2016-2022	Gareayaghi et al.	68195	0.4	0.08	0.03	0.28

*our study

CONCLUSION

As a result; the significant (0.8%) positivity detected in the HBsAg, anti-HCV, anti-HIV and syphilis tests of the donors showed that blood transfusion screening tests were vital. In addition, considering the window period of infections and the pre-seroconversion period, it was concluded that there is a need for methods with high sensitivity and solutions such as avoiding unnecessary transfusions.

In addition, it is thought that it would be beneficial to conduct more comprehensive and continuous epidemiological studies in order to understand the high follow-up and treatment costs of the diseases caused by these factors and to evaluate the sufficiency of the protective and control measures.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of the Health Sciences University, Şişli Hamidiye Etfal Training and Research Hospital, Health Practice and Research Center, Clinical Research Ethics Committee (Date: 24/05/2022, Decision No: 3567).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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The effect of immunosuppressive therapy on the development of ventilator-associated pneumonia in patients with COVID-19

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ABSTRACT

Aim: It remains unclear whether immunosuppressive treatments such as corticosteroids and IL-6 receptor blockers have an effect on the development of ventilator-associated pneumonia (VAP). The aim of this study was to investigate the effect of immunosuppressive therapy on the development of VAP in critically ill patients with COVID-19.

Material and Method: Two hundred thirty five patients with critically ill patients with COVID-19, who were treated in the intensive care unit (ICU) and received mechanical ventilator support, were evaluated retrospectively. VAP development, secondary infections, microorganisms isolated, and resistance patterns were compared between the groups that received and did not receive immunosuppressive therapy, and also the groups that did not receive immunosuppressive therapy, received only corticosteroid, received only tocilizumab, and received corticosteroid plus tocilizumab were compared in the subgroup analysis.

Results: In the immunosuppressive treatment group, VAP development (40.2% vs. 21.2%; p=0.001), secondary infection development (48.4% vs. 29.2%; p=0.003), at least one drug resistant bacteria growth (46.7% vs. 27.4%; p=0.001), extensively-drug resistant (XDR) microorganism growth (89.8% vs. 72.7%; p=0.033) were higher than the group that did not receive immunosuppressive treatment. VAP (53.3%; p=0.004), secondary infection (73.3%; p=0.0002), the growth of bacteria resistant to at least one drug (70%; p=0.0003) were highest in the corticosteroid plus tocilizumab group in the subgroup analysis. In addition, XDR (95.5% vs. 72.7%; p=0.032) and pan-drug resistant (PDR) microorganism growth (31.8% vs. 9.1% p=0.032) were higher in the corticosteroid plus tocilizumab group than the no immunosuppressive therapy group. There was no difference between the groups in terms of mortality (p>0.05).

Conclusion: Immunosuppressive therapy has been found to potentially enhance the risk of VAP and secondary infections in critically ill patients with COVID-19 pneumonia as well as the growth of bacteria resistant to at least one drug, the length of stay in hospital and ICUs. In addition, it has been evaluated that there may be an increase in the growth of XDR and PDR microorganisms when corticosteroid and tocilizumab are used together. Although there was no difference in mortality, using immunosuppressive therapy may require careful use of targeted antibiotics and longer-term antimicrobial therapy.

Keywords: Corticosteroids, COVID-19, IL-6 receptor, ventilator-associated pneumonia

INTRODUCTION

Globally, 525 million people have been affected by the coronavirus disease 2019 (COVID-19) caused by SARS-CoV-2, and approximately 6.3 million people have died since the pandemic began in December 2019 (1). COVID-19 patients may have asymptomatic or mild symptoms, but they may require invasive mechanical ventilation in the intensive care unit (ICU) due to respiratory failure and acute respiratory distress syndrome (ARDS). Approximately 80% of severe COVID-19 patients require oxygen support, and 30-40% require mechanical ventilation support. This increases the likelihood of nosocomial infection, particularly ventilator-associated pneumonia (VAP) (2-4). VAP is defined as pneumonia that develops 48 hours after being connected to a mechanical ventilator in ICU patients with hospital-acquired pneumonia (HAP). According to international guidelines, the incidence of HAP ranges from 5 to 20 per thousand. It is most common in immunocompromised, surgically treated, and elderly patients (5).

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The World Health Organization (WHO) recommends corticosteroids and interleukin-6 (IL-6) receptor blockers for severe or critically ill COVID-19 patients (6). However, the use of these drugs raises concerns of secondary infection adverse effects. Secondary bacterial infection has been reported at varying rates in the literature when IL-6 receptor blockers are used. In the REMAP-CAP study, the rate of secondary bacterial infection in the tocilizumab arm was 0.3% (1/353) (7). In the COVACTA study, serious infection development in the tocilizumab arm was 21% (62/294) (8). Studies on the development of VAP in COVID-19 patients in the literature have mainly focused on microorganisms detected (9, 10). It is unclear whether treatments such as corticosteroids and IL-6 receptor blockers influence the development of VAP.

The aim of this study was to investigate the effect of immunosuppressive therapy on the development of VAP in critically ill patients with COVID-19. Furthermore, it was determined whether there were differences in growing microorganisms and resistance patterns between the groups.

MATERIAL AND METHOD

This study was approved by the Ethics Committee of İstanbul Ümraniye Training and Research Hospital (Date: 26.05.2022, Decision No: 167). All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

This retrospective study included 235 COVID-19 patients over the age of 18 who were treated at İstanbul Sultan 2. Abdülhamid Han Training and Research Hospital between April 2020 and November 2021 and had a positive reverse transcriptase-polymerase chain reaction (PCR). All patients were followed up and treated in the ICU, and all required invasive mechanical ventilation. Patients under the age of 18 who did not enter the ICU and did not receive invasive mechanical ventilation were excluded from the study.

International guidelines recommend obtaining a quantitative culture for the diagnosis of VAP, because various diseases may mimic lung infection on radiological imaging (5, 11). In this study, VAP was diagnosed with clinical and radiological abnormalities, as well as microbiological growth, at least 48 hours after the patient was placed on mechanical ventilation support. As a conclusion, the presence of all of the following was used to determine VAP diagnostic criteria; a) recently detected radiological infiltration and; b) clinically at least one of them (fever >38°C, leukopenia or leukocytosis, increased sputum production or purulence, impaired gas exchange); and c) microorganism growth in quantitative endotracheal aspirate or blood culture (9,12). The patients with a PaO_2/FiO_2 ratio ≤300 and/or requiring mechanical ventilation or high flow oxygen support were given ≥40mg/day corticosteroid therapy for at least 10 days. Tocilizumab was given to patients with a PaO_2/FiO_2 ratio ≤300 and/or requiring mechanical ventilation or high flow oxygen support, persistent fever, persistent C-reactive protein (CRP) and IL-6 elevation, cytokine storm findings such as increased ferritin and D-dimer, lymphopenia, thrombocytopenia, and negative procalcitonin at a dose of 800 mg/day once or 400 mg/day for two consecutive days.

Age, gender, immunosuppressive therapy used (no immunosuppressive therapy, only corticosteroid, only tocilizumab, corticosteroid and tocilizumab together), Acute Physiology and Chronic Health Evaluation II (APACHE II) and Sequential Organ Failure Evaluation Score (SOFA) scores, comorbidities, infection parameters such as leukocyte, neutrophil, lymphocyte, CRP and procalcitonin, and length of stay in ICU, the entire length of hospital stay, the development of VAP, the detection of microorganisms and antibiotic resistance were all recorded. The patients were divided into two groups: those who were given immunosuppressive therapy and those who were not. Furthermore, a comparison was made as a subgroup analysis between four groups: those who did not receive immunosuppressive therapy, those who received only steroids, those who received only tocilizumab, and those who utilized both immunosuppressive agents. *Klebsiella pneumonia*, *Pseudomonas Aeruginosa*, *Acinetobacter Baumanii*, *Staphylococcus Aureus*, and *Streptococcus Pneumonia* were among the microorganisms observed in patients. Multi-drug resistant (MDR) microorganisms were defined as having resistance to at least one drug in at least three antimicrobial categories. Extensively-drug resistant (XDR), a subgroup of MDR, was defined as having resistance to at least one drug in all categories but no resistance in two or fewer categories (susceptibility in one or two antimicrobial categories). Pan-drug resistance (PDR) as a subgroup of MDR and XDR, was defined as having resistance to all agents in all antimicrobial categories (13).

Frequency distributions, percentage, mean, standard deviation, median, minimum and maximum were used as descriptive analyses in the analysis of data in the study. Normality of the data was ensured by Kolmogorov-Smirnov test. ANOVA (Post Hoc. Bonferroni), t test, chi-square test, Fisher's Exact test, Kruskal Wallis H test, Mann Whitney U Test were applied. IBM SPSS ver 20 and Microsoft Excel computer programs were used for data analysis.

RESULTS

This study comprised 235 critically ill patients with COVID-19 who were followed in the ICU and on mechanical ventilation. Eighty-four (35.7%) of the patients were female, whereas 151 (64.3%) were male. The mean age of the patients was 70.67±14.01 years. Sixty (25.5%) of the patients died, and 175 (74.5%) survived. One hundred and twenty-two (51.9%) patients were given immunosuppressive therapy. One hundred and six (45.1%) of the patients received corticosteroids and 46 (19.6%) received tocilizumab. Thirty (12.7%) of patients received corticosteroid and tocilizumab both, whereas 76 (32.3%) received corticosteroid only and 16 (4.3%) received tocilizumab only. The APACHE II score was 60.61±25.4 and the SOFA score was 6.23±3.32. Two hundred fourteen (91.1%) of the patients had comorbid disease, which included pulmonary disease in 70 (29.8%), coronary artery disease in 66 (28.1%), diabetes mellitus in 89 (37.9%), heart failure in 51 (21.7%), renal failure in 41 (17.4%), malignancy in 35 (14.9%), neurological disease in 62 (26.4%) and arrhythmia in 28 (11.9%) patients.

Seventy-three (31.1%) patients had VAP diagnosed in accordance with the protocol, while 162 (68.9%) patients did not have VAP diagnosed. The total length of hospital stay was 14.36±12.83 days, and the length of stay in the ICU was 11.1±9.68 days. Seventy-three (31.1%) patients with VAP had a total of 92 microorganisms present in their blood and tracheal aspirate cultures. Because of the different microorganism growths in a patient, they were evaluated separately. The majority of microorganisms were *Acinetobacter Baumanii*, which was found in 44 (47.8%) of 92 growths. *Klebsiella pneumonia* was found in 35 (38.0%) of the isolates, *Pseudomonas Aeruginosa* in 8 (8.7%), *Staphylococcus Aureus* in 4 (4.3%) and *Streptococcus Pneumonia* in 1 (1.0%). In addition, MDR was found in 88 (95.7%) of the 92 growths, XDR in 77 (83.7%) and PDR in 15 (16.3%) (**Table 1**).

The difference in study variables between the groups of patients who received immunosuppressive therapy (corticosteroid only, tocilizumab only, corticosteroid plus tocilizumab) and those who did not was investigated in this study. The no-immunosuppressive therapy group had a higher SOFA score (7.57±3.37 vs. 5±2.75; p =0.001) and mean age (73.12±12.74 vs. 68.39±14.78 years; p=0.01).

Gender, APACHE II score, and comorbid disease presence did not vary between groups (p>0.05). The heart failure (30.1% vs. 13.9%; p=0.001) and arrhythmia (16.8% vs. 7.4%; p=0.03) were higher in the no-immunosuppressive treatment group. CRP (97.17±82.29 vs. 42.94±60.88; p=0.001), length of stay in the ICU (13.66±10.27 vs. 8.34±8.18 days; p=0.001), and overall hospital stay (17.86±14.34 vs. 10.58±9.7 days; p=0.001) were all greater in the immunosuppressive therapy group than in the no-immunosuppressive treatment group. Mortality rates in both groups were similar (22.1% (n=25) vs. 28.7% (n=35); p=0.25). VAP (40.2% (n=49) vs. 21.2% (n=24); p=0.001) and secondary infection (48.4% (n=59) vs. 29.2% (n=33); p=0.003) were more common in the immunosuppressive treatment group than in the no-immunosuppressive treatment group. In addition, the growth of bacteria resistant to at least one drug was higher in the group receiving immunosuppressive therapy compared to the other group (46.7% (n=57) vs. 27.4% (n=31); p=0.001). When the number of growing microbiological microorganisms was examined between the groups, the number of *Klebsiella pneumonia* (33.3% vs. 40.7%; p=0.486), *Pseudomonas Aeruginosa* (15.2% vs. 5.1%; p=0.100), *Acinetobacter Baumanii* (45.5% vs. 49.2%; p=0.733), *Staphylococcus Aureus* (3% vs. 5.1%; p=0.643) and *Streptococcus Pneumonia* (3% vs. 0%; p=0.179) were similar. While there was no difference in MDR (93.9% vs. 96.6%; p=0.547) or PDR (9.1 vs 20.3%; p=0.161), the number of XDR microorganisms growing was higher in the immunosuppressive therapy group than in the no-immunosuppressive therapy group (89.8% (n=53) vs. 72.7% (n=24); p=0.033) (**Table 1**).

The groups that received no immunosuppressive therapy, corticosteroid only, tocilizumab only, and corticosteroid plus tocilizumab therapy were compared in the subgroup analysis. The highest mean age was 73.12±12.74 in the no-immunosuppressive treatment group, followed by 71.12±15.26 in the corticosteroid only group (p=0.002). The tocilizumab only group had the least APACHE II score (p=0.049), while the corticosteroid plus tocilizumab group had the least SOFA score (p=0.0001). The corticosteroid plus tocilizumab group had the longest overall hospital stay and ICU stay (p=0.0001). VAP was highest in the corticosteroid plus tocilizumab group (n=16, 53.3%), followed by the corticosteroid only group (n=28, 36.8%) and the tocilizumab only group (n=5, 31.3%) (p=0.004).

Secondary infections were most common in the corticosteroid plus tocilizumab group (n=22, 73.3%), followed by tocilizumab only (n=7, 43.8%) and corticosteroid only (n=30, 39.5%) (p=0.0002). Similarly, resistant bacteria were most prevalent in the corticosteroid plus tocilizumab group (n=21, 70.0%), followed by only tocilizumab (n=7, 43.8%) and only corticosteroid (n=29, 38.2%) (p=0.0003). There was no difference in mortality between the groups (p=0.55). Furthermore, there was no difference in subgroup analyses between the groups for growing microorganisms and MDR, XDR and PDR (p>0.05). However, XDR (95.5% (n=21) vs. 72.7% (n=24); p=0.032) and PDR (31.8% (n=7) vs. 9.1% (n=3); p=0.032) were higher in the corticosteroid plus tocilizumab group than the no-immunosuppressive therapy group (**Table 2**).

Table 1. Baseline characteristics of the patients and identified microorganisms

Variables	Overall (n= 235)	No immunosuppressive therapy (n=113)	Immunosuppressive therapy (n=122)	p
Age[a] (years)	70.67±14.01	73.12±12.74	68.39±14.78	0.01*
Female[b]	84 (35.7)	41 (36.3)	43 (35.2)	0.87
APACHE II score[a]	60.61±25.4	59.69±27.75	61.46±23.08	0.60
SOFA score[a]	6.23±3.32	7.57±3.37	5±2.75	0.001*
Comorbidities[b]	214 (91.1)	105 (92.9)	109 (98.3)	0.34
Pulmonary disease[b]	70 (29.8)	33 (29.2)	37 (30.3)	0.85
CAD[b]	66 (28.1)	37 (32.7)	29 (23.8)	0.13
DM[b]	89 (37.9)	46 (40.7)	43 (35.2)	0.39
Hearth failure[b]	51 (21.7)	34 (30.1)	17 (13.9)	0.001*
Kidney disease[b]	41 (17.4)	21 (18.6)	20 (16.4)	0.66
Malignancy[b]	35 (14.9)	18 (15.9)	17 (13.9)	0.67
HT[b]	132 (56.2)	63 (55.8)	69 (56.6)	0.90
Neurological disease[b]	62 (26.4)	35 (31.0)	27 (22.1)	0.12
Arrhythmia[b]	28 (11.9)	19 (16.8)	9 (7.4)	0.03*
CRP[a] (mg/L)	72.09±77.88	42.94±60.88	97.17±82.29	0.001*
Procalcitonin[a] (ng/mL)	4.57±11.47	4.93±11.67	4.27±11.33	0.67
Lymphocyte[a] (10^3/mm^3)	1.15±3.08	1.06±1.25	1.23±4.05	0.68
Neutrophil[a] (10^3/mm^3)	10.28±6.62	10.96±7.61	9.71±5.59	0.16
Ferritin[a] (ng/mL)	1416.67±2327.88	1475.92±3047.16	1369.67±1548.69	0.76
Fever[b] (°C)	128 (54.5)	58 (51.3)	70 (57.4)	0.35
Total LOS[a] (days)	14.36±12.83	10.58±9.7	17.86±14.34	0.001*
ICU LOS[a] (days)	11.1±9.68	8.34±8.18	13.66±10.27	0.001*
VAP[b]	73 (31.1)	24 (21.2)	49 (40.2)	0.001*
Death[b]	60 (25.5)	25 (22.1)	35 (28.7)	0.25
Secondary infection[b]	92 (39.1)	33 (29.2)	59 (48.4)	0.003*
Resistant bacteria[b]	88 (37.4)	31 (27.4)	57 (46.7)	0.001*
Causative microbiology	**Overall MO isolated (n=92)**	**No Immunosuppressive therapy. MO isolated (n=33)**	**Immunosuppressive therapy. MO isolated (n=59)**	**p**
Klebsiella pneumonia[b]	35 (38.0)	11 (33.3)	24 (40.7)	0.486
Pseudomonas aeruginosa[b]	8 (8.7)	5 (15.2)	3 (5.1)	0.100
Acinetobacter baumanii[b]	44 (47.8)	15 (45.5)	29 (49.2)	0.733
Staphylococcus aureus[b]	4 (4.3)	1 (3.0)	3 (5.1)	0.643
Streptococcus pneumonia[b]	1 (1.0)	1 (3.0)	0 (0)	0.179
MDR[b]	88 (95.7)	31 (93.9)	57 (96.6)	0.547
XDR[b]	77 (83.7)	24 (72.7)	53 (89.8)	0.033*
PDR[b]	15 (16.3)	3 (9.1)	12 (20.3)	0.161

Abbreviations: APACHE II, Acute Physiology and Chronic Health Evaluation II; SOFA, Sequential Organ Failure Evaluation Score; CAD, coronary artery disease; DM, diabetes mellitus; HT, hypertension; CRP, C-reactive protein; LOS, length of stay; VAP, ventilator-associated pneumonia; MO, microorganisms; MDR, multi-drug resistant; XDR, extensively drug resistant; PDR, pun-drug resistant. [a]Values are mean±SDs. [b]Values are n (%). *Statistical significance.

DISCUSSION

According to the study's findings, the development of VAP, secondary infection, bacterial growth resistant to at least one drug, length of hospital stay and ICU stay, and XDR microorganism growth were higher in the group using immunosuppressive therapy compared to not using it. There was no difference in mortality between the groups. Furthermore, the development of XDR and PDR was higher in the corticosteroid plus tocilizumab group than in the no-immunosuppressive therapy group in the subgroup analysis.

Hyperinflammatory syndrome is a disease that progresses with a severe cytokine storm and is most commonly caused by viral infections. It is characterized by fever, elevated ferritin, an increase in proinflammatory cytokines such as IL-6, and cytopenia, and can result in manifestations such as ARDS in the lung (14). In cytokine storm, the IL-6 receptor antagonists (tocilizumab, sarilumab) or IL-6 antagonists (siltuximab) cause rapid improvement in lung and hemodynamic parameters (15). In the RECOVERY study, the corticosteroid therapy reduced 28-day mortality in patients requiring oxygen support or invasive mechanical ventilation (16). In the CHIC study, mortality was 65% lower and the requirement for invasive mechanical ventilation was 71% lower in the treatment arm, which included high-dose methylprednisolone and tocilizumab were added if needed, compared to the control group, which did not receive immunosuppressive therapy (17). In our study,

although the SOFA score in the no-immunosuppressive therapy group was higher, there was no difference in mortality between the groups that received and did not receive immunosuppressive therapy.

In the meta-analysis conducted by Ippolito et al. (18), the risk of VAP was 3.24-fold higher in patients with COVID-19 than in individuals without COVID-19. In the COVID-19 group, however, there was no difference in mortality between patients with and without VAP (18). There was no difference in the use of tocilizumab and corticosteroids between groups with and without VAP in the study of Martinez-Martinez et al. (19). According to Roumier et al. (20), VAP development was lower in the tocilizumab arm compared to the control group (8% vs. 26%). There was no difference in VAP development between the dexamethasone group and the non-dexamethasone group (63% vs. 57%) in the study of Gragueb-Chatti et al. (21). Unlike previous studies, VAP development was higher in the immunosuppressive therapy group than in the no-immunosuppressive therapy group in the current study (40.2% vs. 21.2%). The corticosteroid plus tocilizumab group had the highest risk of VAP in the subgroup analysis (53.3%).

Table 2. Baseline characteristics of patients and identified microorganisms based on immunosuppressive therapy group

Variables	No immunosuppressive therapy (n=113)	Only corticosteroid (n=76)	Only tocilizumab (n=16)	Corticosteroid plus tocilizumab (n=30)	p
Age[a] (years)	73.12±12.74	71.12±15.26	64.88±14.32	63.37±12.24	0.002*
Female[b]	41 (36.3)	32 (42.1)	4 (25.0)	7 (23.33)	0.24
APACHE II score[a]	59.69±27.75	65.36±22.8	46.59±22	59.5±21.5	0.049*
SOFA score[a]	7.57±3.37	5.61±2.9	4.88±2.42	3.53±1.87	0.0001*
Comorbidities[b]	105 (92.9)	70 (92.1)	12 (75.0)	27 (90.0)	0.13
Pulmonary disease[b]	33 (29.2)	21 (27.6)	5 (31.3)	11 (36.7)	0.83
CAD[b]	37 (32.7)	19 (25.0)	3 (18.8)	7 (23.3)	0.46
DM[b]	46 (40.7)	25 (32.9)	4 (25.0)	14 (46.7)	0.35
Hearth failure[b]	34 (30.1)	14 (18.4)	2 (12.5)	1 (3.3)	0.008*
Kidney disease[b]	21 (18.6)	16 (21.1)	1 (6.3)	3 (10.0)	0.34
Malignancy[b]	18 (15.9)	16 (21.1)	0 (0)	1 (3.3)	0.04*
HT[b]	50 (44.3)	31 (40.8)	8 (50.0)	14 (46.7)	0.89
Neurological disease[b]	35 (31.0)	24 (31.6)	1 (6.3)	2 (6.7)	0.009*
Arrhythmia[b]	19 (16.8)	8 (10.5)	1 (6.3)	0 (0)	0.064
CRP[a] (mg/L)	42.94±60.88	109.03±85.95	51.91±69.26	91.29±71.65	0.0001*
Procalcitonin[a] (ng/mL)	4.93±11.67	6.42±13.92	1.44±2.95	0.39±0.43	0.064
Lymphocyte[a] (10^3/mm^3)	1.06±1.25	1.53±5.13	0.71±0.28	0.75±0.45	0.564
Neutrophil[a] (10^3/mm^3)	10.96±7.61	9.75±5.69	9.12±4.86	9.91±5.86	0.54
Ferritin[a] (ng/mL)	1475.92±3047.16	1457.22±1780.64	1289.31±1147.69	1193.66±1047.46	0.939
Fever[b], (°C)	58 (51.3)	42 (55.3)	12 (75.0)	16 (53.3)	0.36
Total hospital LOS[a] (days)	10.58±9.7	15.17±9.21	20.75±15.65	23.13±21.44	0.0001*
ICU LOS[a] (days)	8.34±8.18	11.43±7.43	14.25±9.36	19±14.47	0.0001*
VAP[b]	24 (21.2)	28 (36.8)	5 (31.3)	16 (53.3)	0.004*
Death[b]	25 (22.1)	21 (27.6)	6 (37.5)	8 (26.7)	0.55
Secondary infection[b]	33 (29.2)	30 (39.5)	7 (43.8)	22 (73.3)	0.0002*
Resistant bacteria[b]	31 (27.4)	29 (38.2)	7 (43.8)	21 (70.0)	0.0003*
Causative microbiology	**No immunosuppressive therapy. MO isolated (n=33)**	**Only corticosteroid. MO isolated (n=30)**	**Only tocilizumab. MO isolated (n=7)**	**Corticosteroid plus tocilizumab. MO isolated (n=22)**	**p**
Klebsiella pneumonia[b]	11 (33.3)	9 (30.0)	2 (28.6)	13 (59.1)	0.137
Pseudomonas aeruginosa[b]	5 (15.2)	3 (10.0)	0 (0)	0 (0)	0.207
Acinetobacter baumanii[b]	15 (45.5)	15 (50.0)	5 (71.4)	9 (40.9)	0.549
Staphylococcus aureus[b]	1 (3.0)	2 (6.7)	0 (0)	1 (4.5)	0.555
Streptococcus pneumonia[b]	1 (3.0)	0 (0)	0 (0)	0 (0)	0.613
MDR[b]	31 (93.9)	29 (96.7)	7 (100.0)	21 (95.5)	0.890
XDR[b]	24 (72.7)	25 (83.3)	7 (100.0)	21 (95.5)	0.089[1]
PDR[b]	3 (9.1)	4 (13.3)	1 (14.3)	7 (31.8)	0.148[1]

Abbreviations:APACHE II, Acute Physiology and Chronic Health Evaluation II; SOFA, Sequential Organ Failure Evaluation Score; CAD, coronary artery disease; DM, diabetes mellitus; HT, hypertension; CRP, C-reactive protein; LOS, length of stay; VAP, ventilator-associated pneumonia; MO, microorganisms; MDR, multi-drug resistant; XDR, extensively drug resistant; PDR, pun-drug resistant. [a]Values are mean±SDs. [b]Values are n (%). *Statistical significance. [1]XDR and PDR were higher in corticosteroid plus tocilizumab group than no immunosuppressive therapy group (p=0.032)

There was no difference in mortality between the tocilizumab and placebo groups in the randomized controlled study of Stone et al. (22). However, the development of serious infections was lower in the tocilizumab arm compared to placebo (8.1% vs. 17.1%) (22). On the contrary, superinfection was found more frequently in the tocilizumab arm compared to the control group (54% vs. 26%) in the study by Somers et al. (15). In the CoDEX study, there was no difference in secondary infection between dexamethasone and standard treatment groups (21.9% vs. 29.1%) (23). In the study by Naik et al. (24), secondary infection was roughly 5.5-fold greater in the high-dose dexamethasone group than in the tocilizumab group. In current study, secondary infection was more common in the group that got immunosuppressive therapy than in the group that did not (48.4% vs. 29.2%). In the subgroup analysis, it was highest in the corticosteroid plus tocilizumab group (73.3%), followed by tocilizumab only (43.8%) and corticosteroid only (39.5%).

According to the pre-pandemic statistics, *Acinetobacter Baumanii* was responsible for roughly 47% of VAP development in the ICU (25). Giacebbo et al. (26) reported 77 culture positivities (45%) in 171 VAP and COVID-19 patients. *Pseudomonas Aeruginosa* (35%) was found to be the most often isolated microorganism among the growing microorganisms (26). In their study evaluating MDR growth, Baiou et al. (27) discovered Stenotrophomonas maltophilia (24.5%) and *Klebsiella pneumonia* (23.5%) most commonly. Karatas et al. (28) identified *Acinetobacter Baumanii* as the most prevalent respiratory infection pathogen among COVID-19 patients, and concluded that the prevalence of MDR *Acinetobacter Baumanii* increased during the pandemic compared to the pre-pandemic period. In our study, microbiological culture positive was observed in 92 (39.1%) of the patients, with *Acinetobacter Baumanii* (47.8%) being the most common pathogen found. This was followed by *Klebsiella pneumonia* in 38% of the patients and *Pseudomonas Aeruginosa* in 8.7%. There was no relationship between immunosuppressive therapy and microorganisms.

When MDR infections trigger the development of VAP in the ICU, mortality might reach up to 60% (29). Bentivegna et al. (30) evaluated MDR infections from 2017 to 2020 and found a decrease in MDR infections during the pandemic era compared to the pre-pandemic times. This was assumed to be due to hand washing and the usage of personal protective equipment. During the pandemic, however, MDR infections were higher in COVID-19 clinics than in non-COVID clinics (29% vs 19%) (30). Baiou et al. (27) investigated the link between the development of MDR infection and immunosuppressive therapy and found that corticosteroid and tocilizumab treatments were not associated with the development of MDR. In current study, there was no difference in MDR and PDR between groups that got and did not receive immunosuppressive therapy, however the development of XDR was higher in the group that received immunosuppressive therapy compared to those who did not (89.8% vs. 72.7%). In the subgroup analysis, the development of XDR and PDR was higher in the corticosteroid plus tocilizumab group than in those who did not receive immunosuppressive therapy.

The limitations of the study were that it was single-center and retrospective. Furthermore, no microorganisms other than respiratory bacterial pathogens were evaluated in this study.

CONCLUSIONS

Immunosuppressive therapy may increase the development of VAP, the risk of secondary infection, the growth of bacteria resistant to at least one drug, and the length of stay in hospital and ICUs in patients with critically ill COVID-19 pneumonia. Furthermore, when corticosteroid and tocilizumab were used together, it was determined that the proliferation of XDR and PDR microorganisms may be increased. Although there was no difference in mortality between groups, it was determined that when immunosuppressive therapy was used, targeted antibiotics and longer-term antimicrobial therapy might be required.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was approved by the Ethics Committee of İstanbul Ümraniye Training and Research Hospital (Date: 26.05.2022, Decision No: 167).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Conflict of Interest: The authors have no conflict of interest to declare.

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Author Contribution: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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Examining social media and academic social network use, and trends in physician-patient communication via social media: a national study

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ABSTRACT

Introduction: In the history of the internet, social media occupy an exceptional place because they bring about sociological changes and cause things that will influence the course of history. It has become inevitable to conduct a study that examines the changes in the relationship between academic social networks and online patient-physician relationships, which have become widespread in recent years, especially among physicians. This study attempted to address this deficiency.

Material and Method: An online survey was created on Google Forms that included questions about physicians' use of social and academic media networks and their communication habits with online patients. Age, gender, medical specialty and workplace, social media use, academic social networks usage, and relationships with patients via social media were analyzed.

Results: Daily social media usage was significantly associated with age and medical specialty. Participants aged 40-50 and Basic Medic Science Consultants were least likely to use social media. The use of Facebook was the lowest among those under 30 (12.2%). Among those under 30, the use of LinkedIn was deficient (2.0%). Google Scholar was the most frequently used academic social network (38.5%). Surgical specialists were more likely to share medical content. Under 30 and over 50 were more likely to share their medical titles on social media than other groups. The percentage of those who reported having also physically examined the patient during online communication was 64.5%. This high rate is by no means negligible. Patients' most frequent responses to online communication requests were via WhatsApp (80.3%). The under-30 age group was found to have less contact with patients on social media.

Conclusion: According to the results of the study, the use of the academic social network is lower than expected, even among academically active participants. The fact that Facebook usage is significantly lower among those under 30 suggests that Facebook is outdated as a social medium for young physicians. Participants in university hospitals, private clinics, and those under 40 use social media differently than other groups. More online patient communication is an important advance. It is also significant that the number of studies has increased after online communication. If investments are made in this topic, it can be expected that a substantial part of patient-doctor relationships will be handled online soon. However, social media studies wear out quickly, so they should be repeated frequently.

Keywords: Social media, academic social network, physician-patient communication

INTRODUCTION

At the University of California, LA, Charley Kline attempted to establish a connection between two computers through the servers of ARPANET at 10:30 p.m. on October 29, 1969 (1). The attempt, which ended with "LO..." (Login), is considered the first internet connection (first online connection). The Big Bang of the internet was not long ago. However, technological development has led to a sociological situation that exceeds all expectations. Over the past decade, the single front-runner in this regard has been social media and the social life it has shaped. No one underestimates the impact of social media anymore, claiming that it is a virtual environment.

The internet is an online network where computers can communicate. Although initial purpose of internet was military and national security concerns, scientists later developed it with the foresight that it would create an infrastructure for more specific and advanced applications. The additions and developments of the following years are filled with stories about internet use and facilitating access to information. Its availability via mobile data networks and cell phones makes its potential power undeniable. It is

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expected that a technology that has penetrated so far into human life will also influence social life. Therefore, social media is a natural concomitant of the internet. Social media platforms for academics can also be seen as another natural consequence of social media.

The modern social media platforms (Facebook, LinkedIn, Twitter, and Reddit) established after 2003 had a structure that allowed people to reach each other easily. With the new generation of social media, sharing pictures, videos, and audio is more important, such as Instagram, Pinterest, Snapchat, and TikTok, which emerged after 2010 (2). Regardless of these developments, the evolution of Academic Social Networks (ASNs) is older than previously thought. ASNs were a necessity long before social media. However, with the spread of social media, the gap was filled with Researchgate and Academia, founded in 2008 (3).

Although there are several articles on physicians' use of social media, use of ASNs, and communication between patients and physicians via such social media, no paper simultaneously examines social media, ASNs, and online communication with patients. This study aims to fill this gap.

MATERIAL AND METHOD

The study was conducted under the Declaration of Helsinki and approved by the Institutional Ethics Committee of Samsun Training and Research Hospital (Date: 01.12.2021, Decision No: GOKA/2021/19/1). All procedures were carried out under the ethical rules and the principles of the Declaration of Helsinki.

The study data were collected prospectively using a 15-question questionnaire created with Google Forms (4). Invitations were sent to the physicians to participate in the study between January 15, 2022, and February 20, 2022, via WhatsApp, Facebook, and e-mail groups.

Gender, length of service, age (under 30 years, 30-40 years, 40-50 years, over 50 years), medical specialties (General Practitioner (GP), Family Physician (FP), Occupational Medicine Physician (OMP), Basic Medical Science Physician (BMSP), Clinical Medical Science Physician (CMSP), Surgical Medical Science Physician (SMSP), Research Assistant (RA), Academic Staff (AS)), and the institutions and locations where they work (Public Hospital and Health Institution (PHHI), University Clinic (UC), Educational and Research Hospital and Clinic (EEHC), Private Hospitals and Private Clinic (PHPC)) were recorded.

Participants were asked about their daily use of social media (less than 1 hour, 1-2 hours, 2-3 hours, and more than 3 hours). They were asked about their social media accounts (WhatsApp, Facebook, Instagram, YouTube, and Twitter) and academic social networks (LinkedIn, Vumedi, Researchgate, Academia, and Google Scholar). Participants were asked if their social media accounts had a medical title. It was also investigated whether another social media account was used for medical activities.

Likert scale questions were used to examine whether they shared medical information, medical images, and workplace. Communication with patients via social media and responses to their questions were discussed. The social media through which patients were contacted was examined. It was questioned whether the patients with online communication were subsequently reviewed and treated using traditional methods.

Statistical Analysis

It was decided to use only age data because the length of service and age on the form referred to the same demographics. The data from 361 individuals who agreed to participate in the study were transferred to a data table and then statistically were analyzed using the SPSS 26 software (IBM Corp., Armonk, NY, USA).

Frequencies and frequency tables were created for all variables. Descriptive statistics explored. The relationship between the duration of social media use and gender, age, specialization, and workplace was examined. The relationship between the academic social network and social media account usage and gender, age, specialization, and workplace was examined. The relationship between online medical sharing with gender, age, specialty, and workplace was examined. The relationship between gender, age, specialty, and workplace was examined regarding whether they communicated with patients via social media and received care via social media. The Mann-Whitney U test and the Kruskal-Wallis test were used for data analysis. The one-way ANOVA and the Tamhane test were used for post hoc analysis. The relationship between questions with multiple responses and other variables was analyzed by cross-tabulation.

RESULTS

Of the 361 individuals who participated in the study, 163 were women (45.2%), and 198 were men (54.8%)-the distribution by age, specialty, and place of work is shown in **Table 1**. Comparing the data in the Turkish Health Statistics in 2019, (the latest published study) from the Ministry of Health of the Republic of Turkey (5) with the distributions in our research, we can assume that the study series reflects physicians throughout Turkey (**Table 2**). It was found that daily use of social media was significantly related to age (P< .000) and specialty (P=.003) but not to gender (P=.541) and workplace (P=.333). Among the groups, it was found that those aged 40-50 had the lowest daily use of social media. Compared to the other groups, participants under 40 had higher social media use (**Table 3**). Posthoc analyses showed that BMSCs used social media significantly less than the other groups, and those RAs used social media the most, considering their areas of expertise.

Table 1. Percentages of gender, age, specialization, and workplace

Gender	n	%
Female	163	45.20%
Male	198	54.80%
Age		
30↓	49	13.60%
30-40	38	10.50%
40-50	121	33.50%
50↑	153	42.40%
Specialty		
GP,FP,OMP	86	23.80%
BMSP	13	3.60%
CMSP	83	23%
SMSP	102	28.3
RA	56	15.50%
AS	21	5.80%
Workplace		
PHHI	90	24.90%
UC	72	19.90%
EEHC	99	27.40%
PHPC	100	27.70%

Note. General Practitioner (GP), Family Physician (FP), Occupational Medicine Physician (OMP), Basic Medical Science Physician (BMSP), Clinical Medical Science Physician (CMSP), Surgical Medical Science Physician (SMSP), Research Assistant (RA), Academic Staff (AS), Public Hospital and Health Institution (PHHI), University Clinic (UC), Educational and Research Hospital and Clinic (EEHC), Private Hospitals and Private Clinic (PHPC)

Table 2. Comparison of national data with study data

	Study Data		Turkish Health Statistic 2019*	
	n	%	n	%
GF, FP, OMP	86	23.8	46843	29.1
BMSP, CMSP, SMSP, AS	219	60.7	85199	53
RA	56	15.5	28768	17.9
	361		160810	
	n	%	n	%
PHHI, EEHC	189	52.4	97145	60.4
UC	72	19.9	33750	21
PHPC	100	27.7	29915	18.6
	361		160810	

General Practitioner (GP), Family Physician (FP), Occupational Medicine Physician (OMP), Basic Medical Science Physician (BMSP), Clinical Medical Science Physician (CMSP), Surgical Medical Science Physician (SMSP), Research Assistant (RA), Academic Staff (AS), Public Hospital and Health Institution (PHHI), University Clinic (UC), Educational and Research Hospital and Clinic (EEHC), Private Hospitals and Private Clinic (PHPC). * Turkish Health Statistics 2019 (the latest published study) by the Ministry of Health of the Republic of Turkey

Table 3. Age-social media usage crosstabulation

			less than 1 hour	1-2 hour	2-3 hour	over 3 hour	
Age	under 30	n	2	22	20	5	49
		%	4.10%	44.90%	40.80%	10.20%	100.00%
	30-40	n	6	11	15	6	38
		%	15.80%	28.90%	39.50%	15.80%	100.00%
	40-50	n	23	73	17	8	121
		%	19.00%	60.30%	14.00%	6.60%	100.00%
	over 50	n	26	67	29	31	153
		%	17.00%	43.80%	19.00%	20.30%	100.00%
Total		N	57	173	81	50	361
		%	15.80%	47.90%	22.40%	13.90%	100.00%

There was no significant difference between genders when analyzing responses to the question of having medical identities and medical positions (medical titles) on their social media accounts (P=.107). However, there was a significant association between age (P<.000), specialty (P=.027), and work location (P=.050). In posthoc analyses, participants under 30 and over 50 were more likely to report their job titles on social media than other groups (**Table 4**). Among other groups, SMSPs used significantly fewer medical titles than others did. Similarly, PHPC and OMP used significantly fewer medical titles on social media.

Table 4. Age-medical title sharing crosstabulation

			Yes	No	
Age	Under 30	n	15	34	49
		%	30.6%	69.4%	100.0%
	30-40	n	17	21	38
		%	44.7%	55.3%	100.0%
	40-50	n	61	60	121
		%	50.4%	49.6%	100.0%
	Over 50	n	90	63	153
		%	58.8%	41.2%	100.0%
Total		N	183	178	361
		%	50.7%	49.3%	100.0%

The reliability of questions about sharing medical information and medical images on social media accounts was examined and found to have Cronbach α= .648. After reviewing the habit of sharing medical posts on social media, it was found that there was no association with gender (P=.484). They were found to differ significantly by age (P=.006), specialty (P<.000), and workplace (P< .000). Post-hoc reviews found that under-30s posted less medical content on social media than over-40s. According to their specialization, BMSCs and ASs share minor than the others. SMSCs share more with all other groups. CMSPs share more than other groups (compared with RAs, BMSPs, and AS). Groups working in PHPC share more than groups working in EEHC and UCs (**Table 5**).

When examining the responses to the questions about answering medical questions from patients on social media and examining these patients, it was found that there was no association with gender (P=.606). However, it was found that there were differences between age (P <.000), specialties (P <.000), and workplace (P <.000) in responding to patient questions on the internet and the personal relationship between the patient and the physician. 63/361 (17.5%) of physicians reported that they never responded to communication requests from online patients. The under 30-age group was found to have less contact with patients via social media than all other groups. The 30-to-40-year-old group has fewer

relationships with patients via social media than the 40-and-older group. GPs had less contact with patients via social media than CMSPs and SMSPs, and RAs had less contact with patients than other groups. Participants from UCs and EEHC reported less contact with patients online than other groups, and those who worked in PHPC did more than others (**Table 6**). The proportion of those who reported that they also physically examined the patient with communication online was 233/361 (64.5%). This rate is by no means negligible.

Table 5. According to medical specialty, a comparison of physicians' habits of sharing medical information and photos on their social accounts

Multiple comparisons					
	Mean difference	Std. error	Sig.	95% Confidence interval	
				Lower bound	Upper bound
GF, FP, OMP					
BMSP	0.28205	0.13962	0.590	-0.1850	0.7491
CMSP	-0.25301	0.09704	0.140	-0.5414	0.0353
SMSP	-.27124*	0.09139	0.050	-0.5423	-0.0002
RA	0.11905	0.08514	0.932	-0.1346	0.3727
AS	0.23810	0.12125	0.586	-0.1414	0.6176
BMSP					
GF, FP, OMP	-0.28205	0.13962	0.590	-0.7491	0.1850
CMSP	-.53506*	0.14431	0.019	-1.0100	-0.0601
SMSP	-.55329*	0.14057	0.013	-1.0218	-0.0848
RA	-0.16300	0.13659	0.986	-0.6259	0.2999
AS	-0.04396	0.16158	1.000	-0.5633	0.4753
CMSP					
GF, FP, OMP	0.25301	0.09704	0.140	-0.0353	0.5414
BMSP	.53506*	0.14431	0.019	0.0601	1.0100
SMSP	-0.01823	0.09840	1.000	-0.3103	0.2739
RA	.37206*	0.09263	0.001	0.0960	0.6481
AS	.49111*	0.12662	0.005	0.0985	0.8838
SMSP					
GF, FP, OMP	.27124*	0.09139	0.050	0.0002	0.5423
BMSP	.55329*	0.14057	0.013	0.0848	1.0218
CMSP	0.01823	0.09840	1.000	-0.2739	0.3103
RA	.39029*	0.08668	0.000	0.1325	0.6481
AS	.50934*	0.12234	0.003	0.1274	0.8913
RA					
GF, FP, OMP	-0.11905	0.08514	0.932	-0.3727	0.1346
BMSP	0.16300	0.13659	0.986	-0.2999	0.6259
CMSP	-.37206*	0.09263	0.001	-0.6481	-0.0960
SMSP	-.39029*	0.08668	0.000	-0.6481	-0.1325
AS	0.11905	0.11774	0.997	-0.2526	0.4907
AS					
GF, FP, OMP	-0.23810	0.12125	0.586	-0.6176	0.1414
BMSP	0.04396	0.16158	1.000	-0.4753	0.5633
CMSP	-.49111*	0.12662	0.005	-0.8838	-0.0985
SMSP	-.50934*	0.12234	0.003	-0.8913	-0.1274
RA	-0.11905	0.11774	0.997	-0.4907	0.2526

*. The mean difference is significant at the 0.05 level. General Practitioner (GP), Family Physician (FP), Occupational Medicine Physician (OMP), Basic Medical Science Physician (BMSP), Clinical Medical Science Physician (CMSP), Surgical Medical Science Physician (SMSP), Research Assistant (RA).

Table 6. Comparing physicians' habits of communicating and answering online patient questions depending on where they work.

Multiple Comparisons					
	Mean Difference	Std. Error	Sig.	95% Confidence Interval	
				Lower Bound	Upper Bound
PHHI					
UC	.49167*	0.13820	0.003	0.1227	0.8607
EEHC	.39697*	0.11652	0.005	0.0871	0.7068
PHPC	-.63778*	0.11719	0.000	-0.9494	-0.3262
UC					
PHHI	-.49167*	0.13820	0.003	-0.8607	-0.1227
EEHC	-0.09470	0.13992	0.984	-0.4681	0.2787
PHPC	-1.12944*	0.14048	0.000	-1.5043	-0.7546
EEHC					
PHHI	-.39697*	0.11652	0.005	-0.7068	-0.0871
UC	0.09470	0.13992	0.984	-0.2787	0.4681
PHPC	-1.03475*	0.11922	0.000	-1.3516	-0.7179
PHPC					
PHHI	.63778*	0.11719	0.000	0.3262	0.9494
UC	1.12944*	0.14048	0.000	0.7546	1.5043
EEHC	1.03475*	0.11922	0.000	0.7179	1.3516

*. The mean difference is significant at the 0.05 level. Note. Public Hospital and Health Institution (PHHI), University Clinic (UC), Educational and Research Hospital and Clinic (EEHC), Private Hospitals, and Private Clinic (PHPC).

While WhatsApp was the most used by 352/361 (97.5%), Twitter was considered the least used social media by 154/361 (42.7%) (**Table 7**). When the distribution by gender was examined, there was no difference between WhatsApp, Facebook, and Instagram users, but it was found that the use of Twitter and YouTube was more common among men. It was calculated that Twitter was used 1.82 times higher (48/154, 29.4% women, 106/154, 53.5% men) and YouTube 1.3 times higher (94/242, 57.7% women, 148/242, 74.7% men) by men. Facebook usage was lowest among those under 30 (6/49, 12.2%). Among YouTube users, the under-30 age group had the highest frequency at 45/49 (90.8%), while the 40-50 age group had the lowest frequency at 59/121 (48.8%). The percentage of RAs was lowest among Facebook users 10/56 (17.9%). RAs 45/56 (80.4%) and SMSPs 85/102 (83.3%) had higher Instagram usage rates. ASs 17/21 (81.0%) and RAs 53/56 (94.6%) were the most frequent users among YouTube users and Twitter users 34/56 (60.7%). Those working at UCs had the lowest Facebook use, 24/72 (33.3%). University physicians used Instagram 58/72 (80.6%), and YouTube 65/72 (90.3%) were the top usage scores. Twitter was used the least by PHPC participants 26/100 (26.0%).

The most striking thing about using ASN is that Vumedi was never indicated as an answer. Google Scholar was

the most frequently used, 139/361 (38.5%). Those who never used ASN were 158/361 (43.8%). Among men, Researchgate was used 1.72 times higher (21/65, 12.9% women, 44/65, 22.2% men) and Academia 2.34 times higher (19/73, 11.7% women, 54/73, 27.3% of men). Physicians under 30 were particularly low on LinkedIn 1/86 (2.0%). Among those over 50, Researchgate was the least used ASN 19/65 (12.4%). It was found that the under 40 groups used Google Scholar frequently 52/87 (59.77%). 16/56 (28.6%) of RAs and 3/21 (14.3%) of AS did not use ASN. BMSPs use only LinkedIn 7/13 (53.8%) and Google Scholar 13/13 (100.0%). Among those who did not use ASN, the percentage was highest in the group of GP, FP, and OMP 56/86 (65.1%) **Table 8**.

Table 7. Percentages of social media and academic social network usage

	n	%	Digital2022*
Social media			
WhatsApp	352	97.50%	81%
Facebook	202	56.00%	76%
Instagram	251	69.50%	83%
Twitter	154	42.70%	61%
Youtube	242	67.00%	90%
Academic social media			
LinkedIn	86	23.80%	32%
Researchgate	65	18.00%	
Academia	73	20.20%	
Google Scholar	139	38.50%	
Vumedi	0	0.00%	
None	158	43.80%	

Note. *Digital2022 (weaeresocial.com): Turkey Social Media Usage 2022

We can say that UC participants use all ASN accounts except LinkedIn (Researchgate 30/72, 41.7%, Academia 24/72, 33.3%, Google Scholar 53/72, 73.6%). We found that physicians in PHPC ranked last in the usage of Researchgate 4/100 (4.0%) and Academia 11/100 (11.0%).

The percentage of physicians who did not have a separate social media account for medical activities was 252/361 (69.8%). The least preferred account types were YouTube 12/361 (2.4%) and Twitter 9/361 (2.5%). Personal websites 65/361 (18.0%) and Instagram 57/362 (15.8%) were the preferred individual medical social media accounts. Men's preference for medical social media was 2.1 times higher for Facebook (13/46, 8.0% women, 33/46, 16.7% men) and 1.8 times higher for Instagram (18/57, 11.0% women, 39/57, 19.7% men). Those under 40 who did not have an additional medical social media account were 78/87 (89.7%). It was noted that nine individuals with a Twitter account and eight with a YouTube account were between 40 and 50 years old. It was found that there were no medical social media accounts among BMSPs and AS. Only one person had such an account among RAs, and 69/72 (95.8%) of the UC participants had no other social media accounts. 40/100 (40%) of participants in PHPC had personal websites.

The patients' most frequent responses to online communication requests were via WhatsApp 290/361 (80.3%). All nine doctors who interacted with patients on Twitter were over 50 years old and female. CMSPs 76/83 (91.6%) and SMSPs 94/102 (92.2%) were most likely to communicate with patients online. Only half of the RAs prefer to answer patient questions. UC physicians 25/72 (34.7%) and physicians in EEHC 20/99 (20.2%) are least likely to respond.

Table 8. Academic social media usage crosstabulation for speciality

		Speciality-ASN Crosstabulation					
		LinkedIn	Researchgate	Academia	Google Scholar	None	
Specialty							
GF, FP, OMP	n	23	0	7	10	56	86
	%	26.70%	0.00%	8.10%	11.60%	65.10%	
BMSP	n	7	0	0	13	0	13
	%	53.80%	0.00%	0.00%	100.00%	0.00%	
CMSP	n	30	12	11	28	38	83
	%	36.10%	14.50%	13.30%	33.70%	45.80%	
SMSP	n	22	22	34	34	45	102
	%	21.60%	21.60%	33.30%	33.30%	44.10%	
RA	n	1	14	9	38	16	56
	%	1.80%	25.00%	16.10%	67.90%	28.60%	
AS	n	3	17	12	16	3	21
	%	14.30%	81.00%	57.10%	76.20%	14.30%	
Total	N	86	65	73	139	158	361

Note. General Practitioner (GP), Family Physician (FP), Occupational Medicine Physician (OMP), Basic Medical Science Physician (BMSP), Clinical Medical Science Physician (CMSP), Surgical Medical Science Physician (SMSP), Research Assistant (RA), Academic Staff (AS).

DISCUSSION

The main shortcoming of the study is that it targets groups that already use the internet and social media. The Google form used in this study and the way this form is submitted to the relevant pollsters is online, so participants who already use the internet and social media can participate in this study. Therefore, although the study appears to have a weakness in this regard, we do not believe that it is a weakness. We still cannot reach physicians who do not use the internet or do not use social media, and we cannot know if they have an idea about ASN. Nevertheless, it does not seem possible that a physician not already using traditional social media could know about ASN. Therefore, the study has no weak points in this regard.

Comparing the latest published data from the Ministry of Health of the Turkish Republic in 2019 with the distribution in the study group, we think that the physician series in the study reflects the distribution of physicians in Turkey in a national sense, and the data can be considered nationally significant. For this reason, we think that with this study, physicians in Turkey provide information about social media use, ASN use, and online communication with patients across the country.

The wearesocial.com website publishes annual statistics on global internet usage, social media usage, internet advertising, and brands. Looking at the January 2022 data, 67.1% of the world's population (7.91 billion) has at least one phone. In addition, 62.5% are internet users, and 58.4% are social media users. The average daily use of social media in Turkey was calculated to be 2 hours and 27 minutes. It can be concluded that physicians use social media less than the general average, as the option of using social media for 1-2 hours is the most common response with 47.9% (6).

37/49 (75.5%) of those under 30 are RAs, 37/56 (66.1%) of RAs are under 30, and 12/38 (31.6%) are in the 30-40 age group, suggesting that the data derived from those under 30 should be strongly associated with RAs. When determining demographic groups in internet and social media use studies, it is common to examine the (18-29), (30-49), (50-64), and over 65 age groups. However, we found that the age groups under 30, (30-40), (40-50), and over 50, which we compiled according to our study and occupational group, did not match the data in this routine demographic structure. In particular, the data for the 40-50 age group was inconsistent in general social media use studies in the 30-49 age group. We attribute this to the study being conducted with a specific occupational group.

We can assume that the under-30s and RAs clearly state their medical identities and titles on their social media accounts. Physicians in this group have the lowest rate of additional social media accounts for their medical activities. We believe they do not mind disclosing their medical identities on their primary social media accounts. We think the low rate of medical identity and title usage in PHPC is that physicians working there do not see the need to disclose such a medical identity because private hospitals already engage in the promotional activities. This is also supported by the high rates of using additional social media accounts for medical activities in this group. There is no meaningful answer that SMSPs share fewer medical identities and titles. 40% of this group work in private hospitals, which could be an answer for the reasons already explained. However, although 41/102 (40.2%) of CMSPs work in private hospitals, no such underutilization was found. Thus, there is no statistically significant explanation.

Men are 1.3 times more likely to prefer YouTube and 1.82 times more likely to use social media than women (YouTube male 53.9%, female 46.1%, social media usage male 56.4%, female 46.1%); these findings are not consistent with previous findings, but they are significant. Among the under 30 years old (12.2%) and RAs (17.9%), these usage rates are much lower than the use of social media on Facebook in Turkey (76%). We can interpret this to mean that Facebook is now a stale platform for the younger generation of physicians.

Oge et al. (7) and Imran MK et al. (8) published data showing that social media can be used for information sharing and exchange in social sciences. Brown J et al. (9) have pointed out the ethical problems in professional relationships in patients' online communications with physicians after the proliferation of social media. The fact that von Muhlen M et al. (10) published their review paper on social media in 2012 and that only Facebook and Twitter were the main topics means that, given the rapid developments in social media and on the internet, even a 10-year-old article that can be considered new is outdated.

The purpose of academic and educational use of social media is quite different from that of traditional social media. Academic staff, who have long used various social media platforms, began to require more specific social media options when those platforms were insufficient. For this reason, ASN platforms have found many users over the past decade. The platforms that correspond to ASN and have been used more frequently in recent years, Academia, Reserachgate, LinkedIn, Vumedi, and Google Scholar, could be included in the study questions. It is not possible to be a unique platform that meets all needs. Communication and sharing are possible with the forum options at Researchgate. Since LinkedIn is not only a medical and academic network that offers a broader range

of network usage. Instant direct messaging on LinkedIn provides more benefits than other academic network platforms by creating standard networks with other professionals. Google Scholar features easy accessibility to all other acquaintances across the Google platform.

It is remarkable that physicians participating in the study do not use the Vumedi platform, which has more than 450 thousand members among physicians (11) and where educational videos and presentations are shared. It is significant that LinkedIn, a platform specializing only in business relationships and sectoral networks, is hardly used by physicians under 30. This can partly be explained because the platform has a sectoral rather than academic infrastructure. Understandably, the private hospitals and clinics group has very few members, as platforms such as Researchgate and Academia are focused on sharing scientific articles, as shown in the paper by Meishar-Tal et al. (12). Academic concerns are not the primary purpose of private hospitals. It is hugely concerned that two out of seven residents and one out of six faculty members do not use ASN. 18/77 (23.4%) of RAs and AS expected to be closer to academic life do not use ASN. This can be explained in two ways: Awareness of ASN is not yet sufficiently developed, or academic members continue to build their academic networks using traditional methods.

About a third of physicians have a second social media account for their medical activities. Nearly half of surgical physicians require social media accounts for their additional medical activities. It is a well-known fact that surgical physicians have been using social media visually in recent years. Accordingly, the need for other media accounts is higher in this group.

There is a clear boundary between physicians under 40 and physicians over 40 when answering patients' medical questions on social media. Those under 40 are the least responsive group, which can be explained by the high percentage of RAs in this group. The high proportion of PHPC in these groups may explain that CMSPs and SMSPs communicate more with patients online than BMSPs. The fact that commercial interests can explain why PHPC participants share more than other groups.

CONCLUSION

The study shows that the use of ASN is still insufficient. The high rates of online communication between patients and physicians suggest that more social networking will soon be needed in the medical field than expected. The social media behaviors of physicians under 40 and physicians working in university clinics and private hospitals differ significantly from other groups. Studies with broad participation and deeper analysis are needed to confirm this information and conduct detailed analyzes. However, it is clear that the data is valid only for Turkish physicians, and further studies in broader regions are needed. It should be understood that the information in this study will be outdated in ten years at the latest, and such studies should be conducted routinely.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was approved by the Institutional Ethics Committee of Samsun Training and Research Hospital (Date: 01.12.2021, Decision No: GOKA/2021/19/1).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

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Author Contributions: The author declares that he has all participated in the paper's design, execution, and analysis and that he has approved the final version.

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Is it as harmless as it appears? Thoracic traumas caused by Pat-Pat accidents

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ABSTRACT

Aim: This study examines the morbidity and mortality of chest traumas due to Pat-Pat accidents, which is one of the most frequently used motor vehicles in agriculture, especially in mountainous regions in developing countries.

Material and Method: This retrospective study included 57 patients who were followed up in a single center between November 2018 and 2021 for thoracic trauma due to a Pat-Pat accident. Patients' age, gender, position in the vehicle, trauma mechanism, trauma location and time, trauma-related pathologies, treatments, and length of stay in hospital and intensive care unit were examined.

Results: The cases included 44 (77.2%) men and 13 (22.8%) women with a mean age of 49.93±20.9 years. Of the accidents, 54 (94.7%) occurred on rural roads, 35 (61.4%) occurred on weekdays, 29 (50.9%) occurred in spring, and 20 (35.1%) occurred in summer. The cases consisted of 37 (64.9%) drivers and 20 (35.1%) passengers, of which 31 (54.4%) were injured due to collision and 26 (45.6%) were injured due to vehicle overturning and being ejected from the vehicle. All cases had rib fractures, 8 (14%) had sternum fracture, 25 (43.9%) had pneumothorax, 36 (63.2%) had hemothorax, 22 (38.6%) had pulmonary contusion, and 2 (3.5%) had cardiac contusion. While 19 (33.3%) of the cases were discharged after evaluation and treatment in the emergency department, 30 (52.6%) were treated in the ward, and 8 (14%) were treated in the intensive care unit (ICU). Thirty-three patients underwent a surgical procedure. The mean hospital stay was 7.8 days, and the mean ICU stay was 5.47 days. Mortality developed in 3 (5.3%) cases. The rates of ICU admission and mortality were found to be higher in injuries caused by being ejected from the vehicle compared to injuries caused by impact ($p<0.05$).

Conclusion: Pat-Pat accidents cause severe thoracic trauma. In these vehicle accidents with no significant safety precautions, morbidity and mortality are quite high, especially in thoracic trauma caused by ejection from a vehicle.

Keywords: Agriculture vehicles, Pat-Pat, thoracic surgery, trauma

INTRODUCTION

Agricultural vehicle accidents are one of the leading causes of accident-related morbidity and mortality in developing countries such as Turkey (1,2). Simple agricultural tools, which provide great convenience with their use in agricultural production, also cause severe trauma due to the easy access of untrained careless people (3). Accidents involving a vehicle called "Pat-Pat", which is used both in agricultural production and worker transportation, are increasingly observed in harsh natural conditions, especially in rural hilly areas such as the Eastern Black Sea Region of Turkey (4,5).

The Pat-Pat vehicle gets its name from the loud running noise of its engine. In fact, the 2-wheeled front part that houses the engine is a simple digging-riding tool used in gardening (**Figure 1a**). Adding a simple chassis to this engine part, placing a driver's seat, and adding a 2-wheel trailer or chassis to the rear create a simple 4-wheel tractor (**Figure 1b**). There is no surrounding hull or roof structure for safety (4,6). Pat-Pat is difficult to use due to low vehicle stability and handling ability, weak braking system, and simple steering system. There is no active or passive safety equipment to protect passengers against accidents. There is no driver's license requirement for its use, nor is a vehicle license required. In essence, the Pat-Pat is similar to the all-terrain vehicles (ATVs) in the world that were originally designed for agricultural use. ATVs are 4-wheel drive, and vehicle stability is much more advanced than Pat-Pats. In addition, unlike ATVs, Pat-Pats consist of two parts; the 2-wheel engine at the front and the trailer structure at the rear create a high

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level of instability and frequently cause accidents (7). These vehicles cost much less than ATVs, and they can be used for transporting workers or digging, planting, and spraying by attaching different apparatus (4). It is used especially in the Black Sea region as well as in rural areas in other parts of Turkey. Apart from Turkey, it is also used as an agricultural tool in Russia, Pakistan, Afghanistan, and Macedonia (8). Accidents involving this vehicle do not usually occur during its use as an agricultural tool in the garden. Pat-Pat, which is forbidden for travel on the highway, is used intensively in the transportation of workers and cargo, especially during hazelnut cultivation. Lack of safety measures and roof structure, being thrown from the vehicle during the accident, being under the overturned vehicle, and collisions are the most critical injury mechanisms in Pat-Pat accidents. These accident mechanisms cause chest trauma, which causes significant morbidity and mortality. There are few studies on pat-pat accidents in the literature. While some of these studies describe trauma mechanisms, some describe orthopedic injuries. There is no study in the literature on isolated chest trauma, which is a significant cause of morbidity and mortality in pat-pat accidents. This study investigates the morbidity and mortality rates of thoracic injuries in Pat-Pat accidents.

Figure 1. The design of the Pat-Pat vehicle **(a)** The two-wheeled Pat-Pat, which is designed as simple gardening digging-riding equipment. **(b)** A simple chassis, seats, and a 2-wheel trailer are added to the back to obtain a simple four-wheel drive Pat-Pat vehicle. (The vehicle in the photos belongs to the author's father and the author took these photographs himself. The author has the right to use the photographs.)

MATERIAL AND METHOD

This study was approved by the Ordu University Clinical Research Ethics Committee (Date: 05.11.2021, Decision No: 229). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

This study was designed in a tertiary hospital in the Black Sea region of Turkey, where the Pat-pat vehicle is widely used. Approximately 600 traffic accident patients are followed and treated annually in this center, especially during the intense agricultural and tourism months.

All patients with chest trauma brought to the emergency department after a traffic accident between November 2018 and November 2021 were reviewed retrospectively. Cases were reported as pat-pat accidents in epicrisis, thoracic surgery consultation notes, and forensic reports were recorded. The study did not include patients whose required data could not be obtained. The study included 57 patients with thoracic trauma. Cases with extra-thoracic injuries were excluded from the study. Cases were examined in terms of age, gender, whether the accident occurred on weekdays or weekends, seasonal distribution, whether the accident occurred in a rural or central location, the mechanism of injury, whether the injured was a driver or passenger, Revised Trauma Score (RTS) at the time of admission to the emergency department, the rate of continuation of treatment after discharge in the ward or the intensive care unit (ICU), presence of rib and sternum fracture, presence of pneumothorax hemothorax and pulmonary-cardiac contusion, type of treatment and type of surgical intervention, length of hospital stay, duration of work power loss, and mortality rate. The RTS used in the study is a combined scoring system created by adding the systolic blood pressure and respiratory rate parameters to the Glasgow Coma Scale and is frequently used today (9).

Statistical Analysis

Statistical analysis was performed using the "IBM SPSS Statistics for Windows Version 22.0 (Statistical Package for the Social Sciences, IBM Corp., Armonk, NY, USA)" program. Descriptive statistics were presented with frequency and percentage for categorical variables and mean and standard deviations for numerical variables. Independent group analyses were performed using Mann-Whitney U and Student's T-test, and a $p<0.05$ value was considered significant.

RESULTS

Baseline patients' characteristics are given in **Table 1**. Forty-four male and 13 female patients with thoracic trauma after a Pat-Pat accident were included in the study. The mean age of the patients was 49.93±20.9 (minimum-maximum: 18-86). Of the accidents, 35 (61.4%) occurred on weekdays, and 22 (38.6%) occurred on weekends. The distribution of accidents by season was as follows: 29 (50.9%) in spring, 20 (35.1%) in summer, and 8 (14%) in autumn. There was no thoracic trauma due to a Pat-Pat accident in the winter season.

Fifty-four (94.7%) accidents occurred on rural roads, and only 3 (5.3%) happened in the city center. Of the cases, 32 (56.1%) were brought to the emergency department by personal vehicle and 25 (43.9%) by ambulance. 37 (64.9%) drivers and 20 (35.1%) passengers had thoracic injuries due to the accident.

Table 1. Baseline patients' characteristics and Pat-Pat accident distribution due to season, location, and trauma mechanism

Variables	n	% Mean±SD
Age		49.93±20.9
Gender		
Female	13	22.8
Male	44	77.2
Incident location		
Urban	3	5.3
Rural	54	94.7
Season of the incident		
Spring	29	50.9
Summer	20	35.1
Autumn	8	14.0
Day of the week		
Weekday	35	61.4
Weekend	22	38.6
Trauma mechanism		
Collision	31	54.4
Ejection	26	45.6
Location of the injured		
Driver	37	64.9
Passenger	20	35.1
Revised trauma score		
5	5	8.8
6	3	5.3
7	8	14.0
8	41	71.9

Analysis of the mechanism of accidental thoracic injuries revealed that in 31 (54.4%) cases, injuries occurred due to the collision of the Pat-Pat vehicle with another vehicle or object (tree, wall, stone, etc.), and in 26 (45.6%) cases, injuries occurred due to being under the vehicle or being ejected directly from the vehicle due to vehicle overturning.

In terms of RTS, 41 (71.9%) cases scored 8 points, 8 (14%) cases scored 7 points, 5 (8.8%) cases scored 5 points, and 3 (5.3%) cases scored 6 points. After being evaluated in the emergency department, 30 (52.6%) cases were treated in the ward, 8 (14%) cases were admitted to the ICU 19 (33.3%) cases were discharged after evaluation and treatment in the emergency department (**Table 1**).

All cases had rib fractures; there were multiple rib fractures in 51 (89.5%) cases and single rib fractures in only 6 (10.5%) cases. Sternal fractures were detected in 8 (14%) cases. Pneumothorax was detected in 25 (43.9%) and hemothorax in 36 (63.2%) cases. Pulmonary contusion was detected in 22 (38.6%) cases, and cardiac contusion was detected in only 2 (3.5%) cases (**Table 2**).

A total of 24 (42.1%) cases received conservative treatment. A total of 33 (57.9%) patients underwent surgical treatment, 29 (50.9%) received tube thoracostomy, 3 (5.3%) received bleeding control intervention and lung parenchyma repair by thoracotomy, and only 1 (1.8%) received bleeding control intervention by videothoracoscopy (**Table 2**).

Table 2. Distribution of trauma-related injuries and treatments according to patients

	n	%
Rib fracture	57	100
Rib fracture number		
Single	6	10.5
Multiple	51	89.5
Sternum fracture	8	14
Pneumothorax	25	43.9
Hemothorax	36	63.2
Pulmonary contusion	22	38.6
Cardiac contusion	2	3.5
Follow-up		
Ambulatory	19	33.3
Inpatient service	30	52.6
Intensive care unit	8	14.0
Treatment		
Conservative	24	42.1
Surgical	33	57.9
Surgical intervention		
Tube thoracostomy	29	50.9
Thoracotomy	3	5.3
VATS	1	1.8

The average length of stay of the patients in the ICU was 5.47±5.0, and the mean total treatment time was 7.8±4.26 (minimum-maximum: 2-21). Labor force loss due to thoracic injuries as a result of the Pat-Pat accident was 24.21±10.3 days on average. When examined according to the trauma mechanism, the hospitalization duration was 2.55±0.546 and 8.96±9.42 days for injuries caused by impact and being ejected from the vehicle, respectively. The loss of workdays according to injuries due to impact and ejection from the vehicle was 19.68±1.33 and 29.62±2.12 days, respectively. Hospitalization and lost workdays differed significantly according to the trauma mechanism ($p<0.05$).

Mortality was seen in a total of 3 (5.3%) patients. Cases with mortality were male Pat-Pat drivers treated in the ICU, had a first admission RTS of 5, and were traumatized due to being ejected from the vehicle. Injuries caused by ejection from the vehicle resulted in significantly different ICU admission rate ($p=0.001$) and mortality ($p<0.05$).

DISCUSSION

Studies in the literature indicate that the mortality rate of injuries related to tractor and Pat-Pat accidents is high, especially during agricultural activities (1,4,10-12). The Pat-Pat vehicle is used as a simple tractor suitable for narrow and steep terrain conditions in the eastern Black Sea region where the study is carried out. In our study, the mortality rate due to thoracic traumas after the Pat-Pat accident was observed as high as 5.3%. We can attribute this high rate to the vehicle's serious stability problems, insufficient brake system, especially in rural areas such as the mountainous regions of the

eastern Black Sea region with many sharp bends and high slopes, drivers not wearing protective equipment, and the vehicle overload during worker transportation.

Traumas resulting from falling under the vehicle or being thrown from the vehicle in traffic accidents involving agricultural vehicles cause severe injuries and even death (3,4,10,13). Our study determined that injuries in 54.4% of the cases were caused by the Pat-Pat vehicle colliding with another vehicle or object (tree, wall, stone, etc.), and in 45.6%, the injuries resulted from the vehicle overturning or being ejected directly from the vehicle. Three patients with mortality had suffered chest trauma due to accidents involving ejection from the vehicle. In terms of mortality, accidents involving ejection from the vehicle were statistically significantly different from crashes ($p<0.05$). Among the causes of severe trauma are the increase in vehicle power with home-made modifications, the ease of overturning on high-speed winding roads due to its high structure, the absence of safety equipment such as seat belts and airbags, the drivers not wearing personal protective equipment, and the absence of chassis parts on the roof and top protection in the vehicle cabin structure.

The RTS score of 3 cases with mortality was 5, and the RTS score of all 19 cases discharged after the emergency department evaluation was 8. We think that obtaining the correct RTS score is crucial in determining the severity of the patient in the first evaluation made in the emergency department in such accidents, and the correct triage approach contributes to the functioning of the emergency department in cases where trauma patients come in groups.

It has been reported that men (approximately 75%) are mostly affected by traumas caused by tractors and similar vehicles used in agricultural activities (4,12,14). In our study, 77.2% of the patients with thoracic trauma after a Pat-Pat accident were men. Karapolat et al. (4) reported that cases aged 20-40 constituted the majority, while our study had a relatively older population with a mean age of 49.9 years. We think that the higher prevalence of such traumas in middle-aged men is due to the migration of young male adults from rural areas to city centers and shifting from agricultural activities to business lines in city centers.

Some studies reported that Pat-Pat accidents occur very little in winter and frequently in summer and autumn, especially in the months of July, August, and September during intensive hazelnut cultivation (3,4). Karapolat et al. (4) reported that those accidents mostly occur during summer. In our study, the examination of the seasonal distribution of accidents revealed that 29 (50.9%) accidents occurred in spring, 20 (35.1%) in summer, and 8 (14%) in autumn. It is observed that thoracic traumas due to the Pat-Pat accident occured in parallel with the hazelnut and other agricultural activities that started in the post-winter period, especially in the Eastern Black Sea region where our study was conducted, increased in spring and summer, and ended in September during hazelnut harvest.

Existing literature indicates that the majority of Pat-Pat accidents occur on highways in rural areas and to a lesser extent in agricultural areas (3-5). Researchers have attributed the increasing rate of these accidents to the speeds of Pat-Pats and other vehicles on the highways being different and to the increased traffic density (4). Our study found that accidents occurred on highways in rural areas with a high rate of 94%. The Pat-Pat vehicle was designed for activities such as carrying goods in the agricultural field, tilling the garden, and application of agricultural medicine. Yet, their use for transporting workers and goods on highways in rural areas paves the way for accidents. Those who use these vehicles, which require no license plates and drivers' licenses, drive quickly and hastily not to be caught by the security guards because driving these vehicles on the highway is illegal. This results in accidents and serious injuries, and even deaths.

In our study, the average hospital stay of the cases after the Pat-Pat accident was 7.8 days, and the mean duration of disability after trauma was 24.2 days. Pat-Pat accidents were associated with severe traumatic pathologies and mortality. The patients were separated from working life for a long time due to pneumothorax, hemothorax, rib and sternum fractures that developed as a result of thoracic trauma. Previous studies have reported serious economic damage due to the decrease in the workforce in agricultural production as a result of similar accidents (15,16). These accidents can be prevented with necessary legal regulations and serious controls to be applied in the field. The use of Pat-Pat vehicles in traffic is already prohibited by law, but in daily practice, it is clear from the number of accidents that people cannot be prevented from entering the traffic. We think that these accidents and thus injuries and loss of life can be prevented by the strict controls of law enforcement officers on rural roads, especially during hazelnut cultivation.

The limitations of our study are its retrospective design, the low number of cases, the coverage of accidents that took place in a relatively short period of time, the inclusion of only the cases that came to or were brought to the hospital due to the Pat-Pat accident, and not including mortality at the scene of the accident.

CONCLUSION

Pat-Pat accidents cause severe thoracic trauma that results in significant morbidity and mortality. These vehicles, manufactured only for agricultural use and without adequate safety precautions, cause serious injuries when used on highways.

Abbreviations

ATV: All-terrain vehicles, **RTS:** Revised trauma score, **ICU:** Intensive care unit

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was approved by the Ordu University Clinical Research Ethics Committee (Date: 05.11.2021, Decision No: 229).

Informed Consent: Because the study was designed retrospectively, no written informed con-sent form was obtained from patients.

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Original Article

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Does uterine septum resection improve IVF treatment success?

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ABSTRACT

Aim: Uterine septum is the most common type of congenital uterine malformation. The septum has also been suggested as a potential cause of infertility. The role of the septum in infertility and whether the septum can be resected is debatable. We aimed to reveal the results of assisted reproductive techniques in patients with septate uterus according to whether or not resection is performed.

Material and Method: 7790 patients were scanned retrospectively. 110 patients with the uterine septum and unexplained infertility were included in the study. Patients who underwent uterine septum resection were recorded. The clinical pregnancies of patients were compared according to whether or not resection was performed. In addition, patients with complete septum were evaluated according to whether or not resection was performed, and their clinical pregnancies were evaluated.

Results: It was revealed that 79 of the patients with uterine septum underwent septum resection operation. It was determined that 31 patients were not treated. Clinical pregnancy rates were found to be statistically significantly higher in the expectant management group (p=0.02). In addition, comparing the clinical pregnancy rates of the resection and expectant management groups in patients with a complete septum, no significant difference was found between the groups (p=0.134).

Conclusion: In our study, the success of treatment with assisted reproductive techniques did not change after septum resection. Although uterine septum resection is a simple and safe method, it has disadvantages such as the development of adhesions and rupture in the uterus. There is insufficient evidence to perform septum resection in patients with uterine septum prior to infertility treatment.

Keywords: Infertility, IVF, septum resection, uterine septum

INTRODUCTION

Uterine septum is the most common type and accounts for 35% of congenital uterine malformations (1). This situation can vary from a mild midline septum form (arcuate) to a complete septum. (2). Subfertility, pregnancy loss, preterm delivery, and fetal malpresentation may be more common in women with a septate uterus (3). The most common complication is miscarriage. Many studies have found that septum resection improves obstetric outcomes (4-6). Despite the potential benefits of hysteroscopic septum resection, a recent Cochrane review (7) concluded that there is no solid evidence supporting this procedure for women to improve reproductive outcomes due to the lack of published randomized controlled trials (RCTs) comparing hysteroscopic septum resection to expectant management. The main reason for septum resection is that the septum is structurally distinct from the uterine wall. According to a recent review, the endometrial and myometrial tissue in the septum is similar to that in the normal uterine wall. The endometrial lining of the intrauterine septum exhibits lower or similar expression of HOXA genes and transcription factors and lower expression of VEGF receptors than does the endometrium of the uterine wall, according to the same study. (8).

Worldwide, hysteroscopic septum resection improves reproductive outcomes in women of reproductive age who have a septate uterus (7,9). However, the recommended resection treatment is based mainly on observational studies; well-designed prospective randomized controlled studies are unavailable. These studies also provide a low level of evidence. In a recent randomized controlled study with a small number of samples, septum resection was not

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found to be statistically significant (10). The ASRM (2016) guideline recommends septum resection (11). ESHRE (2017) and RCOG (2011) guidelines state that resection should be repeated with other studies in patients with recurrent miscarriages. The uterine septum has also been suggested as a potential cause of infertility (12). Many studies have included uterine septums in the group of idiopathic infertility, so the role of the septum in infertility and whether the septum can be resected is debatable (13-16).

In our study, infertile patients with a septate uterus were filtered retrospectively in a single center. We aimed to reveal the results of assisted reproductive techniques in patients with septate uterus according to whether or not resection is performed.

MATERIAL AND METHOD

Study Design and Population

The study was initiated with the approval of the Gazi University Medical Faculty Clinical Researches Ethics Committee (Date: 01.06.2021, Decision No: 2021-666). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Between January 2000 and December 2021, data from 7790 patients were scanned retrospectively. A total of 196 patients with uterine septum were identified. Patients with infertility factors such as male factor, tubal pathologies, endometriosis, and PCOS were excluded. In addition, eight patients were excluded from the study because they did not complete their treatment. A total of 110 patients with the uterine septum and unexplained infertility were included in the study. The first cycles of each patient were evaluated.

The diagnosis of the uterine septum was made according to the AFS classification system by hysterosalpingography of the patients (16). Uterine septums were divided into two groups complete septum and partial septum. Patients who underwent uterine septum resection were recorded. It was learned from the notes in the file that it was a joint decision of the patient and the physician, depending on the conditions of the day, to whom resection and expectant management were performed. It was seen that the septum resection procedure was performed with the hysteroscopic resectoscope in the operating room.

Treatment Protocol

Age, gravida, duration of infertility, and BMI were examined in the database containing patient records (body mass index). Data were recorded. The patients' ovarian stimulation protocols were learned. Standardized ovarian stimulation protocols were started for the patients, and these protocols included GnRH antagonist, long GnRH agonist, microdose flare-up protocols. These protocols were chosen based on the unique conditions of the patients. 225–300 IU of gonadotropins were used in the stimulation protocols. The patients' hormonal and ultrasonographic measurements were taken into consideration as the gonadotropin doses were adjusted. The Aloka SSD-1000 was used to measure each follicle using a 5 MHz transvaginal probe. Once at least two follicles had reached an average diameter of 18 mm, recombinant HCG (Ovitrelle; Serono) was injected to induce ovulation, and oocytes were retrievaled 36 hours later while the patient was under general anesthesia. IVF/ICSI was used to fertilize mature oocytes. Three to five days after the oocyte retrieval, an embryo transfer (ET) under ultrasound guidance was carried out. From 12 days after ET until a pregnancy test was taken, patients received 200 mg of transvaginal micronized progesterone three times daily. Increased -hCG concentrations and sonographic evidence of an intrauterine gestational sac after ET were used to make the clinical diagnosis of pregnancy.

Outcome Measures

The clinical pregnancies of all patients were compared according to whether or not resection was performed. In addition, patients with complete septum were evaluated according to whether or not resection was performed, and their clinical pregnancies were evaluated.

Statistical Analysis

The Statistical Package for Social Sciences was used for all statistical analyses (SPSS, version 22.0,IBM, USA). The Kolmogorov-Smirnov test and other normality tests were applied to the analysis of normal distribution data. The Student's t-test was used to compare parametric data that were normally distributed. Mann-Whitney Data that did not exhibit a normal distribution were compared using the U test. The differences between categorical data were examined using the chi-square test or the Fischer's exact test. The presentation of continuous variables was as mean standard deviation. Categorical data were presented as a percentage and data with a non-D normal distribution were shown as the median. Statistical significance was accepted as $p < 0.05$.

RESULTS

Between January 2000 and December 2021, the uterine septum was detected in 196 of 7790 infertile patients who applied to our clinic, and the rate of patients with a uterine septum in the clinic was 2.5%. 110 of these patients had unexplained infertility and uterine septum. There were 17 secondary infertile patients and 93 primary infertile patients. Patients suffering from secondary infertility had previously had a single pregnancy. Four of the patients had living

children. Thirteen patients' pregnancies ended in abortion. There were 25 patients with a partial septum and 85 with a complete septum. It was revealed that 79 of the patients with uterine septum underwent septum resection operation. It was determined that 31 patients were not treated. When age, BMI, and infertility duration were compared between the resection and expectant management groups, there was no significant difference (**Table 1**).

Table 1. Comparison of demographic features between the resection and expectant management groups

	Septum resection group	Expectant management group	P value
Age (years) *	31.4±5.7	29.5±6.8	0.151
BMI*	26.6±5.2	26.5±4.9	0.932
Infertility duration (years) *	7.3±5.3	5.5±5.2	0.140

* Data are given as mean±SD, BMI: Body mass index, (p<0.05 was considered significant.)

Resection was performed in 10 patients (40%) out of 25 patients with the partial septum. Resection was performed in 69 (81%) of 85 patients with the complete septum. When the results of assisted reproductive therapy patients were examined, it was found that 35 of 110 patients had clinical pregnancies. In 75 patients, pregnancy was not detected.

It was found that 15 (48.4 %) of the patients with 35 clinical pregnancies were in the non-resection group, while 20 (25.3 %) were in the resection group. Clinical pregnancy rates were found to be statistically significantly higher in the expectant management group (p=0.02) (**Table 2**). In addition, patients with a complete septum were evaluated (n=85). Pregnancy rates of these patients were compared according to whether or not resection was performed. There were 27 patients with clinical pregnancies. Of these, 8 (50%) were in the non-resection group, while 19 (27.5%) were in the resection group. There was no statistically significant difference between the groups in terms of clinical pregnancy rates (p=0.134) (**Table 3**).

Table 2. Comparison of clinical pregnancy rates of resection and expectant management groups in the whole patient group

	Septum resection group	Expectant management group	P value
Clinical pregnancy positive	20 (25.3%)	15 (48.4%)	0.02
Clinical pregnancy negative	59 (74.7%)	16 (51.6)	

Table 3. Comparison of clinical pregnancy rates of resection and expectant management groups in the patients with complete septum

	Septum resection group	Expectant management group	P value
Clinical pregnancy positive	19 (27.5%)	8 (50%)	0.134
Clinical pregnancy negative	50 (72.5%)	8 (50%)	

DISCUSSION

In our clinic, the incidence of the uterine septum in the infertile population was found to be 2.5 percent. In the literature, the incidence is reported to vary between 1% and 15% (9). Especially in the infertile population, the prevalence of uterine septum in the literature varies between 5% and 25% (17). In this study, a statistically significant difference was found between clinical pregnancy outcomes, depending on whether or not septum resection was performed. This difference is that the clinical pregnancy rates were higher in the group that did not undergo treatment. Observational studies show that there is no significant change in clinical pregnancy rates after septum resection and support our results (18, 19) In the literature, there are not enough randomized controlled studies on this subject. A recent randomized controlled study also supports our results (10).

Previous studies have shown that there is a significant increase in viable pregnancy rates with septum resection (9,13). These studies are not randomized controlled trials. Therefore, the necessity of septum resection is presented with weak evidence.

In a recent review by Krishnan et al. (20), it was shown that there is a significant decrease in abortion rates with septum resection. However, this study found no substantial evidence to suggest that hysteroscopic resection improves live birth and clinical pregnancy rates and/or reduces preterm birth. Unlike previous reviews, patients with subfertility and poor obstetric outcomes are included in this review. And two recent studies are up to date (16,21).

In another recent meta-analysis showed that there was a significant decrease in abortions in the group that underwent hysteroscopic septum resection, and there was a significant decrease in the frequency of preterm birth and fetal malpresentation (22). Although many studies have shown that septum resection improves obstetric outcomes, most of them are retrospective studies (23,24).

A recent randomized controlled study by Rikken et al. (10) showed that there was no significant difference in pregnancy loss and preterm delivery rates between the group that underwent septum resection and the group that underwent expectant management.

In our study, it was observed that the success of treatment with assisted reproductive techniques did not change after septum resection. The biggest limitation of our study is that; follow-up of the cases resulting in clinical pregnancy was not performed. Therefore, our data on obstetric outcomes are insufficient. This study's retrospective design, which makes it challenging to access data and potentially biased records, is another drawback.

CONCLUSION

Although uterine septum resection is a simple and safe method, it has disadvantages such as the development of adhesions and rupture in the uterus, as well as being an invasive procedure (9). There is insufficient evidence to perform septum resection in patients with uterine septum before infertility treatment. Randomized controlled studies with large samples are needed.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was initiated with the approval of the Gazi University Medical Faculty Clinical Researches Ethics Committee (Date: 01.06.2021, Decision No: 2021-666).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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The relationship between subclinical hypothyroidism and vitamin D

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ABSTRACT

Aim: Vitamin D (vitD) is primarily responsible for bone formation and mineralization. However, in recent years, it has been suggested that vitD may play a role as an immune modulator in the development of numerous diseases, including autoimmune diseases. It has been observed that there is an association between chronic autoimmune thyroiditis (AIT) and vitD levels. This study aims to investigate whether there are differences in the levels of 25-hydroxy vitamin D [25(OH)D], calcium, and phosphorus in patients with subclinical hypothyroidism (SCH) due to AIT, in patients with antibody-negative subclinical hypothyroidism (ANSCH), and in healthy control subjects.

Material and Method: Data from 50 newly diagnosed patients with SCH (35 of whom AIT) and 50 euthyroid and antibody-negative healthy controls who presented to the Department of Endocrinology and Internal Medicine at our hospital between 2018 and 2020 were retrospectively reviewed. Calcium, phosphorus, and 25(OH)D levels of patients and controls were compared.

Results: Serum 25(OH)D levels were significantly lower in patients compared to controls (16.2±7.8 ng/ml and 20.4±8.2 ng/ml, respectively; p=0.024). Serum levels of calcium (p=0.081) and phosphorus (p=0.712) did not differ between groups. In a subgroup analysis, patients with AIT had significantly lower 25(OH)D values than controls (p=0.009). Compared to controls, 25(OH)D levels were comparable in the ANSCH group (p=0.096). 25(OH)D level was higher in the AIT group than in the ANSCH group (p=0.01).

Conclusion: Our results show that patients with SCH have lower 25(OH)D levels than healthy controls. However, this difference is significant in patients with AIT. It is recommended to screen for vitD deficiency in patients with SCH due to AIT.

Keywords: Chronic autoimmune thyroiditis, 25-hydroxy vitamin D, subclinical hypothyroidism.

INTRODUCTION

Vitamin D (vitD) deficiency is a common health problem in our country and around the world (1). VitD is a steroid hormone whose main function is the formation and mineralization of bone and the balance of calcium (Ca) and phosphorus (P) in the body. However, in recent years, vitD has been shown to play a role as an immune modulator in the development of numerous diseases such as autoimmune diseases, heart disease, cancer, inflammatory bowel disease, diabetes, and rheumatic diseases (2,3). In addition, patients with autoimmune thyroid disease (AITD), including Graves' disease and autoimmune thyroiditis (AIT) have been reported to have lower vitD levels (4). Both vitD and thyroid hormones bind to similar steroid hormone receptors and vitD receptor gene polymorphism has been found to be associated with AITD (5). Studies have demonstrated vitD deficiency in hypothyroidism (4,5). It is suggested that the associated mechanism is inadequate uptake of vitD from the gut, or the body may not activate vitD properly (5) and also autoimmunity in AITDs (4,6). The vit D status of an individual is usually determined by measuring the 25-hydroxyvitamin D level [25(OH)D] because it has a long half-life and its level is regulated within a very narrow range by parathyroid hormone, Ca, and P. If the 25(OH) D level is below 20 ng/ml, vitD deficiency exists (7-9). A serum level of thyroid stimulating hormone (TSH) above the established upper limit of the reference range and a serum level of free thyroxine (fT4) within the reference range is termed subclinical hypothyroidism (SCH) (4). Globally, subclinical hypothyroid dysfunction is more common than overt disease (10). Limited studies discussed the vitD levels in SCH (11). This study aims to investigate

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whether there are differences in 25(OH)D, Ca, and P in patients with SCH due to AIT, in patients with antibody-negative subclinical hypothyroidism (ANSCH), and in healthy controls. In addition, we aimed to observe whether there is a relationship between anti-thyroid peroxidase (anti-TPO) and vitD levels.

MATERIAL AND METHOD

This retrospective study was conducted in University of Health Sciences Antalya Training and Research Hospital, Department of Internal Medicine and Endocrinology. The Ethics Committee of the University of Health Science Antalya Training and Research Hospital approved the study protocol (Date 27.05.2021, Decision No. 7/2). All procedures were performed in accordance with the ethical rules and principles of the Declaration of Helsinki.

Data from patients treated between January 2018 and January 2020 were studied.

The inclusion criteria were as follows:

- 18-70 years old
- Patients with newly diagnosed SCH
- Patients whose history, physical examination, and laboratory tests including TSH, fT4, fT3 (free triiodothyronine), thyroid antibodies, 25(OH)D, Ca, and P were accessible
- Patients in whom thyroid ultrasound had been performed
- Patients who had no history of cardiovascular disease, cancer, osteomalacia, other bone diseases, diabetes mellitus, obesity, inflammatory bowel disease or rheumatologic diseases
- Patients who had no history of levothyroxine use.
- Patients who were not taking Ca or vitD supplements

The exclusion criteria were as follows:

- <18 years old or >70 years old
- Patients with known pre-existing conditions such as cardiovascular disease, malignancies, osteomalacia, other bone diseases, diabetes mellitus, obesity, chronic inflammatory diseases including inflammatory bowel disease, rheumatologic diseases etc.
- Patients with overt hypothyroidism
- Patients who have already been diagnosed with SCH and treated with levothyroxine
- Patients taking Ca or vitamin D supplements
- Smokers or alcoholics

Because the upper limit for TSH in our hospital is 5.6 uIU/ml, SCH was defined as a TSH level of 5.6-10 mU/l when the fT4 concentration was normal. The diagnosis of AIT was based on the positivity of thyroid antibodies (anti-TPO and, if negative, with anti-thyroglobulin antibodies (anti-TG)) and typical ultrasound findings including pseudonodular appearance and hypoechogenic pattern. When anti-TPO is positive, we do not routinely measure anti-TG; therefore, only anti-TPO results were collected and recorded in these patients. Patients with SCH, in whom both anti-TPO and anti-TG were negative, were included in the ANSCH group. Fifty patients with newly diagnosed SCH (35 of whom had AIT) who met the inclusion and exclusion criteria were included in the study. Fifty control subjects aged 18-70 years with no acute or chronic disease, no medications, and no history of thyroid disease with normal TSH, fT3, fT4, anti-TPO, and anti TG levels were included in the study. The TSH, anti-TPO, fT3, fT4, 25(OH)D, Ca, P, and albumin levels of patients and controls were recorded. Because all patients with AIT had anti-TPO positivity, we could not obtain their anti-TG results, so anti-TG was not included in our analysis. For patients with low serum albumin levels, Ca levels were corrected according to the following formula: Corrected Ca (mg/dL)=measured total Ca (mg/dL) + 0.8 (4-patient albumin). The cut-off value for anti-TPO antibodies was assumed to be 10 IU/mL according to the cut-off value of our laboratory. Ca, P, albumin, and other biochemical assays were analyzed by conventional spectrophotometric methods using commercial kits from Beckman Coulter on a Beckman Coulter AU5800 (Beckman Coulter Inc. CA, USA) autoanalyzer. 25(OH) D, TSH, fT3, fT4, and other necessary hormone tests were analyzed on a Beckman Coulter DxI800 (Beckman Coulter Inc. CA, USA) using chemiluminescence methods.

Statistical Analysis

Descriptive statistics were used to determine continuous variables (mean±standard deviation and median, minimum and maximum). The Shapiro-Wilk test was used to determine if the parameters were normally distributed. Statistical analysis was performed using Student's t test and one-way test ANOVA, followed by Tukey's test for multiple comparisons. The Spearman correlation test was used for correlation analyzes. A 'p' value of less than 0.05 was considered statistically significant. Data analysis was performed using IBM SPSS version 20.

RESULTS

The mean age of the patients was 36±8 years and 35 were female. The mean age of the control group was 34±9 years and 33 were female. There was no statistically significant difference between patients with SCH and the control group in terms of gender and age (p=0.741 and p=0.987, respectively). As expected, TSH level was significantly higher in the study population compared to the control group (7.1±1.4 μIU/ml and 2.1±0.7 μIU/ml, respectively;

p=0.006). The mean 25(OH)D level was 16.2±7.8 ng/ml in the SCH group and 20.4±8.2 ng/ml in the euthyroid control group. Serum 25(OH)D level was significantly lower in patients than in controls (p=0.024). The mean serum Ca level was 9.1±0.6 mg/dl in the SCH patients and 9.3±0.5 mg/dl in the controls (p=0.081). Serum P level did not differ between groups (p=0.712). The comparison of study parameters between the study and control groups is summarized in **Table 1**. Patients in the SCH group were divided into the AIT group (n=35) and the ANSCH group (n=15). All patients in the AIT group were anti-TPO positive. In the ANSCH group, both anti-TPO and anti-TG were negative. A one-way ANOVA test was used to compare these two subgroups and the control group (**Table 2**). There was no statistical difference between the groups in terms of sex (p=0.860), age (p=0.145), Ca (p=0.126), and P (p=0.324). There was a statistically significant difference between groups in terms of TSH (p=0.005), anti-TPO (p=0.0001) and 25(OH)D (p=0.012). According to post-hoc analyzes performed to determine the source of significance between groups, TSH level did not differ between AIT and ANSCH patients (p=0.08). TSH level was significantly higher in the AIT group (p=0.001) and in the ANSCH group (p=0.006) than in controls. The 25(OH)D level was similar between the control group and the ANSCH group (p=0.096). The 25(OH)D level was lower in the AIT group than in the control group (p=0.009). The 25(OH)D level was lower in the AIT group than in the ANSCH group (p=0.01) (**Table 2**). In the whole study group, there was an inverse correlation between 25(OH)D and TSH (r=-0.241, p=0.004) and anti-TPO (r=-0.387, p=0.001).

Table 1. Study Parameters between subclinical hypothyroid patients and control group

Parameters	Subclinical hypothyroid patients n (50)	Control group n (50)	p
Male n (%) Female n (%)	15 (30) 35 (70)	17 (34) 33 (66)	0.741
Age (yrs.)	36±8	34±9	0.987
TSH (μIU/ml)	7.1±1.4	2.1±0.7	0.006*
fT3 (ng/dl)	2.9±0.6	2.8±0.3	0.843
fT4 (ng/dl)	1.3±0.3	1.1±0.2	0.921
25(OH)D (ng/ml)	16.2±7.8	20.4±8.2	0.024*
Calcium (mg/dl)	9.1±0.6	9.3±0.5	0.081
Phosphorus (mg/dl)	3.9±0.3	3.8±0.2	0.136

fT3: Free triiodothyronine; fT4: Free thyroxine; 25(OH)D: 25-hydroxy vitamin D, *p<0.05 is statistically significant

DISCUSSION

Our study showed that vitD levels were lower in patients with SCH due to AIT than in the control group. TSH levels were similar in the AIT and ANSCH groups, and vitD levels were lower in the AIT group than in patients with ANSCH. In addition, a negative correlation was observed between vitD levels and anti-TPO. Numerous studies suggest a relationship between AITD and vitD deficiency, considering how vitD regulates inflammatory response and autoimmunity (12-16). Previous research has found that vitD deficiency is one of the features of AITDs, particularly Hashimoto's thyroiditis (HT), and may cause the autoimmune process that leads to HT and hypothyroidism (16). In the study by Tamer et al. (13), 92% of patients with HT had vitD deficiency, and vitD deficiency was significantly higher in patients with HT than in healthy controls. In this study, vitD levels were similar in euthyroid and hypothyroid HT patients. Shin et al. (14) observed lower vitD levels in patients with AITD compared with patients without AITD in their study of 304 patients. They did not show a difference in vit D levels related to thyroid function. Mazokopakis et al. (15) showed inversely relation between anti-TPO and 25(OH)D levels in HT. Bozkurt et al. demonstrated that the severity of 25(OH)D deficiency correlated with the duration of HT, thyroid volume, and antibody levels, suggesting a possible role of 25(OH)D in the development of HT and progression of hypothyroidism (16). Our study also confirms the literature and shows that AIT and VitD deficiency are related independently of thyroid hormone levels. The relationship between vitD and autoimmunity is not clearly established but is likely related to its anti-inflammatory and immunomodulatory functions (15,19). Expression of the nuclear vitD receptor (VDR) and the vitD-activating enzyme 1α-hydroxylase (CYP27B1) has been detected in most immune cells, leading to the idea that vitD may play a role in the pathogenesis of the immune system and autoimmune diseases (20-23). 1-25(OH)D suppresses B cell proliferation, immunoglobulin production, and differentiation into plasma cells and promotes the apoptosis of B cells (20). In the CD4 + T-cell response, vitD directly inhibits the production of Th1 cytokines

Table 2. Study Parameters between patients with subclinical hypothyroidism due to chronic autoimmune thyroiditis, patients with antibody negative subclinic hypothyroidism and control subjects

Parameters	SCH due to AIT n (35)	ANSCH n (15)	Control n (50)	p
Male n (%) Female n (%)	10 (28.5) 25 (71.5)	5 (33.3) 10 (66.7)	17 (34) 33 (66)	0.860
Age (yrs.)	36±8	35±10	34±9	0.145
TSH (μIU/ml)	7.3±1.2	6.8±0.7	2.1±0.7	0.005*&
Anti-TPO (IU/ml)	215 (108-420)	1.2 (0.2-4.6)	0.9 (0.2-4.2)	0.0001*
25(OH)D (ng/ml)	14.2±5.8	18.3±6.1	20.4±8.2	0.012*+
Calcium (mg/dl)	8.9±0.7	9.2±0.4	9.3±0.5	0.126
Phosphorus (mg/dl)	3.9±0.5	3.8±0.3	3.8±0.2	0.324

SCH: Subclinical hypothyroidism; AIT: Chronic autoimmune thyroiditis; ANSCH: Antibody negative subclinic hypothyroidism; Anti-TPO: Anti-Thyroid Peroxidase; 25(OH)D: 25-hydroxy vitamin D,
*One-Way ANOVA results of 3 groups, p<0.05 is statistically significant.
+ Statistical analysis was performed by ANOVA followed by Tukey's multiple comparison test, p=0.096 control group Vs ANSCH group; p=0.009 control group Vs AIT group; p=0.01 AIT groupVs ANSCH group
& Statistical analysis was performed by ANOVA followed by Tukey's multiple comparison test, p=0.08 AIT group Vs ANSCH group; p=0.001 control group Vs AIT group; p=0.006 control group Vs ANSCH group.

(IL2 and IFN-γ) and increases the production of Th2 cytokines (IL-4) (24). In light of these data, recent studies have suggested that vitD supplementation may improve autoimmunity. These studies reported a decrease in anti-TPO levels after cholecalciferol supplementation in HT patients with vitD deficiency (15,25). Mazokopakis et al, reported a significant decrease in anti-TPO levels after 4 months of oral vitD supplementation in patients with vitD deficiency (15). Another study from our country showed that vitD treatment can reduce the development of hypothyroidism in patients with HT (26). Some literature studies have shown that hypothyroidism is associated with vitD deficiency in addition to autoimmunity (4-6). Kim D found that patients with overt hypothyroidism had lower 25(OH)D levels than euthyroid individuals or patients with SCH with or without HT. In this study, researchers found that lower serum 25(OH)D levels were associated with higher TSH levels (6). A few studies have also found an inverse association between 25(OH) D and TSH levels or disease severity in HT, suggesting a link between poor vitD status and progressive thyroid destruction (6,16,27,28). We did not include patients with overt hypothyroidism in this study, so we can not definitively state whether there is a relationship between TSH and vitD. However, we found that vitD levels in SCH patients without AIT were similar to those in euthyroid healthy controls, and SCH patients with positive antibody had lower vitD levels than SCH patients with negative antibody.

The limitations of the study are the small number of patients and its retrospective nature. There may be missing data because the medications taken by the patients and concomitant diseases are recorded retrospectively via the computer system. Patients not currently taking vitD were included in the study, but if they had recently taken vitD and then stopped, this may have affected patients' vitD levels. Seasonal variation in blood collection may have affected the results because patient samples were not collected in a single season. We did not include euthyroid and overt hypothyroid patients with AIT in the study. If we included this group of patients, the association between vitD and hypothyroidism and autoimmunity could have been more clearly demonstrated.

CONCLUSION

Our results show that patients with SCH are more likely to have lower vitD levels than the control population, and this finding was statistically significant in anti-TPO(+) patients. VitD levels can be measured in patients with SCH and anti-TPO positivity. New studies are needed to investigate the effects of vitamin D deficiency on bone and other systems in this patient population.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was initiated with the approval of the University of Health Sciences Antalya Training and Research Hospital Clinical Researches Ethics Committee (Date: 27.05.2021, Decision No: 7/2).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Comparison of high performance liquid chromatography and turbidimetric inhibition immunoassay methods for measurement of hemoglobin A1c

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ABSTRACT

Aim: Hemoglobin A1c is a valuable parameter for the diagnosis and follow-up of its diabetes mellitus since its biological variation is low, does not require preparation before the test, is not affected by acute stress, and has high preanalytical stability. HbA1c measurement by HPLC has been determined as the reference method by National Glycohemoglobin Standardization Program (NGSP) in USA; after that The International Federation of Clinical Chemistry (IFCC) defined another reference method which could be related with NGSP. In our study, we aim to compare the two NGSP-certified methods of HbA1c, which are high-performance liquid chromatography (HPLC) and turbidimetric inhibition immunoassay (TINIA).

Material and Method: HbA1c levels of the patients were measured using two HPLC and one TINIA method in three different hospitals (Lab A, Lab B (Both are HPLC), and Lab C (TINIA), in which Lab A was served as a reference). Because of the lower precision values of LabB, we firstly conducted a method comparison study of 40 volunteers (Group 1). After that, corrective and preventive activities carried out and the precision values in LabB reached the desired range. Following this, another method comparison study consisting of 60 new volunteers (Group 2) was conducted. The statistical flow of this study complied with Clinical Laboratory Standards Institute (CLSI) EP09-A3; Precision studies, Blant-Altman and Passing Bablok regression analysis were performed.

Results: The percentage of the mean difference between the two HPLC methods (LabA and LabB) was 3.1%. After corrective and preventive actions had been taken, the mean difference between the two HPLC methods decreased to 2.0%. A decrease in systematic bias was found in our study. Two HPLC methods can be used interchangeably in both Group 1 and Group 2. In Group 1; 95% CI of intercept and slope were found as (-1.41 to -0.30) and (1.03 to 1.22), respectively. In Group 2; 95% CI of intercept and slope were found as (-1.33 to -0.31) and (1.01 to 1.17), respectively. HPLC and TINIA methods could not be used interchangeable without affecting patient results and outcome in both Group 1 and Group 2.

Conclusion: Our study concluded that TINIA and HPLC methods could not be used interchangeably without affecting patient results and outcome. Because of the methodology that clinical laboratories are used to, clinicians and clinical biochemists should collaborate on managing diabetes mellitus regarding diagnosis, treatment, and follow-up.

Keywords: Diabetes mellitus, HPLC, immunoturbidimetry, HbA1c

INTRODUCTION

HbA1c (Hemoglobin A1c) molecule is formed by Maillard reaction where N-terminal valine of the β chain reacts with glucose to the aldimide (Schiff base) and performs an Amadori rearrangement to the stable ketoamine (1). Measurement of HbA1c is essential for the evaluation of retrospective glycemia. It is well known that the measurement of HbA1c reflects the mean value of blood glucose for 6-8 weeks and is also related to late complications of diabetes mellitus (2).

HbA1c is a valuable parameter for the diagnosis and follow-up of its treatment since its biological variation is lower compared to fasting plasma glucose and/or 2 hour plasma glucose in both within (CVI) and between subject (CVG) variation (CVI and CVG values for HbA1c and plasma glucose are 1.6% and 5,0% and 7.1% and 8.1%; respectively) (3), does not require preparation before the test, is not affected by acute stress, and has high preanalytical stability (4).

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High-performance liquid chromatography (HPLC) is a reference method to standardize other routine procedures with long-term validity, accuracy, and stability (5,6). Other methods for measurements of HbA1c are immunologic methods, affinity chromatography, capillary electrophoresis, and enzymatic methods (7,8).

HbA1c was defined as a diagnostic criteria of diabetes mellitus, after the International Expert Committee's report that was published in 2009 (4). HbA1c measurement by HPLC has been determined as the reference method by National Glycohemoglobin Standardization Programme (NGSP) in USA; after that The International Federation of Clinical Chemistry (IFCC) defined another reference method which could be related with NGSP (9,10).

In our study, we aim to compare the two NGSP-certified methods of HbA1c, which are high-performance liquid chromatography (HPLC) and turbidimetric inhibition immunoassay (TINIA).

MATERIAL AND METHOD

This study was approved by the Ordu University Clinical Researchers Ethics Committee (Date: 17.06.2022, Decision No: 2022/149). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Study Design

One hundred patients from Ordu University Hospital diagnosed with prediabetes or diabetes were admitted to the study. HbA1c levels of the patients were measured using two HPLC (Adams HA8180V (Arkray Inc, Kyoto, Japan)) and one TINIA method (Cobas, Roche Diagnostics GmbH, Mannheim, Germany) in three different hospitals (Lab A, Lab B, and Lab C, in which Lab A was served as a comparative laboratory) in Ordu, a city in Turkey. Lab A and Lab B used the HPLC method with the same kit, Lab C used the immunoturbidimetric method). The study group was grouped into two subgroups; Group 1 consisted of 40 patients, and Group 2 consisted of 60 patients. Main reason of forming Group1 and Group 2 is to evaluate the corrective and preventive actions. LabB were facing problems regarding both internal quality control (recorded lower precision values (3.5%) than we expected) and patient results. As a result of this, Group 1 was formed to show that whether a systematic and/or random error in LabB; so method comparison study which was taken place with 40 patient samples were completed . According to the EP09-A3 guideline, 40 patient samples were enough to perform for method comparison study. In the group 1, because of the method comparison study's results were not efficient, preventive and corrective actions (implementing new calibration, new kit and requested service care) were performed. After the preventive and corrective actions had been taken, another method comparison study (Group 2) was planned to observe the effects of preventive and corrective actions.

Procedures

HPLC (Adams Arkray 8180T)

This system is a fully automated HbA1c analyzer using reverse-phase cation exchange chromatography. The system can handle both whole blood and manually diluted samples. Four microlitres of whole blood are automatically sampled, hemolyzed, and injected into the column. Hb molecules elute with inorganic phosphate buffers (80A, 80B, and 80CT) from low to high polarity. Upon elution, sample components pass through the spectrophotometric detector, where fractions are monitored with dual-wavelength detection (420 and 500 nm). (11) This autoanalyzer complies with the latest Diabetes Control and Complications Trial (DCCT) reference method, which is stated by National Glycohemoglobin Standardization Programme (NGSP).

TINIA (Cobas c501, Roche Diagnostics)

In this method, sample pretreatment to remove labile HbA1c is not necessary. The HbA1c determination is based on the TINIA for hemolyzed whole blood. HbA1c in the sample reacts with anti-HbA1c antibody to form soluble antigen-antibody complexes. Since the specific HbA1c antibody site is present only once on the HbA1c molecule, formation of insoluble complexes does not take place. The polyhaptens react with excess anti-HbA1c antibodies to form an insoluble antibody-polyhapten complex which can be determined turbidimetrically. % hemoglobin A1c is measured using a ratio (12).

Medical decision limit was stated as 6,5%, and desirable imprecision and bias for HbA1c were determined as 0,6% and 1,2%, respectively; according to the EuBIVAS (3)

Lab A and Lab B used the HPLC method, with the coefficient of variation (CV%) <1%; Lab C used the immunologically based method, with the <3%CV. Lab A and B's linearity range is 4-15% and 4.2-20.2% for Lab C.

Statistical Methods

Statistical analyses were performed using IBM SPSS Statistics Ver.22, MedCalc® Statistical Software version 20.009 (MedCalc Software Ltd, Ostend, Belgium; https://www.medcalc.org; 2021) and GraphPad Prism version 8.0.0 for Windows, GraphPad Software, San Diego, California USA, www.graphpad.com".

Evaluation of normality of HbA1c values were performed with Kolmogorov-Smirnov test.

The statistical flow of this study, which has complied with Clinical Laboratory Standards Institute (CLSI) EP09-A3,

has three steps; Precision studies which were found within biological variation limits were performed in the first step, and comparisons of mean and the differences of the mean values that were performed with Blant-Altman analysis in the second step, Passing Bablok analysis were performed in the last step.

RESULTS

Mean HbA1c and SD (Error Bars) of the laboratories in Group 1 and Group 2 are shown in **Figure 1**.

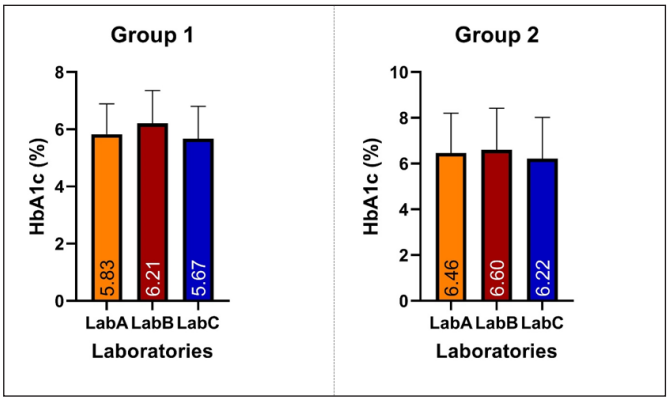

Figure 1. Mean HbA1c and SD Values of the Laboratories in Group 1 and Group 2

Comparisons of the median HbA1c values of the laboratories in Group 1 and Group 2 are shown in **Table 1**

In the **Table 1**, median values of LabB and LabC are compared with the LabA, which is selected as "comparative laboratory"

Table 1. Comparisons of the median HbA1c values of the laboratories in Group 1 and Group 2

	LabA Median (Minimum-Maximum)	LabB Median (Minimum-Maximum)	LabC Median (Minimum-Maximum)	p*	p**	p***
Group 1 (n:40)	5.80 (5.30-11.00)	5.40 (4.60-10.40)	5.50 (4.90-10.20)	<0.01	<0.01	<0.01
Group 2 (n:60)	5.80 (4.60-12.40)	5.80 (4.70-13.00)	5.55 (4.00-12.20)	0.30	<0.01	<0.01

p*: Mann Whitney U test of LabA and LabB, p**: Mann Whitney U test of LabA and LabC, p***: Mann Whitney U test of LabB and LabC

In Group 1; median HbA1c value of LabC is significantly lower than LabA and LabB (p<0.01 for both, respectively).

In Group 2; median HbA1c value of LabC is significantly lower than LabA and LabB (p<0.01 for both, respectively).

In Group 2, there was no difference between median values of LabA and LabB (p:0.30)

In group 1, there is a positive significant correlation between LabA vs Lab B (r: 0.99, p<0.001 and 95% CI: 0.99 to 0.99) and LabA vs LabC (r:0.99, p <0.01 and 95% CI: 0.98 to 0.99).

In group 2, there is a positive significant correlation between LabA vs Lab B (r: 0.99, p<0.001 and 95% CI: 0.99 to 0.99) and LabA vs LabC (r:0.99, p <0.01 and 95% CI: 0.98 to 0.99).

Blant-Altman analysis of LabA vs LabB and LabA vs LabC in Group 1 are shown in **Figure 2A and 2C**, respectively. Scatter plots of LabA vs LabB and LabA vs LabC in Group 2 are shown in **Figure 2B and 2D**, respectively.

As it is shown in **Figure 2A-D**; Blant-Altman plot demonstrating that confidence interval of the mean bias which is shown as the green line in the **Figure 2A-D** do not include zero value, that is indicative of systematic bias.

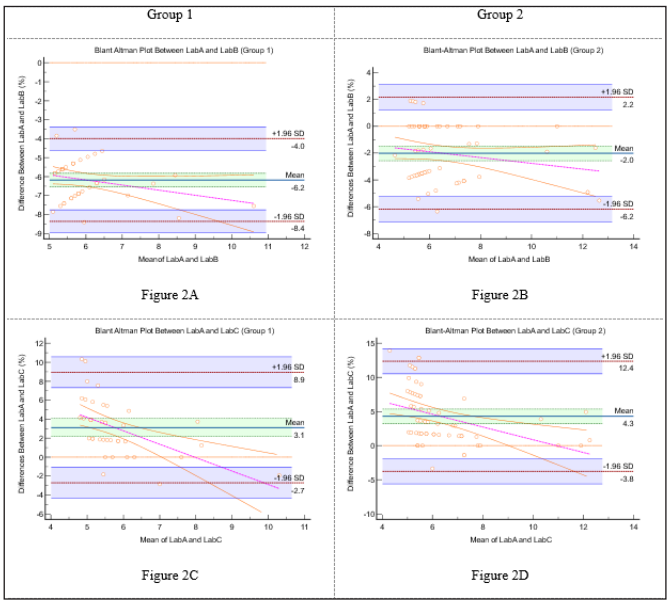

Figure 2. Blant Altman Plot for the Laboratories in Group 1 and Group 2

Passing-Bablok Regresyon analysis and equations of laboratories are shown in **Figure 3**.

Regression analysis of LabA vs LabB and LabA vs LabC in Group 1 are showed in **Figure 3A and 3C**, respectively. Regression analysis of LabA vs LabB and LabA vs LabC in Group 2 are showed in **Figure 3B and 3D**, respectively.

In Group 1;

Regression equation of LabA and LabB is found as y=-0.02 +1.06 x. 95% CI of intercept and slope were found as (-0.24 to 0.40) and (1.00 to 1.10), respectively.

Regression equation of LabA and LabC is found as y=-0.07 +1.16 x. 95% CI of intercept and slope were found as (-1.41 to -0.30) and (1.03 to 1.22), respectively.

In Group 2;

Regression equation of LabA and LabB is found as y=-0.06 +1.03 x. 95% CI of intercept and slope were found as (-0.25 to 0.15) and (1.00 to 1.06), respectively.

Regression equation of LabA and LabC is found as y=-0.75 +1.08 x. 95% CI of intercept and slope were found as (-1.33 to -0.31) and (1.01 to 1.17), respectively.

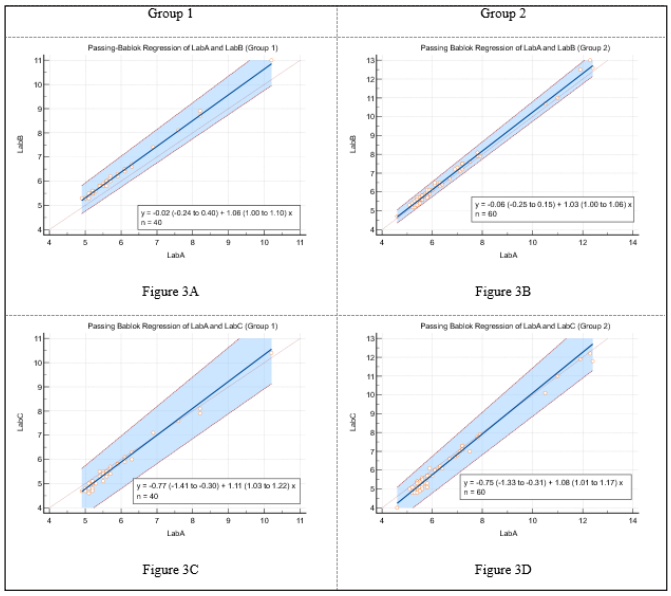

Figure 3. Passing-Bablok Regression of Laboratories in Group 1 and Group 2

DISCUSSION

It is widely stated that complications of diabetes are related to patients' long-term glycemia. Therefore, the measurement of HbA1c is a standard method to evaluate the long-term glycemic control of the patients (5, 13). Due to the relatively higher cost of implementing a reference method, like HPLC in the clinical laboratory, method comparison studies that aim to compare HPLC and the other methods should be performed.

Our study concluded that TINIA and HPLC methods could not be used interchangeably without affecting patient results and outcome in both Group 1 and Group 2. In the literature, there are studies that TINIA is a reliable method with high imprecision and accuracy (14-16). In this article, the authors used correlation analysis to evaluate the method comparison. However, according to the CLSI EP09-A3 guideline (17), Blant-Altman analysis and Passing Bablok or Deming Regression analysis could be performed for the method comparison study. Contradictive findings can be attributed to differences in sample preparations, internal quality control rules, etc.

In the passing-bablok analysis, significance testing is performed by examining the confidence intervals for a and b in the equation of y=ax+b. Null hypothesis H0 is accepted if confidence intervals for a and b's include 0 and 1, respectively (18).

Our first hypothesis was that lower precision in the HPLC method in LabB affected the results shown in Group 1. The percentage of the mean difference between the two HPLC methods (LabA and LabB) was 3.1%. The maximum allowable bias for HbA1c is 1.2% (3). After corrective and preventive actions had been taken, the mean difference between the two HPLC methods decreased to 2.0%. A decrease in systematic bias was found in our study. To summarize, it can be concluded that harmonization among hospitals within defined periods can be helpful for the management of patients with diabetes mellitus.

According to the Passing Bablok analysis, two HPLC methods can be used interchangeably in both Group 1 and Group 2. In Group 1; 95% CI of intercept and slope were found as (-1.41 to -0.30) and (1.03 to 1.22), respectively. In Group 2; 95% CI of intercept and slope were found as (-1.33 to -0.31) and (1.01 to 1.17), respectively. Because of the fact that 95% CI of intercept values did not included 0 and slope values did not included 1; we could conclude that However, HPLC and TINIA methods could not be used interchangeable without affecting patient results and outcome in both Group 1 and Group 2.

In our study, mean HbA1c values with HPLC methods are relatively higher than with TINIA. These results are consistent with the literature (15, 19-21). This finding is also essential, because the TINIA method is widely used in clinical laboratories due to its relatively lower cost and is easily applicable. Clinicians should keep in mind this conclusion for the management of patients with diabetes. Furthermore, turnaround time is also important parameters that should be kept in mind. Turnaround time of HPLC method is much faster than immunoturbidimetric method. Therefore, clinical biochemists should not be validate of the HbA1c results without evaluating related analytes (Glucose etc).

Cost is an important parameter in the clinical biochemistry laboratories. According to the social security instution communique on healthcare practices, HPLC method is approximately 4.5x expensive, comparing the TINIA method (22).

Our study aims to show the importance of preventive and corrective actions and harmonization steps for evaluating the HbA1c levels in three hospitals. This is the main advantage of our study. However, we could not analyze repeated measurements for each method and could not perform appropriate sampling. These are the main drawbacks of our study.

CONCLUSION

Clinicians and clinical biochemists should collaborate on managing diabetes mellitus regarding diagnosis, treatment, and follow-up.

ETHICAL DECLERATIONS

Ethics Committee Approval: This study was approved by the Ordu University Clinical Researchers Ethics Committee (Date: 17.06.2022, Decision No: 2022/149).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

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

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All the authors declare that they have all participated in the design, statistical evaluation and writing and that they have approved the final version.

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Original Article

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Effect of oral estrogen supplement on gonadotropin-induced intrauterine insemination: A retrospective cohort study

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ABSTRACT

Aim: The effect of estrogen on gonadotropin-induced intrauterine insemination (GI-IUI) is not well studied. Furthermore, risk factors for clinical pregnancy rates are not clearly defined. This study aimed to evaluate the effects of oral estrogen supplementation and clinical pregnancy rates on GI-IUI.

Material and Method: Patients treated with primary and secondary infertility were retrospectively analyzed between September 2016 and September 2019. IUI session was performed after ovarian stimulation with human chorionic gonadotropin. Patients were supplemented with a vaginal gel containing progesterone and oral estrogen (Group A) or only the vaginal gel (Group B). The differences between the groups in pregnancy rate and endometrial thickness and the risk factors associated with clinical pregnancy were determined as primary and secondary outcomes, respectively.

Results: A total of 112 couples were evaluated, where the mean age for females was 31.3±6.1 years. Group A and Group B had 33 (29.5%) and 79 patients (70.5%), respectively. Duration of infertility, number of follicles, and endometrial thickness were significantly different between the two groups. The rate of pregnancy was significantly higher in Group A (51.5%) than in Group B (19%) (p=0.001). There were significant differences between positive and negative pregnancy cases in terms of age, type and duration of infertility, estradiol level, motile sperm number and morphology, number of follicles, and endometrial thickness. The follicle count and estradiol levels were significant risk factors for clinical pregnancy.

Conclusion: Estrogen has a positive effect on pregnancy rates in GI-IUI. The follicle number and estradiol level can be used as a risk factor for IUI.

Keywords: Infertility, intrauterine insemination, gonadotropins, estrogen, clinical pregnancy

INTRODUCTION

Intrauterine insemination (IUI) is preferred, especially in cases of unexplained infertility or cases with mild to moderate endometriosis and/or infertility associated with the mild male factor (1,2). The IUI cycles performed using different agents cause an increase in estrogen levels with the effect of formed ovarian follicles, thereby the thickening of the endometrium (1). In cases where IUI was performed using clomiphene citrate, it has been reported that the endometrial thickness induced by the anti-estrogenic effect of clomiphene at the endometrial level was lower than the thickness induced by natural cycles (3-5). In contrast, it has been speculated that endometrial thickness will not be adversely affected in gonadotropin-induced IUI since injectable gonadotropins do not have a regulatory effect on estrogen (1). However, the possible relationships between gonadotropin-induced IUI, estrogen level, and endometrial thickness have not been studied in detail.

Estrogen is known to positively affect the pregnancy-related endometrial thickness, cervical mucus, and uterine blood flow (6). Therefore, it may be possible to prevent events such as impaired endometrial development and decreased uterine blood flow, especially in IUI, with exogenous estrogen support.

The studies available in the literature addressed the effect of estrogen in IUI performed using clomiphene citrate with or without ethinyl estradiol. In one of these studies, Gerli et al. (7) demonstrated that the use of clomiphene citrate and ethinyl estradiol caused an increase in endometrial thickness. In another study, Unfer et al. (8) reported that even low doses of ethinyl estradiol have a

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significant effect on endometrial thickness, histological matching, and morphological characteristics of the endometrium. On the other hand, the effect of estrogen in gonadotropin-induced IUI has not been adequately studied. In this context, the objective of this study is to investigate the effect of oral estrogen supplementation on pregnancy outcomes in IUI performed with injectable gonadotropin and determine the variables that affect clinical pregnancy rates in gonadotropin-induced IUI.

MATERIAL AND METHOD

Research Design

This study has been designed as a retrospective study of patients who underwent infertility treatment with IUI between September 2016 and September 2019. The study was carried out in accordance with the ethical standards set forth in the Declaration of Helsinki. This study was approved by Van Training and Research Hospital Clinical Research Ethics Committee (Date: 09.01.2020, Decision No: 2020/01). Written informed consent was not obtained from the patients due to the study's retrospective nature.

Population and Sample

The study population comprised the couples with no pregnancy despite having regular sexual intercourse at least twice a week for at least one year, couples in which the female partner has been shown to have at least one tube passage and peritoneal distribution by hysterosalpingography performed in the last two years, couples in which the male partner had no azoospermia, and couples with unexplained primary and secondary infertility. Demographic and clinical data of all patients who were treated for infertility in the clinic where this study was conducted were concurrently recorded in a computerized recording system from the time of admission to the end of the treatment. Obstetric and gynecological backgrounds and menstrual cycle patterns of the patients were investigated. All patients underwent a physical examination. Additionally, the necessary laboratory tests, i.e., thyroid-stimulating hormone (TSH), prolactin, and testosterone tests, were performed in order to detect endocrine and metabolic diseases, if any. Patients with infertility duration of 10 years or longer, patients with a basal follicle-stimulating hormone (FSH) level of >10 IU/L and a basal estradiol (E2) level of >80 pg/mL on the 2nd or 3rd day of menstruation, patients with additional endocrine and metabolic diseases, and patients with solid or cystic mass detected on transvaginal ultrasound (TVUS) were excluded from the study. All male patients were evaluated with urology examination and at least two spermiogram tests performed at different times. Couples with severe male factor were excluded from the study. In the end, 112 primary and secondary infertile couples were included in the study sample (**Figure 1**).

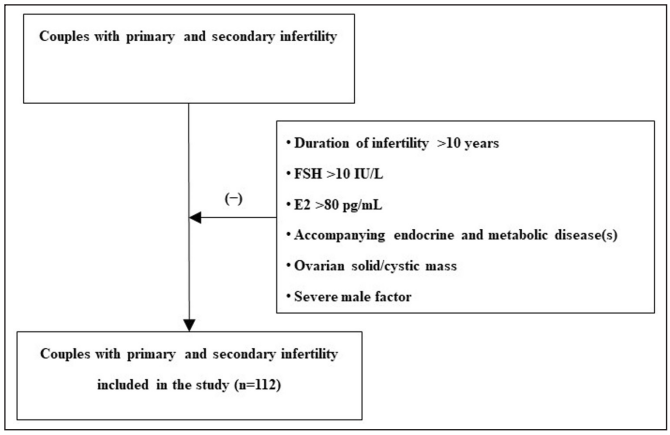

Figure 1. Flow chart of the study.

Ovarian stimulation and IUI protocol

Ovarian stimulation was performed on the 3rd day of menstruation in patients with regular cycles or on the 3rd day of presumed menstruation in oligomenorrhea patients without regular cycles. 50 to 150 IU of recombinant FSH (GonalF®; Merck Serono, Italy, Puregon®; Merck-Sharp & Dohme, Australia) was administered to the patients, taking into account their age, body mass index (BMI), ovarian reserve status, and response to previous treatments, if any. TVUS and serum E2, luteinizing hormone (LH), and progesterone measurements were employed for cycle monitoring. Ovarian follicle detection and endometrial thickness (mm) measurements were performed by TVUS.

In cases with a follicle size of 18 mm and above and spontaneous LH surges, a single IUI session was performed at the 36th hour following the administration of 10.000 IU human chorionic gonadotropin (hCG) (Pregnyl®; Organon, the Netherlands) intramuscularly or 250 µg recombinant hCG (Ovitrelle®; Merck-Serono, Italy) subcutaneously. The vulva was washed with physiological saline solution during the procedure, and then 0.5 mL of sperm was injected into the intrauterine cavity using a soft insemination catheter (Ainseblue-R RI. Mos., Italy). The procedure was terminated after 15 minutes of resting in the supine position.

Luteal phase support was provided with micronized vaginal gel (Crinone 8% vaginal gel, Merck Pharmaceuticals, Bedfordshire, England) containing 90 mg progesterone and continued until the 12th week in patients who developed pregnancy. Oral estrogen supplementation [Estrofem (once daily 2 mg tablet) Novo Nordisk, İstanbul, Turkey] was given to some of the patients, according to the decision of the primary researcher. The patients were divided into two groups according to whether they were administered oral estrogen therapy: patients who were administered vaginal progesterone gel and oral estrogen therapy (Group A) and patients who were administered only vaginal progesterone gel (Group B).

Data Collection

Patients' age (years), type of infertility (primary or secondary), duration of infertility (years), FSH (IU/L) and E2 (pg/mL) levels, antral follicles detected by TVUS (n), and endometrial thickness (mm) measured on the day of administration of hCG were recorded. The total progressive motile sperm count (TPMSC) (n/mL) in men and the percentage (%) of sperms with normal sperm morphology according to Kruger criteria were recorded in the database. Couples were evaluated in two subgroups according to their TPMSCs: couples with>10 million progressive motile sperms per mL and couples with <10 million progressive motile sperms per mL. In addition, couples were divided into two subgroups according to the male partner's percentage of sperms with normal sperm morphology: couples who had >4% sperms with normal morphology and couples who had <4% sperms with normal morphology. Pregnancy was assessed with hCG measurement on the 14th day after the IUI application and confirmed clinically by the presence of a heartbeat in TVUS performed at the 7th week on average.

Statistical Analysis

The primary outcomes of the study were the differences between the treatment groups in pregnancy rates and endometrial thickness, whereas the secondary outcomes were the risk factors that affected the success of clinical pregnancy. The descriptive research data were tabulated as mean±standard deviation or median (interquartile range) values in the case of continuous variables and expressed as numbers and percentages in the case of categorical variables. The Kolmogorov-Smirnov test was used to determine whether the numerical variables conformed to the normal distribution. In the comparisons between the groups, the independent samples t-test was used in cases where numerical variables conformed to the normal distribution, and the Mann-Whitney U test was used in cases where numerical variables did not conform to the normal distribution. The Pearson's chi-squared test or the Fisher's exact test was used for comparisons between categorical variables by groups. The univariate and multiple logistic regression models were used to investigate the risk factors affecting the pregnancy test results. The results of the univariate and multiple logistic regression analyses were given in terms of odds ratio (OR) values within 95% confidence interval (CI). Statistical analyzes were performed with the Jamovi 1.0.7 (Jamovi Project, version 1.0.7, 2019, retrieved from https://www.jamovi.org) and JASP 0.11.1 (Jeffreys's Amazing Statistics Program, version 0.11.1, retrieved from https://jasp-stats.org) software packages. Probability (p) values of <0.05 were deemed to indicate statistical significance in all statistical analyses.

RESULTS

A total of 112 couples were evaluated within the scope of the study. The mean age of female patients was 31.3±6.1 years. Primary infertility was detected in 87 (77.7%) of the couples. The median duration of infertility was 5 (min.:3, max:7) years.

There were 33 (29.5%) patients in Group A and 79 (70.5%) patients in Group B. There was no significant difference between the groups in terms of age, infertility type, FSH, and E2 levels (p>0.05 for all cases) (**Table 1**). However, the median duration of infertility in Group A, the study group, was significantly shorter than in Group B [3 (min.3-max.6) years vs. 6 (min.5-max.7) years, respectively] (p=0.022). Additionally, the rate of couples with TPMSC>10 million/mL and the rate of couples with sperms with normal sperm morphology >4% were significantly higher in Group B than in Group A (p=0.022 and p=0.036, respectively). In Group A, the median number of antral follicles was determined as 5 (min.:3, max.:6), and the median endometrial thickness was determined as 7.0 (min.:6, max.:9) mm, indicating a significant difference between the groups in favor of Group A (p=0.013 and p=0.004, respectively). The rate of couples with positive pregnancy was 51.5% (17 couples out of 33 couples) in Group A and 19% (15 out of 79 couples) in Group B, indicating a significant difference between the groups in favor of Group A(p=0.001) (**Table 1**). The overall clinical pregnancy rate in the study sample was determined as 28.6% (32 out of 112 couples).

Table 1. Demographic and clinical characteristics of the study group (Group A) and the control group (Group B).

	Group A (n=33)	Group B (n=79)	p
Age (years)[a]	30.5±5.7	31.5±6.2	0.414
Type of infertility (%)[b]			0.154
Primary	29 (87.9)	58 (73.4)	
Secondary	4 (12.1)	21 (26.6)	
Duration of infertility (years)[c]	3.0 [3.0- 6.0]	6.0 [5.0- 7.0]	0.022
FSH (IU/L)[a]	7.9±1.5	7.5±1.3	0.204
E2 (pg/mL)[a]	39.0±14.3	37.1±11.5	0.489
TPMSC (%)[b]			0.022
<10 million/mL	11 (33.3)	10 (12.7)	
>10 million/mL	22 (66.7)	69 (87.3)	
Sperms with normal morphology (%)[b]			0.036
<4%	11 (33.3)	11 (13.9)	
>4%	22 (66.7)	68 (86.1)	
Number of antral follicles[c]	5.0 [3.0-6.0]	3.0 [2.0-4.0]	0.013
Endometrial thickness (mm)[c]	7.0 [6.0-9.0]	6.0 [5.0-7.0]	0.004
Pregnancy (%)[b]			0.001
Negative	16 (48.5)	64 (81.0)	
Positive	17 (51.5)	15 (19.0)	

[a]Mean±standard deviation (independent samples t-test), [b]Number (%) (Pearson's chi-squared test), [c]Median [interquartile range] (Mann-Whitney U test), FSH: follicle-stimulating hormone. TPMSC: total progressive motile sperm count. E2: estradiol

There were significant differences between cases with positive and negative pregnancy in terms of age, infertility type, infertility duration, E2 level, TPMSC, sperm morphology, antral follicle count, and endometrial thickness (p<0.05 for all cases) (**Table 2**). The positive pregnancy rate was significantly higher among young female patients and in cases with primary infertility, short infertility duration, and low E2 levels. The rate of couples with TPMSC>10 million/mL and normal sperm morphology >4% was higher among the couples with positive pregnancy. In female patients with and without positive pregnancy, the median number of antral follicles was 6 and 3, and the median endometrial thickness was 9.0 mm and 6.0 mm, respectively (p<0.001).

Table 2. Demographic and clinical characteristics of patients with and without clinical pregnancy.

	Clinical pregnancy		
	Negative (n=80)	Positive (n=32)	p
Age (years)[a]	32.4±6.5	28.3±3.2	<0.001
Type of infertility (%)[b]			0.020
Primary	57 (71.2)	30 (93.8)	
Secondary	23 (28.7)	2 (6.2)	
Duration of infertility(years)[c]	6.0 [5.0-7.0]	3.0 [2.0-3.0]	<0.001
FSH (IU/L)[a]	7.7±1.4	7.3±1.3	0.173
E2 (pg/mL)[a]	40.6±13.1	30.3±5.5	<0.001
TPMSC (%)[b]			0.003
<10 million/mL	21 (26.2)	0 (0.0)	
>10 million/mL	59 (73.8)	32 (100.0)	
Sperms with normal morphology (%)[b]			0.002
<4%	22 (27.5)	0 (0.0)	
>4%	58 (72.5)	32 (100.0)	
Number of antral follicles[c]	3.0 [2.0-4.0]	6.0 [5.0-7.0]	<0.001
Endometrial thickness (mm)[c]	6.0 [5.0- .0]	9.0 [8.0-10.0]	<0.001

[a]Mean±standard deviation (independent samples t-test), [b]Number (%) (Pearson's chi-squared test or Fisher's exact test), [c]Median [interquartile range] (Mann-Whitney U test), FSH: follicle stimulating hormone, TPMSC: total progressive motile sperm count, E2: estradiol

The univariate logistic regression analysis revealed that age, infertility type, duration of infertility, E2 levels, and the number of antral follicles were significant risk factors for pregnancy (**Table 3**). The multiple logistic regression analysis of these risk factors revealed that the number of antral follicles [4.47 (2.4-8.32), p<0.001] and low E2 levels [0.87 (0.79-0.96)pg/mL, p=0.004] were significant risk factors for clinical pregnancy.

DISCUSSION

The results of this study indicated that oral estrogen supplementation in gonadotropin-induced IUI was a significant factor in achieving clinical pregnancy. In addition, it has been determined that the number of antral follicles and E2 levels may also be considered positive risk factors in achieving clinical pregnancy.

It was reported in the literature that the use of estrogen in the IUI treatment positively affected the pregnancy rates (7,8). For instance, in the IUI study conducted by Moini et al. (6) on infertile polycystic ovarian patients, a success rate of 29% was obtained in the group where clomiphene citrate and low dose (0.05 mg) ethinyl estradiol were used compared to 10% in the group where only clomiphene citrate was used. Similarly, the clinical pregnancy rates with and without the use of estrogen were determined as 51.5% and 19.5% in this study. In another study, a significant improvement was detected in endometrial thickness (12-15 mm) with the use of ethinyl estradiol together with clomiphene citrate. In the said study, increasing the ethinyl estradiol dosage from 0.02 mg to 0.05 mg did not yield any significant difference in results (8). It has been reported that the endometrial thickness was>6 mm in each case that was administered IUI along with ethinyl estradiol and that the pregnancy rate in these cases was significantly better (18.75% vs. 6.25%) (7). This positive effect of ethinyl estradiol could not be demonstrated in Moini's study (6); however, this result was attributed to the effect of estrogen on endometrial thickness (7). In comparison, the endometrial thickness was significantly higher in the estrogen-administered group (Group A) than in the control group (Group B). As a matter of fact, the higher pregnancy rate observed in Group A was attributed to higher endometrial thickness.

In a systematic review, it was demonstrated that the administration of additional estrogen to progesterone as luteal phase support for in vitro fertilization, one of the assisted reproductive treatment methods, increased the clinical pregnancy rates by 1.66 times (9). Additionally, it has been speculated that phytoestrogen supplementation would positively affect pregnancy rates (10). However, further prospective studies are needed to determine the net effect of estrogen support on assisted reproductive treatment methods since the studies available in the

Table 3. Univariate and multiple logistic regression analysis for pregnancy development

	Model 1			
	Univariate		Multiple	
	OR (%95 CI)	p	OR (%95 CI)	p
Age (years)	0.86 [0.79- 0.95]	0.002	0.93 [0.79- 1.08]	0.328
Type of infertility (secondary/primary)	0.17 [0.04- 0.75]	0.020	0.08 [0- 2.89]	0.170
E2(pg/mL)	0.91 [0.87- 0.96]	<0.001	0.87 [0.79- 0.96]	0.004
Number of antral follicles	4.4 [2.56- 7.56]	<0.001	4.47 [2.4- 8.32]	<0.001
Duration of infertility(years)	0.23 [0.13- 0.39]	<0.001	-	-

Dependent variable: clinical pregnancy. OR: odds ratio, CI: confidence interval, E2: estradiol.

literature on the assisted reproductive treatment methods and risk factors for the clinical pregnancy, though they are detailed, do not provide clearly defined numerical values. The studies that investigated the relationship between the number and size of antral follicles and pregnancy rates also do not provide clearly defined limit values. In the study of Sun et al. (11), it was reported that the highest pregnancy rates were obtained in cases with a follicle size between 19 and 21 mm and that the lowest pregnancy rates were obtained among cases with a follicle size larger than 21 mm. On the other hand, Merviel et al. (12) reported >16 mm as the optimum follicle size for higher pregnancy rates. In comparison, a limit value was not determined in this study; instead, the evaluations were made based on the comparison of the results of measurements between the groups investigated within the scope of the study.

In a study conducted by Liu et al. (1), no positive correlation was found between endometrial thickness and pregnancy rates in cases administered gonadotropin-induced IUI. Similarly, the meta-analyses available on the subject did not reveal a positive correlation between endometrial thickness and clinical pregnancy rates in cases administered gonadotropin-induced IUI (13). In contrast, some studies reported low pregnancy rates among cases with an endometrial thickness of <6 mm (14). In comparison, although the multiple regression analysis conducted within the scope of this study did not reveal endometrial thickness as one of the significant positive risk factors for the clinical pregnancy, the estrogen-administered group (Group A) and cases where clinical pregnancy was achieved had significantly higher endometrial thicknesses. Additionally, the subgroup analyses revealed significantly more clinical pregnancies in cases with endometrial thickness between 10.5 mm and 13.9 mm, indicating the non-linear relationship between endometrial thickness and clinical pregnancy. The fact that no significant difference was found in endometrial thickness between the estrogen-administered group and the control group in other studies available in the literature was attributed to the small sample size employed in these studies and the non-linear relationship between the pregnancy rate and the endometrial thickness (1).

In a study conducted by Bakas et al. (2) on the effect of changes in estrogen levels observed during the IUI process on clinical pregnancy rates, it was determined that the percent change observed in estrogen levels between the 6th and 10th day following the start of the IUI process was less in cases with clinical pregnancy than in other cases, which was thus suggested as a predictor for clinical pregnancy. Similarly, it was reproducibly shown that the pregnancy rates were positively correlated with the estrogen levels (>500 pg/mL) observed during hCG administration but not with the E2 levels measured on the 3rd day of the cycle (12). In parallel, in a prospective study, no correlation was found between E2 levels and pregnancy rates (15). In comparison, only the baseline FSH and E2 levels before the start of the IUI treatment were investigated in this study, whereas the change in estrogen levels or the estrogen levels during hCG administration were not. Consequentially, it was determined that the E2 levels of the cases with clinical pregnancy were significantly lower. In addition, low E2 levels were detected as a risk factor in the multiple regression analysis. Further prospective studies may clarify the reciprocal interaction between the number and size of antral follicles and the hormone levels of patients in the context of the medical treatment to be administered for the stimulation of the ovaries.

Limitations of the Study

There were some limitations to this study. First, detailed clinical data of the patients could not be obtained due to the study's retrospective design. Secondly, the relatively small number of couples included in the study may be deemed as another limitation.

CONCLUSION

The findings of the study indicated that estrogen supplementation in cases who were administered gonadotropin-induced IUI positively affected the pregnancy rates. In addition, the number of antral follicles and E2 levels were found as risk factors for IUI success. Further large-scale prospective studies are needed to corroborate the results of this study.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was approved by Van Training and Research Hospital Clinical Research Ethics Committee (Date: 09.01.2020, Decision No: 2020/01).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have participated in the design, execution, and analysis of the paper, and that they approved the final version.

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Red cell distribution width, eosinophil-to-lymphocyte ratio and platelet-to-lymphocyte ratio can predict the development of infection and the number of antibiotics used in elderly patients undergoing revision total knee arthroplasty

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ABSTRACT

Introduction: We aimed to evaluate the relationship between complete blood parameters reported to be associated with inflammation and development of complications, length of hospital stays and the number of antibiotics used in elderly patients undergoing revision total knee arthroplasty (rTKA).

Material and Method: Our retrospective study was conducted in a single center and included 72 older patients who underwent rTKA operations. We recorded patients' firs day preoperative, first day postoperative and 45th day postoperative whole blood parameters.

Results: It was found that the development of postoperative infection and the number of antibiotics used were higher in patients with low preoperative Hb values and high platelet-to-lymphocyte ratio (PLR) rates. In patients with high RDW value and high eosinophil-lymphocyte ratio (ELR) one day after surgery, both the development of infection (P=0.002, P=0.002) and the number of antibiotics used during follow-up were found to be significantly higher (P<0.001, P<0.001). When the laboratory parameters were evaluated 45 days after the operation, it was determined that the RDW (P=0.001, P=0.001) and ELR (P=0.039, P<0.001) elevations continued in the patients who developed infection and used multiple antibiotics. CRP and ESR values one day and 45 days after surgery were also found to be significantly associated with the development of infection and the use of multiple antibiotics.

Conclusion: RDW, ELR and PLR parameters may be as important as CRP and ESR in predicting the development of infection and the number of antibiotics used in elderly patients undergoing rTKA.

Keywords: Eosinophil-to-lymphocyte ratio, platelet-to-lymphocyte ratio, red cell distribution width, revision total knee arthroplasty

INTRODUCTION

Total Knee Arthroplasty (TKA) has been widely used as a treatment for gonarthrosis in recent years. In parallel, revision total knee arthroplasty (rTKA) procedures are also increasing. Revision TKA is generally applied for infection, aseptic loosening, polyethylene wear, instability, pain/stiffness, osteolysis, and malposition/misalignment (1,2). According to the Canadian Institute for Health Information (CIHI), between 2012 and 2017, out of 84.770 operations (excluding 35.945 patella operations), the most frequent indication for rTKA was infection (3). It was also shown that infection was the most common reason for early TKA failures in a retrospective study of 781 patients who underwent rTKA (4).

Knee arthroplasty surgery, like other orthopedic surgeries and trauma, causes changes in blood parameters, mainly neutrophil and lymphocyte rates (5-7). Blood parameters such as platelet count, neutrophil count, mean platelet volume (MPV), red cell distribution width (RDW), neutrophil-to-lymphocyte ratio (NLR), and platelet-to-lymphocyte ratio (PLR), which are easily accessible, have been evaluated in several knee surgery studies. Platelet count, MPV, and NLR have been reported to be better markers than erythrocyte sedimentation rate (ESR) and

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C-reactive protein (CRP) in showing inflammation and joint infection (8,9). In a study, serum IL-6, TNF-a, NLR, and PLR were measured just before the operation and on the first and third days postoperative. Stress response was found to be higher in those with high preoperative NLR (10). RDW is a parameter that shows anisocytosis and is routinely calculated in whole blood tests. High RDW is associated with mortality in many internal and surgical diseases other than anemia (11-13). In patients who underwent revision arthroplasty, those with high preoperative RDW values were reported to have worse post-revision optimal results (14).

In our study, we compared the pre- and postoperative whole blood parameters of elderly patients who underwent rTKA in our clinic with the development of complications, especially infection, the number of antibiotics used, and the length of hospital stay. Our aim was to evaluate the relationship between whole blood parameters known to be associated with inflammation, especially RDW, NLR, PLR, eosinophil-lymphocyte ratio (ELR), and the development of postoperative complications, length of hospital stay, and the number of antibiotics in elderly patients undergoing rTKA. The current literature contains no studies of the rTKA patient group in this context.

MATERIAL AND METHOD

The study was carried out with the permission of the Aydın Adnan Menderes University Faculty of Medicine Non-interventional Clinical Researches Ethics Committee (Date: 15.09.2020, Decision No: 2020/165). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Patients

This study is a single-center, retrospective study. The study was planned after obtaining the ethics committee approval from the institution. Seventy-two patients over 65 years of age were included. Patients who underwent total knee revision due to infection were included in the study. The operations were performed by a single physician.

Patients with iron, vitamin B12, folic acid deficiency and Hb value below 11 g/dL, and patients with malignancy, rheumatological disease, severe liver and kidney disease were not included in the study. Patients who developed complications during the operation and needed blood transfusion were excluded from our study.

In our routine revision procedure, infected total knee prostheses are removed and are replaced with antibiotic spacers. Antibiotic treatment is managed with CRP, ESR, and microbial culture follow-ups. Antibiotics are stopped when CRP falls below 10. A total knee prosthesis revision is performed in cases where the CRP level remains below 10 for at least two months. If there is evidence of infection after the operation, prophylactic antibiotics are started. Treatment is revised according to the results of the antibiogram, and combination treatments are used if necessary.

Postoperative complications were recorded in our study. We also recorded the number and type of antibiotics used to treat infections, as well as hospitalization time. If there was no bleeding, drainage, or other complications in the operation area, the patient was discharged after 3-4 days.

Method

Each patient's body mass index (BMI) was calculated and recorded. Venous blood samples taken for whole blood and biochemical tests were collected in VACUETTE tubes (Greiner Bio-One, Monroe, NC, USA). A complete blood count was performed within 60minutes and measurements were made with a Mindray Auto Hematology Analyzer BC - 6800 (Shenzhen Mindray Bio-medical Electronics, Shenzhen, China) using standard methods and reagents. ESR was measured by the Westergren method and CRP titers were measured using standard reagents on a Beckman-Coulter DXC 800 system analyzers. The normal reference range is 0-20 mm/h for ESR and 0-5 mg/dL for CRP.

We recorded patients' one-day preoperative, first day postoperative, and 45th day postoperative blood parameters: Hb, hematocrit (Hct), red blood cell (RBC), MPV, RDW, white blood cell (WBC), MPV, neutrophil, lymphocyte, monocyte, eosinophil, basophil, and thrombocyte results. NLR, MLR, PLR, ELR, and BLR were calculated. The average values and standard deviations of all blood parameters at the three different times of testing were calculated (**Table 1**). The parameters obtained from the blood tests were compared with complications, antibiotic use, and length of hospital stay.

Statistical Analysis

Statistical analyses were conducted using the Statistical Package for Social Sciences (SPSS) program, 22nd version. Quantitative data are presented as mean±standard deviation. Individuals were divided into two groups according to gender, three groups according to smoking and alcohol use, six groups in terms of having additional disease, three groups according to developing complications, three groups according to the anesthetic agent method applied, and four groups according to the anesthetic method. After testing the suitability of the quantitative data to normal distribution with the Kolmogorov-Smirnov method, a Spearman Correlation Test was conducted to determine the relationship between data that were not suitable for normal distribution. The Mann-Whitney U test was applied between quantitative data that did not show normal distribution and dichotomous data. During the statistical evaluation of normally distributed quantitative data, one-way analysis of variance (ANOVA) and then

Tukey honestly significant difference tests were used to examine the differences between groups. During the statistical evaluation of quantitative data that did not show normal distribution, the Kruskal Wallis method and then the Pairwise Comparisons test were used to examine the differences between groups. Pearson Chi-Square and Fisher Chi-Square tests of independence were used for categorical data. A P-value less than 0.05 was considered statistically significant.

Table 1. Pre- and postoperative whole blood parameters, CRP and ESR average values.

	Preoperative day one (Mean±SD)	Postoperative day one (Mean±SD)	Postoperative day 45 (Mean±SD)
Hemoglobin (g/dL)	11.799±1.915	10.239±1.479	10.211±1.876
Hematocrit (%)	37.04±5.586	32.247±4.315	31.968±5.230
RBC (m/uL)	4.497±0.6712	3.909±0.608	3.904±0.764
RDW (%)	15.407±2.258	15.81±2.940	15.858±2.311
WBC (mm^3)	11.295±5.610	8.475±2.871	9.147±4.407
Neutrophil (mm^3)	5.166±2.289	8.725±5.308	6.661±4.103
Lymphocyte (mm^3)	2.176±0.765	1.767±0.709	1.692±0.831
Monocyte (mm^3)	0.590±0.292	0.614±0.254	0.611±0.287
Eosinophil (mm^3)	0.186±0.149	0.164±0.294	0.163±0.230
Basophil (mm^3)	0.040±0.021	0.031±0.015	0.033±0.017
Thrombocyte (mm^3)	319.63±157.12	288.15±128.76	276.14±109.34
MPV (fL)	9.933±1.297	9.811±1.154	9.807±1.174
NLR	2.503±1.415	5.718±3.779	5.001±4.225
MLR	0.293±0.191	0.390±0.220	0.440±0.302
ELR	0.095±0.085	0.098±0.165	0.093±0.133
BLR	0.025±0.035	0.027±0.061	0.024±0.211
PLR	154.035±84.367	180.875±82.305	192.54±113.830
CRP (mg/L)	8.372±17.266	9.676±28.373	7.291±13.162
ESR (mm/h)	27.18±25.32	21.09±44.81	21.17±28.78

RBC: Reed blood cell, RDW: Red cell distribution widths, WBC: White blood cell, MPV: Mean platelet value, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, NLR: Neutrophil-to-lymphocyte ratio, MLR: Monocyte-to-lymphocyte ratio, ELR: Eosinophil-to-lymphocyte ratio BLR: Basophil-to-lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio

RESULTS

Of the 72 patients included in the study, 54 were women (75%). The mean age of the patients was 69.99±4.82 years (range 65-81) and mean BMI was 30.49±3.64 kg/m^2 (range 24-38). Fifty-five of the patients (76%) had chronic diseases such as hypertension, diabetes mellitus, coronary artery disease or cerebrovascular disease. Out of 72 patients, 58 had no history of smoking or alcohol use (81%), while 14 had a history of smoking and/or alcohol use. The average hospital stay was 5.1±2.6 days. There were a variety of postoperative complications: seventeen patients developed infections and seven patients experienced embolic events (deep vein thrombosis in three patients, pulmonary embolism in two patients, myocardial infarction in one patient, and a cerebrovascular accident in one patient).

In patients with postoperative infections; ampicillin + sulbactam, ciprofloxacin, cefazolin sodium, sodium fusidate, cephalexin monohydrate, clindamycin, cefixime, gentamicin, rifambisin, trimethoprim + sulfamethoxazole, levofloxacin, metronidazole, and teicoplanin group antibiotics were used and revised according to culture results and follow-up.

The age, gender, BMI, smoking and/or alcohol use, anesthetic method, and anesthetic agent variables did not affect the length of hospital stays, the development of complications, or the number of antibiotics used. It was observed that the duration of hospitalization was longer in patients with one or more comorbidities (P=0.019). Comorbidities did not affect the development of complications, or the number of antibiotics used.

We separately evaluated the relationships between the duration of hospitalization, development of complications, and the number of antibiotics used to the whole blood parameters, ESR, and CRP values measured one day before, one day after, and 45 days after the operation.

Evaluation of whole blood parameters measured one day before the operation

When the laboratory parameters one day before the operation were evaluated, our data revealed that the postoperative infection development and the number of antibiotics used were both higher in those with low Hb values (P=0.046, P=0.008). In patients with high preoperative platelet counts and PLR rates, both postoperative infection development (P=0.014, P<0.001) and the number of antibiotics used (P=0.001, P<0.001) were observed to be higher. No significant relationship was found between any other parameters (**Table 2**).

Evaluation of whole blood parameters measured one day after the operation

When the laboratory parameters one day after the operation were evaluated, it was found that the development of infections and the number of antibiotics used in follow-up were both significantly higher in those with high RDW values (P=0.002, P<0.001). There were also positive correlations between eosinophil count and ELR, and both development of infection (P=0.001, P=0.002) and the number of antibiotics used (P=0.001, P<0.001, P<0.001). It was observed that the number of antibiotics

used was higher in those with high neutrophil counts and NLR (P=0.026, P=0.003). On the first postoperative day, it was observed that the development of infection (P=0.009, P=0.003) and the number of antibiotics used (P<0.001, P<0.001) were higher in patients with high ESR and CRP. No significant relationship was found between any other parameters (**Table 2**).

Evaluation of whole blood parameters measured 45 days after the operation

When the laboratory parameters were evaluated 45 days after the operation; RDW (P=0.001, P=0.001), eosinophil count (P=0.006, P<0.001) and ELR (P=0.039, P<0.001) parameters were still high in patients who developed infection and overused antibiotics. At day 45, CRP and ESR were also significantly positively correlated with the development of infection (P=0.023, P=0.016) and the number of antibiotics used (P=0.008, P=0.043) (**Table 2**).

DISCUSSION

Whole blood tests can be easily performed routinely in most centers. Various whole blood parameters have been shown to be useful in predicting and evaluating some postoperative problems. In this study, inflammation-related parameters such as CRP, ESR, NLR, PLR, MLR, ELR, BLR and RDW were evaluated in elderly patients undergoing total knee replacement revision surgery. The relationships between these parameters and the development of postoperative complications, the number of antibiotics used, and the length of stay in the hospital were evaluated. The relationship between the parameters in our study and inflammation has been evaluated in different patient groups in many previous studies. Our study is the first study evaluating these parameters in the rTKA patient group.

In several previous studies, the effect of age, gender, BMI, and duration of the operation was evaluated, and it was found that these parameters affect the development of complications (such as infection, prosthetic failure, and thromboembolism) after primary and revision knee surgeries (15-17). In other studies, age, gender, and BMI were not found to be related to operation time or the development of complications (18,19). Out of the factors that were evaluated in our study, age, gender, BMI, alcohol use, smoking, anesthetic method, and anesthetic agent were not found to be associated with the development of infections, number of antibiotics, or the length of hospital stays. It was observed that patients with comorbidities were hospitalized longer. These results are among the important findings of our study. The low number of patients without comorbidity may have caused the lack of a significant relationship with the other two factors. The high number of alcoholic/non-smoker patients may also have caused the absence of a significant relationship.

Table 2. Comparison of pre- and postoperative whole blood parameters, CRP, and ESR and length of hospitalization, infection and number of antibiotics used (p values).

	Preoperative day one p value			Postoperative day one p value			Postoperative day 45 p value		
	Hospital stay	Infection	Antibiotic count	Hospital stay	Infection	Antibiotic count	Hospital stay	Infection	Antibiotic count
Hemoglobin (g/dL)	0.178	0.046*	0.008*	0.982	0.820	0.952	0.605	0.835	0.629
Hematocrit (%)	0.062	0.114	0.367	0.998	0.581	0.276	0.682	0.929	0.244
RBC (M/mcL)	0.058	0.083	0.950	0.607	0.170	0.196	0.935	0.298	0.203
RDW (%)	0.083	0.066	<0.108	0.085	0.002*	<0.001*	0.260	0.001*	0.001*
WBC (mm³)	0.522	0.387	0.538	0.593	0.354	0.120	0.608	0.387	0.745
Neutrophil (mm³)	0.718	0.293	0.413	0.560	0.252	0.026*	0.643	0.372	0.603
Lymphocyte (mm³)	0.455	0.624	0.340	0.360	0.414	0.127	0.298	0.954	0.749
Monocyte (mm³)	0.058	0.072	0.114	0.276	0.243	0.286	0.191	0.262	0.524
Eosinophil (mm³)	0.990	0.388	0.145	0.955	0.001*	<0.001*	0.679	0.006*	<0.001*
Basophil (mm³)	0.188	0.312	0.200	0.987	0.083	0.181	0.095	0.062	0.343
Thrombocyte (mm³)	0.278	0.014*	0.001*	0.463	0.086	0.101	0.635	0.060	<0.091
MPV (fL)	0.589	0.062	0.436	0.792	0.435	0.058	0.752	0.886	0.090
NLR	0.989	0.229	0.096	0.605	0.213	0.003*	0.832	0.509	0.775
MLR	0.590	0.058	0.072	0.377	0.729	0.521	0.626	0.394	0.352
ELR	0.393	0.351	0.086	0.692	0.002*	<0.001*	0.653	0.039*	<0.001*
BLR	0.093	0.572	0.454	0.370	0.226	0.329	0.318	0.153	0.062
PLR	0.507	<0.001*	<0.001*	0.901	0.624	0.210	0.430	0.170	0.086
CRP (mg/L)	0.781	0.087	<0.121	0.509	0.003*	<0.001*	0.953	0.023*	0.008*
ESR (mm/h)	0.946	0.104	<0.234	0.504	0.009*	<0.001*	0.729	0.016*	0.043*

*P<0.05. RBC: Reed blood cell, RDW: Red cell distribution widths, WBC: White blood cell, MPV: Mean platelet volume, CRP: C-reactive protein, ESR: Erythrocyte sedimentation rate, NLR: Neutrophil-to-lymphocyte ratio, MLR: Monocyte-to-lymphocyte ratio, ELR: Eosinophil-to-lymphocyte ratio, BLR: Basophil-to-lymphocyte ratio, PLR: Platelet-to-lymphocyte ratio

RDW is routinely measured during a complete blood count. Aali-rezaie et al. (14) investigated the relationship between preoperative RDW levels and mortality and length of hospital stays after hip and knee revision arthroplasty. They found a significant correlation between preoperative high RDW values and mortality, in-hospital complications, and re-hospitalization within 90 days. In our study, no significant correlation was found between the RDW values on the first day before the operation, the development of complications, the length of hospital stays and the number of antibiotics used. However, high RDW values on the first postoperative day, and 45 days postoperative were found to be significantly associated with both the development of infection and the number of antibiotics used. This significant relationship detected with high RDW value was also in parallel with high CRP and ESR values. These findings, which we found in rTKA patients, are among the important results of our study. With RDW follow-ups that will start immediately after the operation, the development of infection can be predicted and it can give an idea about the number of antibiotics that may be required. In addition, RDW can be as valuable an indicator as CRP and ESR in monitoring the treatment response to infection.

Previous studies suggest that, in patients undergoing total knee and hip revision surgery, platelet counts and MPV should be considered for the diagnosis of periprosthetic joint infection; high platelet counts are an important additional test for the diagnosis of deep surgical site infections after open internal fixation; and perioperative PLR and NLR may be important in predicting deep vein thrombosis (DVT) induced by total joint Arthroplasty (8,20). In our findings, it is seen that high platelet counts and high PLR detected preoperatively are important in predicting the development of postoperative infection. These results suggest that PLR may be more important than CRP and ESR. The development of infections, the number of antibiotics and the length of hospital stay were not significantly associated with MPV and NLR before and after surgery. When our findings are evaluated together with the findings of previous studies, the high PLR detected before the operation seems to be an important parameter in predicting the development of postoperative infection.

Chawla et al. (10) evaluated IL-6, TNF-alpha, NLR, and PLR values pre and postoperatively in 50 patients who underwent primary TKA. IL-6, NLR, and PLR values increased on the first postoperative day and decreased on the third day. They concluded that preoperative risk stratification can be done using preoperative IL-6 and NLR measurements and then appropriate measures can be taken to improve postoperative results. In our study, in which rTKA patients were evaluated and more patients were recruited; It has been determined that preoperative high PLR may be important in predicting the development of postoperative infection. There was no significant relationship between pre- and postoperative NLR. Studies in which more patients are evaluated in different patient groups can be planned.

In addition to whole blood parameters such as RDW, NLR, PLR and MPV, parameters such as ELR and BLR have been investigated in relation to many inflammatory clinical conditions and cancers (21-23). A study conducted by Kökoğlu et al. (24) evaluated whether there was a relationship between patient satisfaction and NLR, PLR, ELR, and BLR in patients who underwent septoplasty. They reported that ELR and BLR can be used to predict patient satisfaction and for patient selection after septoplasty and inferior conchae reduction. Akkececi et al. (25) evaluated the relationship between disease activity and whole blood parameters and inflammation markers in patients with Takayasu Arteritis. They detected significantly higher ESR, CRP, RDW, NLR, PLR, and MLR, and significantly lower MPV in patients with Takayasu arteritis. They found no significant relationship to lymphocytes, monocytes, eosinophil, basophil, ERL, or BLR. In the postoperative evaluations in our study, high eosinophil and ELR ratios were significantly associated with the development of infection and the number of antibiotics used. This result is one of the important findings of our study. As with RDW, follow-up of ELR immediately after surgery can predict the development of infection and give an idea of the number of antibiotics that may be required. In addition, ELR can be as valuable an indicator as CRP and ESR in monitoring treatment response to infection. A significant correlation was found between the number of antibiotics used and the patients with high NLR values on the first postoperative day. No significant relationship was found between NLR and the other two parameters. These findings can be evaluated in different patient groups with new studies involving more patients. In our study, no significant relationship was found between MLR and BLR and complications, number of antibiotics and length of hospital stay.

It was found that patients with low Hb values on the first day preoperatively had a higher rate of postoperative infection and required more antibiotics. This finding shows the importance of adequate preoperative erythrocyte replacement in geriatric patients with low Hb values.

Some whole blood parameters, which have been shown to be associated with inflammation in many previous studies, were evaluated for the first time in the elderly orthopedic patient group. The limitation of this study is that it was performed in a single center and it was retrospective. We thought that the high rate of post-operative infection and slightly longer hospital stay may

be due to the advanced age of our patient group and the high number of patients with comorbidities. The inclusion of patients who underwent revision due to infection in the study may also have caused this situation. In studies involving more patients in different orthopedic patient groups, the relationship of RDW, PLR, ELR, NLR, MLR, BLR whole blood parameters with the development of infection, the number of antibiotics used and the length of hospital stay can be evaluated.

CONCLUSION

In elderly patients who underwent revision knee surgery, high RDW and ELR values detected one day after the surgery may help to predict the development of infection and antibiotic use that may occur during follow-up. These parameters can be as valuable as CRP and ESR in monitoring treatment response to infection. In this patient group, high PLR detected one day before the operation is also an important parameter in predicting the development of postoperative infection.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of the Aydın Adnan Menderes University Faculty of Medicine Non-interventional Clinical Researches Ethics Committee (Date: 15.09.2020, Decision No: 2020/165).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

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Original Article

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The effect of serum 25-hydroxy vitamin D levels on malignancy in exophytic thyroid nodules

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ABSTRACT

Aim: The increase in the incidence of thyroid cancer brings about research of new risk factors. In this study, we aimed to investigate the effect of vitamin D status on malignancy in exophytic nodules.

Material and Method: Two hundred and sixteen patients with exophytic thyroid nodules were included in the study. All patients' thyroid nodule ultrasonographic features, fine needle aspiration biopsy cytology results, rate of surgery and surgery histopathological results were recorded. Vitamin D levels were analyzed and patients were divided into two groups as vitamin D sufficient groups (vitamin D≥20 ng/ml) and vitamin D deficient group (vitamin D<20 ng/ml).

Results: Malignancy rate was significantly higher in the vitamin D deficient group (%19 vs %8.7; p=0.03). There were no significant difference between two groups in terms of demographic characteristics and ultrasonographic features including diameter, hypoechoic nature, having irregular border and microcalcifications.

Conclusion: In exophytic nodules, vitamin D deficiency increases malignancy risk. Determining vitamin D levels may be useful in patients with exophytic nodules.

Keywords: Thyroid nodule, thyroid malignancy, exophytic nodule, vitamin D

INTRODUCTION

Thyroid cancer is the most common malignant tumor in the endocrine system with a rising incidence worldwide over the past decades (1). The factors increasing the risk of thyroid cancer include exposure to radiation to the head and neck, sex, age, iodine deficiency or excess, and family history of thyroid cancer (2,3). Although there are many studies on risk factors and mechanisms in thyroid cancer to date, the number of studies on new risk factors that will explain the dramatic increase in recent years are rather new. Lately, one of the most important potential risk factors suggested by researchers is vitamin D deficiency (4-8).

Epidemiological studies point to a relationship between vitamin D deficiency and cancer risk and alterations in vitamin D levels are involved in the growth regulation of tumours (9,10). The association between vitamin D deficiency and breast, colon, prostate, and pancreatic cancers has been reported (9). In recent years, the number of studies investigating the relationship between thyroid cancer and vitamin D has increased. The data obtained show that low vitamin D is associated with an increased risk of thyroid cancer (11).

Ultrasonographic findings considered as signs for malignancy in a thyroid nodule include the presence of microcalcification, interrupted margin calcification, hypoechogenicity, irregular margin, taller than wide shape and intra-nodular vascularization (2,12-18). In addition, the presence of pathological lymphadenopathy (LAP) and ultrasonographic findings of extrathyroidal extension are considered as signs of malignancy (2,19,20). In recent years, studies on ultrasonographic findings that increase the risk of malignancy have been increasing. There are studies showing an increased risk of malignancy in exophytic nodules (21-23). Exophytic nodule is identified as a nodule that makes a prominent angle with the adjacent thyroid capsule or a nodule that sticks out of the normal thyroid boundary/outline (24).

In this study, we aimed to determine the effect of vitamin D levels on malignancy in exophytic thyroid nodules.

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MATERIAL AND METHOD

All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. Ethics approval has been taken from the Dışkapı Yıldırım Beyazıt Training and Research Ethics Committee (Date: 22.07.2019, Decision No:68/11)

Subjects

Two hundred and sixteen patients with exophytic nodules were included in this retrospective cross sectional study. All patients were followed up in the Endocrinology outpatient clinic of Diskapi Yildirim Beyazit Training and Research Hospital between January 2017 and May 2022. Patients with chronic kidney diseases and patients with a previous history of thyroidectomy and radiotherapy to the head and neck region were also excluded from the study.

Laboratory

TSH and vitamin D levels were noted. Vitamin D cut-off level defining deficiency was 20 ng/ml.

Imaging, Fine Needle Aspiration Biopsy (FNAB) and Surgery

Thyroid ultrasonography (US) and thyroid fine needle aspiration biopsies were performed by Endocrinology and Metabolic Diseases specialists. Hitachi (Hitachi, Japan; EUB 7000) US device with 13 MHz linear probe was used for thyroid US evaluation. Thyroid parenchymal heterogeneity, number of nodules, nodule dimensions (width, depth, and height), nodule properties and localization within the thyroid gland were recorded for each patient. Nodules disrupting the natural course of the thyroid capsule border outward by forming a prominent angle were defined as exophytic nodules.

Thyroid FNAB was US-guided and done by experienced endocrinology specialists. The nodules to be biopsied were decided by following the European Thyroid Association (ETA) guidelines. Patients with malignancy or suspected malignancy cytopathology results underwent surgical treatment.

Cytopathology and Histopathology

Bethesda classification system was used for the cytological diagnoses. The results were reported as benign, atypia of undetermined significance/follicular lesion of undetermined significance (AUS/FLUS), follicular neoplasm/suspicious for follicular neoplasm (FN), suspicious for malignancy and malignant. Patients with non-diagnostic cytology results underwent repeated FNAB after 3 months and adequate biopsy results were considered valid in repeated biopsies. Non-diagnostic results were not included in the study.

Post-operative histopathological results were classified as benign and malignant according to the WHO thyroid cancer classification. In malignant nodules, tumor type, size and histopathological features were documented.

Statistical Analysis

Normal distribution of the variables were determined via visual (histograms, probability plots) and analytic methods (Kolmogorov-Smirnov/Shapiro-Wilk's test). The Mann-Whitney U test was performed to compare non-normally distributed numeric variables. The Chi-square test or Fisher's exact test (when Chi-square test assumptions do not hold due to low expected cell counts) was used to compare the proportions in different groups. The continuity correction was used when the expected count was between 5 and 25. Medians and 25-75% quartile ranges were given for non-parametric parameters. Numbers and percentages were given for categorical variables. A p-value less than 0.05 was considered statistically significant.

RESULTS

As demonstrated in demographic characteristics shown in **Table 1**, there was no difference between two groups in terms of age, sex and TSH levels (normal value range: 0.27-4.2 mIU/L). Ultrasonographic features including diameter, hypoechoic nodule ratio, nodules that have irregular borders and microcalcifications did not reveal any statistically significant difference between two groups. Cytology results are given in **Table 2**. Suspicious for malignancy and malignancy cytology result ratios were higher in the vitamin D deficient group but the results did not reach a statistically significant level (p=0.12). Surgery ratio and histopathological results are given in Table 3. The ratio of the patients who underwent surgery was similar in both groups (p=0,05). Malignancy rate in the vitamin D deficient group was significantly higher (p=0,03). All malignant nodules histopathological results were reported as thyroid papillary carcinoma.

Table 1. Demographic and ultrasonographic characteristics of the patients

	Vitamin D Sufficient Group (n=69)	Vitamin D Deficient Group (n=147)	P value
Age (years)	50.12 (47.58-52.66)	51.39 (48.10-54.68)	0.20
Female. n (%)	62 (89.9)	121 (82.3)	0.15
TSH. mIU/L	1.61 (1.01-2.72)	1.72 (1.11-2.58)	0.11
Ultrasonograpic Features			
Diameter (mm)	17 (12-21)	15 (11-21)	0.06
Hypoechoic nodule n (%)	16 (23.2)	32 (21.8)	0.81
Irregular border n (%)	2 (2.9)	12 (8.2)	0.14
Microcalcification n (%)	3 (4.3)	9 (6.1)	0.59

TSH: Thyroid stimulating hormone

Table 2. Cytology results of the patients

	Vitamin D Sufficient Group (n=69)	Vitamin D Deficient Group (n=147)	P value
Cytology			0.12
Benign, n (%)	56 (81.2)	118 (80.3)	
AUS/FLUS	9 (13)	11(7.5)	
FN/suspicious of FN, n (%)	1 (1.4)	0 (0)	
Suspicious of malignancy, n (%)	1 (1.4)	11 (7.5)	
Malignant, n (%)	2(2.9)	7 (4.8)	

AUS/FLUS: atypia of undetermined significance/follicular lesion of undetermined significance; FN: follicular neoplasm

Table 3. Surgery ratio and malignancy rate of the patients

	Vitamin D Sufficient Group (n=69)	Vitamin D Deficient Group (n=147)	P value
Surgery n (%)	6 (8.7)	28 (19)	0.05
Malignancy (+) n (%)	3 (4.3)	21 (14.3)	0.03

DISCUSSION

In our study, we found that vitamin D deficiency increases the risk for malignancy in patients with exophytic nodules. The signaling role of vitamin D deficiency in the pathogenesis of breast, colon, prostate, and pancreatic cancers has been suggested in literature (9). However, such pathways are not clear for thyroid cancer yet. Several studies showed that the potential antineoplastic effects of vitamin D may include apoptosis (25), paused cell cycle (7,26), inhibited proliferation, promoted differentiation (26,27) and reduced inflammatory response (28). Despite all these data, the role of vitamin D deficiency in carcinogenesis remains controversial. Although many studies have suggested that higher serum vitamin D levels might protect against thyroid cancer (5, 6, 11,29,30), some other studies did not indicate the same results (4, 31-35). In this study, cytology results did not reach a statistically significant level but looking at the statistically significant histopathological results data, our study suggests that vitamin D deficiency increases the risk of malignancy. With further studies in the future, as the data on this subject increases, the importance of the role of vitamin D may become more clearer.

In our study, the malignancy rate in exophytic nodules was found as 11.1% which is higher than the malignancy prevalance (5% for women and 1% for men) indicated by guidelines (2). The incidence of thyroid cancer has been increasing in recent years. Current risk factors have become insufficient to explain this rise. Therefore, in recent years, new risk factors in thyroid cancer have been the subject of researchers. One of the most important evaluation methods in thyroid nodules is thyroid US. Among the thyroid US findings, the features that increase the risk of malignancy are well defined in literature. However, studies on possible additional criteria have been increasing lately. The examination of exophytic nodules is one of these new research areas. Our study supports that the risk of malignancy increases in exophytic nodules. We think that the importance of exophytic nodules will increase as more studies and data are published on this subject.

This study has its limitations as it had a retrospective design, not all histopathological features were recorded and long-term follow-ups have not been evaluated. Also, increasing the number of cases may lead to more statistically significant results including cytology. However, we think that the results of this study will contribute to the literature since there is a considerably growing attention on this subject.

CONCLUSION

Vitamin D deficiency in exophytic nodules increases the risk of malignancy. When evaluating thyroid nodules, it will be useful to assess these two features in addition to the existing risk factors. With the increase in studies on this subject, vitamin d deficiency in exophytic nodules may take place in the literature as a modifiable risk factor.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Dışkapı Yıldırım Beyazıt Training and Research Hospital, Noninvasive Clinical Ethics Committee (Date:22.07.2019, Decision No: 68/11).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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Original Article

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Controversies in neonatology: The efficacy of inhaled nitric oxide in preterm infants with persistent pulmonary hypertension

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ABSTRACT

Introduction: There is limited and conflicting information in literature regarding use of inhaled nitric oxide (iNO) in preterm infants. In this study we examined the characteristics of preterm infants with persistent pulmonary hypertension (PHT) who responded and did not respond to iNO therapy .

Material and Method: We retrospectively reviewed data of infants <34 weeks of gestational age with hypoxic respiratory failure that received iNO for PHT after being diagnosed with severe respiratory distress syndrome after birth. The data of responders and non-responders to iNO therapy were compared.

Results: Twenty-five infants were included in our study. Twelve (48%) had a positive response to iNO administration for PHT. As an antenatal characteristic, oligohydramnios was significantly higher in responders [5 (41.7%) vs 0%, p=0.015] and mortality rate was lower (66% vs. 100%, p=0.039). The SpO_2/FiO_2 ratio before iNO treatment predicted the response to iNO in preterm neonates with PHT. The ROC analysis yielded an area under curve AUC for SpO_2/FiO_2 ratio before iNO of AUCSpO_2/FiO_2before was 0.756; 95% CI, 0.554-0.959; P=0.03. A cut-off value of 79 point by the SpO_2/FiO_2 ratio before iNO treatment predicted the response to iNO treatment with 83% sensitivity and 70% specificity.

Conclusion: In infants born <34 weeks gestation, response to iNO in PHT has a significant effect on improving survival. The presence of oligohydramnios may be an important factor in prediction of positive response. SpO_2/FiO_2 ratio can be useful for estimating the effectiveness of iNO.

Keywords: Nitric oxide therapy, premature, pulmonary hypertension

INTRODUCTION

Nitric oxide (NO), a naturally produced lipophilic endogenous free radical, is synthesized by nitric oxide synthase from the amino acid L-arginine (1). Endogenous NO, which has a biological half-life of a matter of seconds, is produced by venous and arterial endothelial cells, inflammatory cells (macrophages, neutrophils), smooth muscle cells, epithelial cells, fibroblasts as well as non-cholinergic and non-adrenergic cells. Nitric oxide relaxes the smooth muscles of both the pulmonary vessels and bronchi and therefore has a role in the control of pulmonary artery pressure and bronchial tone (2).

In cases of unsuccessful intrauterine to extrauterine transition, persistent increased pulmonary vascular resistance leads to higher risk of mortality and morbidity and is clinically characterized by hypoxemic respiratory failure (HRF) due to persistent pulmonary hypertension of the newborn (PPHN) (3). Hyaline membrane disease, sepsis, and pulmonary hypoplasia may be the underlying etiopathogenetic factors. Inhaled nitric oxide (iNO) has been demonstrated to increase survival in hypoxemic term or near term infants by reducing the need for use of extracorporeal membrane oxygenation (ECMO) (4,5).

The incidence of PPHN per 1000 live births ranges 1.2–4.6 in Asian countries, compared to 1.8–1.9 in the USA. The higher incidence in Asian countries may be due to prematurity, neonatal infection, and low-middle income as well as lack of treatment options such as iNO and ECMO (6).

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Many studies have failed to demonstrate the benefit of iNO in preterm infants born before 34 weeks of gestation, possibly due to insufficient standardization of patients and lack of disease stratification (7). However, there are cohorts of preterm infants < 30 weeks gestation with PHT demonstrating that iNO decreases fraction of inspired oxygen (FiO_2) and oxygenation index (OI) leading to a rapid recovery of oxygen levels (8,9).

Herein, we evaluated the effectiveness of iNO with regards to acute oxygenation and clinical status in premature infants with confirmed acute PHT.

MATERIAL AND METHOD

Approval was granted by the Ethics Committee of University of Health Sciences Zeynep Kamil Maternity and Children's Research and Training Hospital (Date: 03.07.2018, Decision No: 30). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Participants were enrolled in this retrospective cohort study that was performed at a tertiary neonatal intensive-care unit (NICU) (65 incubators and over 1200 newborn admissions per year) between January 2013 and July 2018.

Medical data of infants hospitalized in the NICU were evaluated and preterm infants born before 34 weeks of gestational age undergoing iNO therapy indicated for acute PHT were enrolled for evaluation. Exclusion criteria were: (1) iNO used for bronchopulmonary dysplasia (BPD), (2) Having congenital, genetic or cardiac anomalies (excluding patent ductus arteriosus), (3) transfer to or from NICU.

Patients' demographic characteristics (gestational age, birth weight, gender), perinatal (Preterm prolonged rupture of membranes [PPROM], oligohydramnios, antenatal steroids, delivery mode, Apgar scores), and neonatal characteristics (iNO initiation and duration time, echocardiography findings, ventilation strategy, number of surfactant doses, duration of invasive ventilation, concurrent use of inotropes, intraventricular hemorrhage [IVH], sepsis [confirmed by positive blood culture], necrotizing enterocolitis [NEC], development of BPD, and the overall survival) were recorded. Preterm prolonged rupture of membranes was defined as rupture of membranes >18h before 37 weeks of gestation and before the onset of labor (10). BPD was defined in accordance with criteria set by the National Institute for Child Health and Development (11). For IVH, the Papile cranial ultrasound classification was used (12). For the classification of rethinopathy of prematurity (ROP) and the definition of NEC, the standardized international criteria and the modified Bell criteria were used respectively (13,14).

Acute PHT diagnosis was either established by echocardiographic evaluation prior to or during the first 24 hours of iNO initiation or decision was made by clinical findings of hypoxemic failure, defined by need for FiO_2 >70% with pre/post-ductal saturation difference ≥10% (9). Echocardiographic evaluation was performed by a pediatric cardiologist and demonstration of PHT was made by measuring the peak velocity of tricuspid regurgitation (TR max). Modified Bernoulli equation was used to convert Doppler derivated velocity to pressure between the right ventricle and right atrium=4×TRmax2. By adding right atrial pressure (5 mmHg) to this pressure gradient systolic pulmonary arterial pressure was calculated. Hence, we diagnosed PHT when systolic pulmonary arterial pressure was >40 mmHg or it was ≥ systolic systemic arterial pressure. In the absence of a TR max measurement diagnosis of PHT was made according to right-to-left shunt via ductus arteriosus and/or foramen ovale, with or without flattening or bowing of the septum into the left ventricle at end-systole (8). Persistent pulmonary hypertension (PPHT) was defined with increased mean pulmonary pressure and right to left or bidirectional shunt at patent ductus arteriosus [PDA] and/or patent foramen ovale [PFO] level) as detected on echocardiography (6).

The standard approach to the infants with PHT in our hospital includes conventional or high- frequency oscillation ventilation, surfactant treatment, sedation plus use of inotropes as required (generally initially dopamine and dobutamine). iNO for acute PHT is administered as part of the standard care of infants > 34 weeks' gestation. Additionally, iNO was used in children <34 weeks of gestation that did not respond to treatment with surfactant and appropriate ventilation with conventional or high-frequency oscillation ventilation under consultant discretion. Lung expansion improvement is determined by the decrease in the fraction of inspired oxygen with the saturation target of 91-95% in the case of respiratory distress syndrome. Severe RDS was diagnosed when infants required FiO_2 >0.50 to maintain PaO_2 >50 mmHg after surfactant treatment and despite mechanical ventilation at mean airway pressure (MAP) >12 cmH_2O (8).

Blood oxygen saturation level (SpO_2) and fraction of inspired oxygen (FiO_2) was used for monitoring of neonates. The ratio of these two parameters SpO_2/FiO_2 ratio was used in the management of oxygenation status and defined as follows; SpO_2/FiO_2 ratio before iNO and SpO_2/FiO_2 ratio after iNO treatment. Pre-ductal SpO_2 calculations in all patients were recorded.

Inhaled nitric oxide was commenced using a dose of 20 parts per million (ppm). For this study, patients with a

reduction in the FiO_2 by 20% within 3h of commencing iNO therapy were defined as "positive responders" and those in which FiO_2 increased, remained unchanged or reduced by <20% were defined as "negative responders". If clinical response was not observed to hypoxemia, the dose of iNO was increased to 40 ppm, since NO improvement to oxygenation is largely dose dependent and higher doses of iNO (20-80ppm) may cause progressive pulmonary vasodilatation (15-17). Blood gases and levels of methemoglobin were analyzed prior to initiation of iNO therapy and every 4h thereafter. Complete blood count was also performed within 48 h of starting iNO. Acute PHT was defined as "early" if it developed within the first 72 h of life, or "late" if it developed thereafter. For the weaning process in positive responders, iNO was lowered initially to 5 ppm by decreasing 2 ppm every 4h, then slowly to 1 ppm. In negative responders, weaning was achieved by decreasing to 5 ppm by lowering 5 ppm every 15 minutes, and then by 1 ppm every 15 minutes thereafter. Echocardiographic controls of the infants who respond to iNO and survived has been done during iNO and inotrope therapies.

Study Outcomes

To evaluate the treatment effect of iNO therapy, the primary outcome was established as the difference in FiO_2 requirements caused by iNO therapy (≥20% in infants < 34 weeks, as based on the current definitions). Additionally, neonatal characteristics, IVH, BPD, ROP, and NEC were compared according to positive and negative response.

Statistical Analysis

Data was analyzed using IBM SPSS Statistics for Windows (IBM Corp. Released 2017, Version 25.0. Armonk, NY, USA). Patient characteristics were reported using descriptives. Continuous variables were expressed as mean±standard deviation (SD) or median [Interquartile range (IQR)]. Normality of data for continuous variables was tested with the Shapiro-Wilks test and compared with either the unpaired Student's t-test or Mann-Whitney U test. Categorical data were expressed as n (%) and compared using Fisher's exact test. Subgroups of preterm infants that received iNO were compared using the paired Student's t-test or Mann-Whitney test. Receiver operating characteristic (ROC) analysis was used to evaluate the reliability of the SpO_2/FiO_2 ratio in predicting responding to iNO treatment. Area under the curve (AUC) and reliability data were reported with 95% confidence interval (CI). Cut-off values showed the highest sensitivity. P<0.05 were considered to be statistically significant.

RESULTS

The records of 61 infants born < 34 weeks of gestation who were diagnosed with PHT were reviewed for eligibility. Of these, 30 infants were found to receive iNO during the study period. Those excluded from the study were: iNO administered for chronic PHT (n=2), transferred while receiving iNO (n=1), congenital heart disease (n=1) and congenital anomalies (n=1). Following the exclusion of these patients, twenty-five infants with an average gestational age of 28.3±3.2 weeks and birth weight of 1147±635 gr were included in the study. **Figure 1** shows the CONSORT diagram for the study.

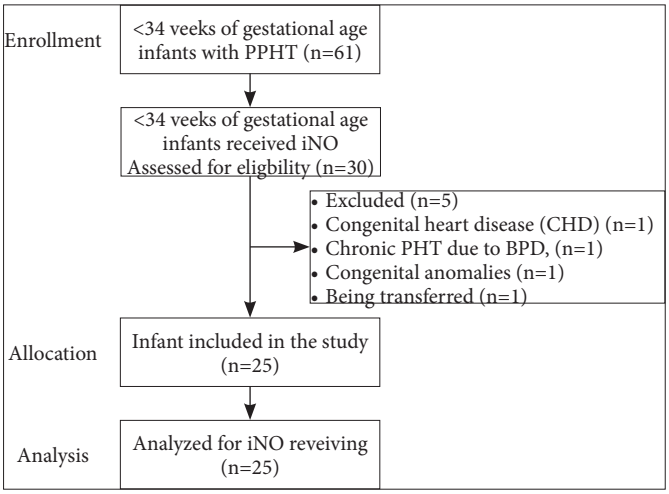

Figure 1. CONSORT chart for selection of eligible infants in the study

Echocardiographically proven acute PHT existed in 23 (92%) infants, while the remaining 2 infants' (8%) diagnosis was clinically based on the presence of ≥10% difference between pre-ductal and post-ductal saturation. However, PHT has been echocardiographically proven to exist in both of these patients after iNO initiation. Moderate-to-large PDA existed in 9 (36%) infants, while 8 (72.7%) of them were <28weeks of gestation. Infants responded to iNO with moderate-to-large PDA (16.7% vs 53.8%, p=0.053) were not significantly different from non-responders.

Twelve infants (48%) were found to be positive responders. Oligohydramnios [5 (41.7%) vs 0%, p=0.015] and mortality [8 (66%) vs 13 (100%), p=0.039] were significantly different between positive versus negative responders.

iNO was administered for a median of 2.5 days in positive and 1 day in negative responders (p=0.016) with maximum median iNO dose of 20 and 40 (p=0.001) for positive and negative responders, respectively. pH in blood gas analysis 4h after commencement of iNO was determined to be significantly increased in

positive responders (7.29±0.14 vs 7.13±0.21, p=0.044). SpO_2 before iNO and 1h after iNO was found to be significantly higher in the positive versus negative responders [80 (78-83) vs 70 (70-80), p=0.025] and [(90 (88-94) vs 72 (70-80), p<0.001)]. SpO_2/FiO_2 ratio was significantly increased in positive versus negative responders [115 (110-126) vs.77 (71-86), p<0.001)] 1 h after iNO commencement. There was a lower mortality rate in the positive versus negative responders [8 (66%) vs 13 (100%), p=0.039]. Patients' characteristics such as gestational age, gender, antenatal steroids, PPROM, early PHT, number of surfactant doses, Apgar values, HFOV application, SpO_2, pH value before iNO were found to be not statistically significantly related with respect to positive response to iNO. Factors found to have a statistically significant difference between positive and negative responders are shown in **Table 1**.

Fifteen infants (60%) were diagnosed with early and 10 (40%) with late acute PHT. Preterm infants having early-onset acute PHT were initiated on iNO at a median of 2 (2-2) days compared to 6 (4-12.5) days (p<0.001) in the late group. Patients with late acute PHT had higher pH in blood gasses analyses before iNO administration compared with the early acute PHT group (7.11±0.17 vs 7.27±0.19, p=0.044). No difference was detected in any other parameter between the groups with regards to survival. There was no significant difference between SpO_2/FiO_2 ratios in early and late PHT after iNO [110 (74-120) vs. 97 (83.5-107.5), p=0.739) (**Table 2**).

Patients with early acute PHT were analysed and their data is shown in **Table 3**. Eight (53%) of these infants had a positive response. Antenatal characteristics and the neonatal morbidities were similar between positive and negative responders except for a significantly higher rate of SpO_2 before and 1h after iNO in the positive responders when compared to the negative responders ([80 (80-85) vs 70 (65-70), p=0.003] and [90 (90-97) vs 70 (65-72), p=0.001]) respectively. In blood gas analysis, positive responders with early acute PHT had a significantly higher pH compared to non-responders (7.30±0.16 vs 7.02±0.17, p= 0.007) 4 h after iNO. Preterm infants with early-onset PHT had higher SpO_2/FiO_2 ratio before and 1h after iNO in the positive responders when compared to the negative responders which was significantly higher [80 (80-85) vs 70 (65-70) p=0.003] and [119 (112-137), vs 74 (68-77), p=0.001] respectively.

Table 1. Antenatal and neonatal characteristics of preterm neonates with acute pulmonary hypertension (aPHT) treated with inhaled nitric oxide (iNO); comparisons between positive and negative responders

Patient Characteristic	Positive responders	Negative responders	p
Gestational age, weeks, mean±SD	28.75±3.5	27.85±3.1	0.504
Birth weight, g, median (IQR)	1012 (715-1995)	911 (582-1240)	0.479
Gender, male, n (%)	8 (66)	6 (46)	0.428
Mode of delivery, CS, n(%)	10 (83)	10 (76)	1.000
PPROM, n (%)	3 (25)	5 (13)	0.387
Oligohydamnios, n (%)	5 (41)	0	0.015*
Antenatal steroids n(%)	4 (33)	5 (38)	1.000
Apgar 1st minute, median (IQR)	4 (3-5)	4 (3-4)	0.321
Apgar 5th minute, median (IQR)	6 (6-8)	6 (6-6)	0.081**
Surfactant doses, median (IQR)	2 (1-2.75)	2 (1-2.5)	0.863
HFOV application prior to iNO, n(%)	6 (50)	7 (53)	1.000
Invaziv ventilation duration, days, median (IQR)	7 (3-26.5)	9.54 (3-12)	0.460
iNO max dose, ppm, median (IQR)	20 (20-35)	40 (40-40)	<0.001*
Erarly iNO, n (%)	8 (66)	7 (53)	0.688
iNO initiation, days, median (IQR)	2 (2-4)	3 (2-10)	<0.001*
iNO duration, days, median (IQR)	2.5 (2-4.75)	1 (1-2)	0.016*
Methemoglobinemia >1.8%, n(%)	3 (25)	2 (15)	0.645
Sepsis, n(%)	1 (8)	3 (23)	0.593
Thrombocytopenia <150000/mm, n (%)3	4 (33)	1 (7)	0.160
IVH III-IV, n (%)	4 (33)	2 (15)	0.378
ROP, n (%)	2 (50)	0	
BPD, n (%)	4 (66)	1 (100)	1.00
NEC, n (%)	2 (16)	4 (30)	0.645
Inotropes (Dopamine, dobutamine, adrenaline), n (%)	12 (12)	13 (13)	0.483
pH prior to iNO, mean±SD	7.16±0.20	7.18±0.19	0.763
pH 4 h after iNO, mean±SD	7.29±0.14	7.13±0.21	0.044*
SpO_2 prior to iNO, median (IQR)	80 (78-83)	70 (70-80)	0.025*
SpO_2 1 h after iNO, median (IQR)	90 (88-94)	72 (70-80)	<0.001*
SpO_2/FiO_2 prior to iNO, median (IQR)	80 (69-85)	70 (65-77)	0.0025*
SpO_2/FiO_2 1h after iNO, median (IQR)	115 (110-126)	77 (71-86)	<0.001*
Mortality, n (%)	8 (66)	13 (100)	0.039*

SD: Standart deviation; IQR: Interquartile range; PPROM: Premature prolonged rupture of membranes; CS: Caesarean section; iNO: Inhaled nitric oxide; HFOV: High frequency oscillatory ventilation; IVH: Intraventricular haemorrhage; BPD: Bronchopulmonary dysplasia; SpO_2: Blood oxygen saturation level; *p<0.05: Statistically significant results are shown in bold and italic font type.

Table 2. Comparison of baseline characteristics in patients with early or late acute PHT

Patient Characteristics	Early PHT (n=15)	Late PHT (n=10)	p
Gestational age, weeks, mean±SD	28.87±3.4	27.40±2.9	0.283
Birth weight, g, median (IQR)	1160 (730-1820)	702 (605-1192)	0.090
Gender, male, n (%)	9 (60)	5 (50)	0.697
Mode of delivery, CS, n (%)	13 (65)	7 (35)	0.358
PPROM, n(%)	3 (20)	5 (50)	0.194
Oligohydamnios, n (%)	4 (26)	1 (10)	0.615
Antenatal steroids, n (%)	4 (26)	5 (50)	0.397
Apgar 1st minute, median (IQR)	4 (3-5)	4 (3-4)	0.858**
Apgar 5th minute, median (IQR)	6 (6-7)	6 (5-6)	0.187
Surfactant doses, n (%)	2 (1-2)	2 (1.75-3)	0.266
HFOV application prior to iNO, n (%)	9 (60)	4 (40)	0.428
Duration of invasive ventilation, days, median (IQR)	3 (2-8)	9 (5.5-17)	0.026*
iNO initiation, days, median (IQR)	2 (2-2)	6 (4-12.5)	<0.001*
iNO duration, days, median (IQR)	2 (1-2)	2 (1-5)	0.64
Methemoglobinemia >1.8, n (%)	2 (13)	3 (30)	0.358
Sepsis, n (%)	2 (13)	2 (20)	1.000
Thrombocytopenia<150000/mm, n (%)3	4 (26)	1 (10)	0.615
IVH III-IV, n (%)	4 (26)	2 (20)	1.000
ROP, n (%)	1 (33)	1 (100)	1.00
BPD, n (%)	4 (80)	1 (50)	1.000
NEC, n (%)	0	6 (60)	0.001
Inotropes, n (%)	12 (80)	9 (90)	0.626
pH prior to iNO, mean±SD	7.11±0.17	7.27±0.19	0.044*
pH 4 h after iNO, mean±SD	7.17±0.21	7.26±0.16	0.284
SpO_2 prior to iNO, median (IQR)	80 (70-80)	77 (73-80)	0.909
SpO_2 1 h after iNO, median (IQR)	88 (70-90)	84 (79-90)	1.00
SpO_2/FiO_2 prior to iNO, median (IQR)	80(70-80)	77(73.75-80)	0.909
SpO_2/FiO_2 1h after iNO, median (IQR)	110 (74-120)	97 (83.5-107.5)	0.739
Mortality, n (%)	12 (80)	9 (90)	0.626

SD: Standart deviation; IQR: Interquartile range; PPROM: Prolonged rupture of membranes; CS,caesarean section; iNO: Inhaled nitric oxide; HFOV: High frequency oscillatory ventilation; IVH: Intraventricular haemorrhage; BPD: Bronchopulmonary dysplasia; SpO_2: Blood oxygen saturation level. *p<0.05: Statistically significant results are shown in bold and italic font type.

Table 3. Comparison between positive and negative responders in patients with early acute PHT

Patient Characteristics	Positive responders (n=8)	Negative responders (n=7)	p
Gestational age, weeks, mean±SD	29±3.2	28±3.8	0.662
Birth weight, g, median (IQR)	1012 (760-2093)	1200 (700-1820)	0.908
Gender, male, n (%)	6 (75)	3 (57)	0.315
Mode of delivery, CS, n (%)	7 (87)	6 (85)	1.000
PPROM, n (%)	1 (12)	2 (28)	0.569
Oligohydamnios, n (%)	4 (50)	0	0.077
Antenatal steroids, n (%)	2 (25)	2 (28)	1.000
Apgar 1st minute, median (IQR)	4 (3-6)	4 (3-4)	0.352
Apgar 5th minute median (IQR)	7 (6-8)	6(6-6)	0.062
Surfactant doses, mean±SD	2±1	1.43±1	0.334*
HFOV application prior to iNO, n (%)	3 (37)	3 (42)	1.00
Invaziv ventilation duration, days, median (IQR)	5.5 (2.25-37.25)	3 (2-5)	0.287
iNO initiation, days, median (IQR)	2 (1.25-2)	2 (2-2)	0.370**
iNO duration, days, median (IQR)	2 (1.25-3.5)	1 (1-2)	0.216
iNO max dose, ppm, median (IQR)	20 (20-40)	40 (40-40)	0.066
Methemoglobinemia >1.8, n (%)	1 (12)	1 (14)	1.000
IVH III-IV, n(%)	3 (37)	1 (14)	0.569
pH prior to iNO, mean±SD	7.13±0.21	7.09±0.12	0.641*
pH 4 h after iNO, mean±SD	7.30±0.16	7.02±0.17	0.007*
SpO_2 prior to iNO, median (IQR)	80 (80-85)	70 (65-70)	0.003*
SpO_2 1 h after iNO, median (IQR)	90 (90-97)	70 (65-72)	0.001*
SpO_2/FiO_2, median (IQR)	80(80-85)	70(65-70)	0.003*
SpO_2/FiO_2, median (IQR)	119 (112-137)	74 (68-77)	0.001*
Mortality, n (%)	5 (62)	7 (100)	0.2

SD: Standart deviation; IQR: Interquartile range; PPROM: Prolonged rupture of membranes; CS,caesarean section; iNO: Inhaled nitric oxide; HFOV: High frequency oscillatory ventilation; IVH: Intraventricular haemorrhage; BPD: Bronchopulmonary dysplasia; SpO_2: Blood oxygen saturation level. *p<0.05: Statistically significant results are shown in bold and italic font type.

The SpO_2/FiO_2 ratio before iNO treatment predicted the response to iNO in preterm neonates with PHT. The ROC analysis yielded an area under curve AUC for SpO_2/FiO_2 ratio before iNO treatment of AUCSpO_2/FiO_2 before as 0.756; 95% CI, 0.554-0.959; P=0.03 (**Figure 2**). A cut-off value of 79 point by the SpO_2/FiO_2 ratio before iNO treatment predicted the response to iNO treatment with 83% sensitivity and 70% specificity. The predictive value of SpO_2/FiO_2 ratio before iNO treatment for responding to iNO treatment was higher than the value of blood gas pH. The ROC analysis yielded an AUC for SpO_2/FiO_2 ratios and pH values before iNO treatment as AUCSpO_2/FiO_2 ratio before= 0.756; 95% CI, 0.554-0.959, P=0.03; AUCpH before=0.497; 95% CI, 0.257-0.737, P=0.978, respectively (**Figure 3**).

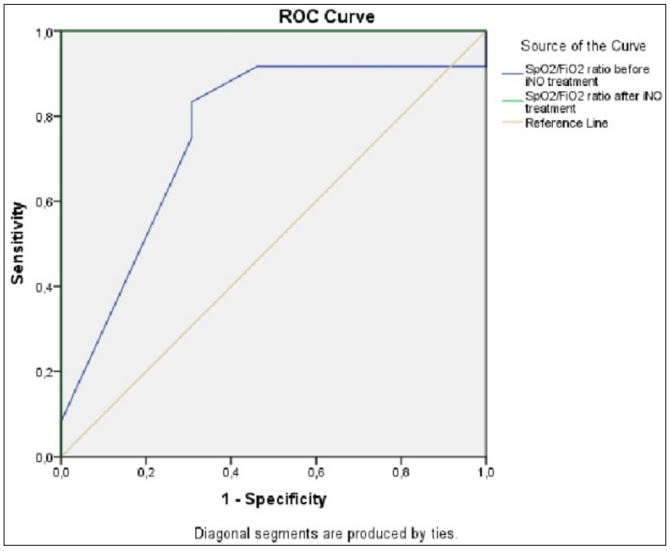

Figure 2. The ROC analysis of SpO_2/FiO_2 ratios before iNO treatment for predicting of Positive Responders in Neonates with PHT. The blue line indicates the SpO_2/FiO_2 ratios before iNO treatnemt. The ROC analysis yielded an AUC for SpO_2/FiO_2 ratios before iNO treatment $AUC_{SpO2/FiO2ratios\ before}$=0.756; 95% Cl, 0.554-0.959, P=0.03.

CL: Confidence interval, iNO: Inhaled nitric oxide, ROC: Receiver operating characteristic curve, SpO_2/FiO_2 ratio PHT: Pulmonary hypertaension

DISCUSSION

Although the indications for iNO treatment in preterm children are vague, our study suggests that it is useful in leading to improvement of HRF in preterm infants with acute PHT.

The rate of mortality in premature infants is severely affected by the presence of respiratory distress syndrome (RDS). Mortality related to RDS in preterm infants changes according to gestational age and ranges from 50-100% (8). Surfactant therapy, advanced ventilation techniques and continuous positive airway pressure have significantly reduced pulmonary morbidity in extremely preterm infants (18). When not treated, RDS may lead to right-to-left extrapulmonary shunting and/or intrapulmonary shunting due to pulmonary hypertension and poor ventilation-perfusion matching respectively (19-21). Preterm infants<28 weeks of gestation have 50-70% moderate- to- large ductal shunt that can increase pulmonary blood pressure and flow and decrease lung compliance stated as 72.7% in our study. On the other hand the presence of an open ductus can regulate extreme elevations in pulmonary arterial pressure as a pop-off valve (22,23).

On the other hand, transition to postnatal circulation may be averted by impaired pulmonary vascular development leading to PHT and HRF (9). This could be the explanation for positive responders to iNO in our study. PPHN is a clinical syndrome that occurs when there is a failure to initiate or sustain the transition to extrauterine life. Affected infants have elevated pulmonary vascular resistance, pulmonary arterial pressure, and right-to-left shunts at atrial and ductal levels at varying degrees (24,25).

In 1999, the FDA approved iNO for use in term and near term infants with PPHN. iNO, a potent, selective pulmonary vasodilator, combines with hemoglobin to form methemoglobin in the intravascular space, preventing systemic vasodilation (selective effect). Additionally it reduces ventilation perfusion mismatch by diverting pulmonary blood to adjacent dilated pulmonary arterioles to only ventilated alveoli (26). Furthermore, iNO can provide a decrease in pulmonary vascular remodeling and lung inflammation by improving airway structure (27,28).

Recent data on the incidence for PHT in term and late preterm infants is reported to be 1-2 per 1000 live births. The exact incidence and clinical features of PHT in extremely premature infants is not known although it is proposed that iNO may be useful in hypoxic respiratory failure secondary to a vascular etiology. Previous cohorts have reported that oligohydramnios and PPROM were more common among infants with early-onset pulmonary hypertension (EOPAH). Infants with EOPAH presenting with HRF have been shown to respond well to iNO treatment (29). As literature on PHT primarily centres on preterm infants with moderate or severe BPD, data on EOPAH and HRF is scarce. Despite significant evidence demonstrating that iNO has no effect on reducing the morbidity or mortality of extremely premature neonates, the off-label prescription of iNO is increasing (30,31). On the other hand, evidence suggests that iNO can be beneficial for a selected subpopulation of extremely premature neonates (32,33). A recent cohort stated that preterm neonates with acute PHT who had positively responded to iNO were associated with a survival benefit (34).

Although the number of preterm infants with positive or negative responses to iNO were approximately equal to each other, iNO response was associated with survival benefit. The inconsistency of response to iNO we observed may be due to several factors. Although the number of neonatal and perinatal characteristics such as birth weight, antenatal corticosteroids Apgar values and use of inotropes were found to be statistically insignificant, this may be due to the small sample size of our study and differences in severity of illness between the positive and negative responders.

Tworetzky et al. (35) reported that an initial dose of 20 ppm for iNO led to oxygen improvement with maximum pulmonary vasodilation with no serious side effects. In our study, the median maximum dose for iNO was observed to be 20 (20-35) among positive responders, compared to 40 (40-40) in negative responders. Inhaled nitric oxide has a good safety profile used at 20ppm, while lower doses may be equally as effective as higher doses. Moreover, higher doses do not provide any additional advantages. The absolute contra-indication of iNO is methemoglobinemia (36,37).

Due to proinflammatory cytokines and endogenous iNO attenuation, infants born with PROM tended to have pulmonary developmental disruptions (38). Pulmonary hypoplasia due to oligohydramnios led to pulmonary hypertension related to pulmonary vascular remodelling in the majority of infants (39). We found olygohydramnios as a significant factor related with a positive response to iNO in our study. In pregnancies complicated by PPROM and olygohydramnios, very low nitrite and nitrate levels were associated with HRF. During iNO initiation nitrite and nitrate concentrations increased (32). Also this finding is consistent with the literature that olygohydramnios and early acute PHT development were the most significant factors related to a positive response (9,40).

iNO therapy leads to an acute decrease in FiO_2 requirement and although the response in pulmonary hypoplasia is similar to that seen in other pulmonary disorders, the mortality rate remains very high in this group. On the other hand, although the mechanism of late-onset PHT is ambiguous, several mechanisms including hypoxia and inflammation have been suggested (41).

Our study aimed to present clinicians with possible indicative factors such as oligohydramnios that have been found to be linked to a positive response to iNO and its continued treatment. Also, it is important to note that our study sheds light on the association of lower mortality rate and positive response to iNO among preterm infants.

Oxygenation index (OI) is an essential indicator in managing neonates with hypoxic respiratory failure (HRF) and pulmonary hypertension (PH). However, the use of the OI is limited by the need for an arterial catheter in each patient. Different studies reported use of oxygen saturation index (OSI) and SpO_2/FiO_2 as non-invasive markers in the management of patients with respiratory failure. In comparison to OI is SpO_2/FiO_2 ratio is a practical non-invasive indicator obtained from mechanical ventilation settings and pulse oxymetry. This indicator does not require repeated samples from the arterial catheter while allowing continuous monitoring of oxygenation status (42). We would especially like to emphasize that SpO_2/FiO_2 ratio can be useful for estimating the effectiveness of iNO despite other invasive oxygenation indices. The SpO_2/FiO_2 ratio has advantages such as being an easy and non-invasive index that can be used at bed-side.

There are some limitations to our study. Firstly, the study was a retrospective analysis and includes a small sample size. Secondly, changes in OI and arterial-alveolar oxygen ratio were not reported as this data was not available for all patients.

CONCLUSION

Overall, our results indicate that infants < 34 weeks gestational age with PHT responded well to iNO with a significant proportion of survival. The use of iNO in the presence of PHT due to oligohydramnios can be a rescue therapy in preterm infants. SpO_2/FiO_2 ratio may be useful for estimating the effectiveness of iNO despite other invasive oxygenation indices. Controversies and confusion remain regarding the care of preterm infants with PHT. There is a need for clarity on the natural history of this diverse disease for early detection of at-risk patients for developing new approaches for diagnosis and treatment.

ETHICAL DECLARATIONS

Ethics Committee Approval: Approval was granted by the Ethics Committee of University of Health Sciences Zeynep Kamil Maternity and Children's Research and Training Hospital (Date: 03.07.2018, Decision No: 30).
Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.
Referee Evaluation Process: Externally peer-reviewed.
Conflict of Interest Statement: The author has no conflicts of interest to declare.
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Author Contributions: The author declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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The factors affecting sexual satisfaction and sexual myths in married women: A prospective study

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ABSTRACT

Aim: The cultural differences and taboos of the society affect women's sexual life and sexual relationship qualities and their belief in sexual myths. In addition, women's demographic characteristics and living conditions are also effective on sexual myths. Our aim in this study is to analyze the frequency of believing in sexual myths in married women and to examine the factors affecting sexual life and sexual relationship qualities.

Material and Method: Married women between the ages of 18-55 in the tertiary gynecology and obstetrics clinic were included in the study, prospectively. Demographic characteristics of women such as age, marital status, marriage and employment status, monthly income, family structure and number of children, sexual life and sexual relationship qualities and sexual myths were compared. The Golombok-Rust Inventory of Sexual Satisfaction (GRISS) Scale, which is used to evaluate the quality of sexual intercourse and sexual dysfunctions, and The Sexual Myth Scale to determine the status of having sexual myths were used.

Results: Overall 171 married women were included in the study. The median age was 35 years (IQR 29-43) and 60.8% (n=104) were university graduates and 20.5% (n=35) were graduate/doctoral graduates. The rate of believing in sexual myths among married women was 21.4%. As the age of the women increased, the frequency of sexual intercourse decreased ($p<0.005$); age of married women had no effect on sexual myths. It was determined that both women with an university or higher education and those with a low level of education have myths; it was seen that education level did not make a difference on sexual myths. While unemployed women were more indifferent to sexual life than working women ($p=0.02$); work status had no effect on sexual myths. All of the women with extended family structure stated that only the time allotted for sexual intercourse during making love is sufficient; 27.1% of women with nuclear family structure stated that this period was not enough ($p=0.037$).

Conclusion: Age, family structure, education level and employment status of married women affect their sexual life, sexual relationship qualities and belief in sexual myths.

Keywords: Sexual myth, sexual life, GRISS, sexuality, sexual satisfaction

INTRODUCTION

The satisfactory sexual life is one of the major component of quality of life and health and the factors which affecting the individual's health, negatively affect women sexual life (1,2). It contains the physical and psychological characteristics and social conditions which affect sexuality and human behavior (1,2). The World Health Organization (WHO) has declared sexual health and sexual functions for women as a basic human right. Especially in the recently published reports, it has been reported that sexual disorders seen in women cause morbidity and decrease in women's quality of life (3).

Sexual dysfunctions are defined as sexual desire disorders and psychophysiological changes that occur in the sexual response cycle (4). Also, it is reported that the frequency of sexual dysfunctions in women varies between 25% and 63% in the literature (5,6). The most important cause of sexual problems is ignorance and/or misinformation about sexual matters and false beliefs about this issue (6). The most common cause of misinformation is sexual myths (7). Although there are social differences, sexual myths that show significant similarities in every society are also widely accepted by societies (8). Sexual satisfaction, on the other hand, can be defined as the mood expressed by people during and after sex. Sexual satisfaction, such as relationship satisfaction and self-esteem, is also considered to be determinant for the continuity of marriage in married individuals.

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Our primary aim is to analyze the frequency of believing in sexual myths in married women and to investigate the effect of sexual myths on sexual satisfaction in women.

MATERIAL AND METHOD

This study was approved by the Demiroğlu Bilim University Clinical Researches Ethics Committee (Date: 22.02.2022, Decision No: 2022-04-03). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Patients Selection

Married women between the ages of 18-55 in the tertiary gynecology and obstetrics clinic were prospectively included in the study. After giving written or verbal information about the study, consent was obtained from the patients who agreed to participation.

Data Collection and Assessment of Patients

Demographic characteristics of women participating in the study such as age, marital status, marriage and employment status, monthly income, family structure and number of children were recorded. The female form of the Golombok-Rust Inventory of Sexual Satisfaction (GRISS) Scale, which consists of 28 questions and is generally used to evaluate the quality of sexual intercourse and sexual dysfunction, was used to measure the variable of sexual satisfaction in women (8). The sexual myth scale (CMI), consisting of 28 questions, was used to determine the participants' status of having sexual myths (9). In this scale, age and sexuality, sexual behavior, masturbation, sexual orientation, sexual violence, sexual intercourse, sexual satisfaction and gender were evaluated. Responses of the participants to both questionnaires were analyzed.

Statistical Analysis

Data were analyzed using the program SPSS 25.0 (IBM, Armonk, NY: IBM Corp.). Mean±standard deviation for parametric tests in presenting continuous variables; For non-parametric tests, the median (interquartile range, IQR) and categorical variables were expressed as numbers and percentages. In comparison of independent group differences, one-way analysis of variance provided parametric test assumptions; Kruskal Wallis analysis of variance was used when parametric test assumptions were not met. The $p<0.05$ was considered statistically significant in all analyzes.

RESULTS

Overall 171 married women between the ages of 18-55 were included in the study. The median age of the women was 35 years (IQR 29-43) and 60.8% (n=104) were university graduates and 20.5% (n=35) were graduate/doctoral graduates (**Table 1**). The family structures, duration of marriage, number of children, employment status and monthly income of the women are shown in **Table 1**.

The results of the GRISS, which evaluates the sexual life and sexual relationship qualities of married women participating in the study, and the sexual myths evaluation form that evaluates sexual myths are given in **Tables 1** and **2.**

Table 1. Family structures, educational status and monthly salary of the women participating in the study.

	n	%
Education		
Primary school	2	1.2
Middle School	5	2.9
High school	25	14.6
University	104	60.8
Master's and Doctorate	35	20.5
Working		
Yes	116	67.8
No	55	32.2
Salary / monyhly (TL)		
1-3000	29	17.0
3000 - 5000	52	30.4
5000 – 10000	60	35.1
>10000	30	17.5
Children		
0	43	25.1
1	68	39.8
2	42	24.6
≥3	18	10.5
Family status		
Core	158	92.4
Large	13	7.6
The duration of marriage (year)		
0-1	21	12.3
1 - 5	44	25.7
5 - 10	39	22.8
10 - 20	40	23.4
> 20	27	15.8

"Do you have sexual intercourse more than twice a week?" It was observed that the answers given to the question "always, often and sometimes" were more frequent as the age of the women got younger ($p=0.005$). However, "do you ever go without sex for a week?" As for the question of "always, often and sometimes" as the age of the women increased, it was determined that the answers were more ($p<0.001$). Also, "do you avoid having sex with your partner?" In the question, it was observed that as the age of the women got younger, the answers of "never and rarely" were significantly higher ($p=0.005$). The answers to the other questions did not differ significantly according to the age of the women.

Table 2. Golombok - Rust Sexual Satisfaction Scale and women's responses

	Never [n, (%)]	Rarely [n, (%)]	Sometimes [n, (%)]	Often [n, (%)]	Always [n, (%)]
Are you indifferent to sexual life?	37 (21.6)	50 (29.2)	66 (38.6)	16 (9.4)	2 (1.2)
Do you ask your partner what he likes and dislikes about your sexual relationship?	20 (11.7)	26 (15.2)	46 (26.9)	53 (31.0)	26 (15.2)
Do you ever have no sexual intercourse for a week? (except menstrual days, illness)	25 (14.6)	40 (23.4)	60 (35.1)	36 (21.1)	10 (5.8)
Are you easily aroused sexually?	5 (2.9)	22 (12.9)	39 (22.8)	92 (53.8)	13 (7.6)
Do you think the time you and your partner spend for foreplay (such as kissing, caressing) is enough?	11 (6.4)	22 (12.9)	32 (18.7)	76 (44.4)	30 (17.5)
Do you think that your own genitals are too narrow for your partner's genitals to enter?	127 (74.3)	15 (8.8)	22 (12.9)	6 (3.5)	1 (0.6)
Do you avoid having sex with your partner?	74 (43.3)	43 (25.1)	40 (23.4)	10 (5.8)	4 (2.2)
Can you reach satisfaction (orgasm) during sexual intercourse?	12 (7.0)	18 (10.5)	31 (18.1)	77 (45.0)	33 (19.3)
Do you enjoy hugging and stroking your partner's body?	6 (3.5)	14 (8.2)	24 (14.0)	50 (29.2)	77 (45.0)
Do you find your sexual relationship with your partner satisfactory?	11 (6.4)	14 (8.2)	26 (15.2)	72 (42.1)	48 (28.1)
If necessary, can you insert your finger into your genitals without discomfort or pain?	75 (43.9)	19 (11.1)	19 (11.1)	29 (17.0)	29 (17.0)
Do you mind touching and caressing your partner's genitals?	99 (57.9)	26 (15.2)	30 (17.5)	9 (5.3)	7 (4.1)
Do you get annoyed when your partner wants to have sex with you?	88 (51.5)	50 (29.2)	25 (14.6)	5 (2.9)	3 (1.8)
Do you think it is not possible for you to reach satisfaction (orgasm)?	86 (50.3)	49 (28.7)	29 (17.0)	5 (2.9)	2 (1.2)
Do you have sexual intercourse more than 2 times a week?	21 (12.3)	23 (13.5)	70 (40.9)	45 (26.3)	12 (7.0)
Can you tell your partner what you like and dislike about your sexual relationship?	11 (6.4)	15 (8.8)	27 (15.8)	61 (35.7)	57 (33.3)
Can your partner's genitals enter your genitals without discomfort?	4 (2.3)	11 (6.4)	16 (9.4)	75 (43.9)	65 (38.0)
Do you feel that love and affection are lacking in your sexual relationship with your partner?	109 (63.75)	25 (14.6)	20 (11.7)	9 (5.3)	8 (4.7)
Do you enjoy having your partner touch and caress your genitals?	7 (4.1)	12 (7.0)	21 (12.3)	50 (29.2)	81 (47.4)
Have you ever refused to have sex with your partner?	35 (20.5)	75 (43.9)	46 (26.9)	11 (6.4)	4 (2.3)
Can you reach satisfaction (orgasm) when your partner stimulates your clitoris during foreplay?	11 (6.4)	18 (10.5)	59 (34.5)	64 (37.4)	19 (11.1)
Is the time allotted only for sexual intercourse during making love enough for you?	13 (7.6)	5 (2.9)	25 (14.6)	99 (57.9)	29 (17.0)
Do you feel disgusted by what you do during lovemaking?	114 (66.7)	33 (19.3)	22 (12.9)	1 (0.6)	1 (0.6)
Do you think that your own genitals are narrow enough to prevent the penetration of your partner's genitals?	129 (75.4)	14 (8.2)	18 (10.5)	6 (3.5)	4 (2.3)
Do you like when your partner loves and caresses you?	3 (1.8)	2 (1.2)	13 (7.6)	36 (21.1)	117 (68.4)
Do you have wetness on your genitals during lovemaking?	1 (0.6)	3 (1.8)	18 (10.5)	73 (42.7)	76 (44.4)
Do you enjoy the moment of sexual intercourse?	4 (2.3)	3 (1.8)	14 (8.2)	62 (36.3)	88 (51.5)
Do you reach satisfaction (orgasm) at the time of sexual intercourse?	9 (5.3)	20 (11.7)	32 (18.7)	74 (43.3)	36 (21.1)

When the sexual myths and the age of the women were compared, it was found that the answers to the view "homosexuality is a disease" increased significantly as the age of the women increased (p=0.045). In contrast, there was no significant difference between other sexual myths and the age of the women.

The education levels of the women, their sexual lives and sexual intercourse qualities were compared. While the majority of women's sexual lives and sexual intercourse qualities do not differ according to their education level; it was observed that only women with a lower education level than university felt the lack of love and affection more during their sexual relations (p=0.016).

When sexual myths and education levels of women are compared, 42.4% of those with a university or higher education level do not agree with the myth that "homosexuality should be treated"; 18.8% of those whose education level was below the university did not agree with this myth (p=0.016). In addition, only 6.5% of those with a university education level and above agree with the myth that "housework is the duty of women"; 25.0% of those with an education level below the university agreed with this myth (p=0.001) (**Table 4**).

The working status had an effect on women's sexual life and sexual relationship qualities. Only 8.6% of working women state that they are mostly indifferent to sexual life; this rate was 14.5% in unemployed women *(p=0.020)* (**Table 5**). However, the frequency of not reaching orgasm during sexual intercourse was found to be 18.9% in working women and 10.9% in non-working women (p=0.038) (**Table 5**). Also, "Have you ever refused to have sex with your partner?" 10.3% of working women answered the question "often and always"; this rate was 5.4% in unemployed women (p=0.010) (**Table 5**).

Table 3. Sexual myths evaluation form and women's responses.

	Never [n, (%)]	Disagree [n, (%)]	Indecisive [n, (%)]	Partially agree [n, (%)]	Absolutely agree [n, (%)]
Homosexuality is a disease.	38 (22.2)	26 (15.2)	40 (23.4)	37 (21.6)	30 (17.5)
Homosexuality should be treated.	33 (19.3)	32 (18.7)	47 (27.5)	29 (17.0)	30 (17.5)
Homosexuals are harmful to society.	59 (34.5)	43 (25.1)	37 (21.6)	13 (7.6)	19 (11.1)
Homosexual men act like women.	20 (11.7)	35 (20.5)	45 (26.3)	51 (29.8)	20 (11.7)
The sexual orientation of the individual can be understood from his external appearance (clothing, speech, behavior).	20 (11.7)	35 (20.5)	22 (12.9)	77 (45.0)	17 (9.9)
Men are more successful than women in tasks that require intelligence, such as math.	72 (42.1)	56 (32.7)	11 (6.4)	21 (12.3)	11 (6.4)
Housework is women's duty.	115 (67.3)	36 (21.1)	3 (1.8)	15 (8.8)	2 (1.2)
Men's decisions are more realistic/logical than women's.	83 (48.5)	56 (32.7)	5 (2.9)	23 (13.5)	4 (2.3)
Men are more competitive than women	65 (38.0)	46 (26.9)	31 (18.1)	22 (12.9)	7 (4.1)
Women need help	110 (64.3)	41 (24.0)	1 (0.6)	17 (9.9)	2 (1.2)
Being a man is more valuable than being a woman.	131 (76.6)	32 (18.7)	2 (1.2)	2 (1.2)	4 (2.3)
It is not right for older people to have sexual intercourse.	86 (50.3)	58 (33.9)	16 (9.4)	7 (4.1)	4 (2.3)
Sex life ends with aging	66 (38.6)	57 (33.3)	21 (12.3)	22 (12.9)	5 (2.9)
Entering menopause (cessation of menstruation) ends a woman's sexual life.	85 (49.7)	55 (32.2)	17 (9.9)	13 (7.6)	1 (0.6)
Being young is essential for a satisfying sex life.	66 (38.6)	63 (36.8)	19 (11.1)	19 (11.1)	4 (2.3)
Every stage of sexual intercourse should be under the control of the man.	91 (53.2)	60 (35.1)	8 (4.7)	11 (6.4)	1 (0.6)
A woman should act according to her husband's wishes during sexual intercourse.	95 (55.6)	52 (30.4)	7 (4.1)	16 (9.4)	1 (0.6)
It is a woman's duty to please her husband in sexual intercourse.	101 (59.1)	51 (29.8)	6 (3.5)	9 (5.3)	4 (2.3)
Masturbating leads to the development of physical diseases.	70 (40.9)	56 (32.7)	31 (18.1)	9 (5.3)	5 (2.9)
Masturbating causes psychological problems.	72 (42.1)	48 (28.1)	27 (15.8)	21 (12.3)	3 (1.8)
Sexual intercourse without the consent of one of the spouses is not considered "rape"	87 (50.9)	43 (25.1)	23 (13.5)	12 (7.0)	6 (3.5)
Boys do not become victims of rape.	129 (75.4)	34 (19.9)	2 (1.2)	3 (1.8)	3 (1.8)
Raped boys become homosexual when they grow up	66 (38.6)	50 (29.2)	42 (24.6)	12 (7.0)	1 (0.6)
Women cause sexual violence with their appearance / clothing	102 (59.6)	44 (25.7)	11 (6.4)	13 (7.6)	1 (0.6)
Sexuality means sexual intercourse (sexual intercourse).	47 (27.5)	68 (39.8)	18 (10.5)	30 (17.5)	8 (4.7)
Sexual intercourse is essential for spouses to experience sexual pleasure.	27 (15.8)	52 (30.4)	25 (14.6)	39 (22.8)	28 (16.4)
Sexual intercourse must necessarily result in orgasm (pleasure / satisfaction).	21 (12.3)	43 (25.1)	22 (12.9)	48 (28.1)	37 (21.6)
Women can reach orgasm only through "sexual intercourse"	47 (27.5)	68 (39.8)	22 (12.9)	25 (14.6)	9 (5.3)

Table 4. The comparison of married women's education levels and attitudes towards sexual myths

	Education levels		*p
	<University	≥University	
Homosexuality should be treated			0.016
Agree	15	44	
Disagree	6	59	
Women need help			0.033
Agree	7	12	
Disagree	25	126	
Women should act according to the wishes of the man in sexual intercourse			0.044
Agree	6	11	
Disagree	23	124	
Housework is women's duty			0.001
Agree	8	9	
Disagree	23	128	
It is a woman's duty to please her husband in sexual intercourse			0.049
Agree	4	9	
Disagree	25	127	
Masturbating causes physical illness			0.040
Agree	5	9	
Disagree	18	108	
Women get orgasm through intercourse			0.021
Agree	11	23	
Disagree	17	98	

*p value <0.05 is considered significant.

Table 5. The effect of working status on women's sexual life and sexual relationship qualities.

	Working status		*p
	+	-	
Indifferent sexual life, [n, (%)]	10/116 (8.6)	8/55 (14.5)	0.020
Not reaching orgasm during sexual intercourse, [n, (%)]	22/116 (18.9)	6/55 (10.9)	0.038
"often and always" refused to have sex with your partner? [n, (%)]	12/116 (10.3)	3/55 (5.4)	0.005

*p value <0.05 is considered significant.

The monthly income of the women and their sexual life and sexual relationship qualities were compared. To the question posed to the women, "Is the time allotted only for sexual intercourse during lovemaking is sufficient for you", none of those with a monthly income of 10000 TL or more received the answer "never and rarely"; 16.3% of women whose income was less than 10000 TL gave the answer "never and rarely" (p=0.011). No difference was found between other sexual life and sexual intercourse statuses and monthly income differences of women. In addition, it was observed that there was no relationship between women's monthly income levels and their attitudes towards sexual myths.

When the sexual life and sexual relationship characteristics and whether the women have children or not, the rate of indifference towards sexual life of women without children is 25.6%; 58.6% of women with at least one child were found to be indifferent to sexual life (p=0.048) (**Table 6**). However, "do you ever go without sex for a week?" 27.9% of women without children and 10.2% of women with at least one child answered "never" (p=0.032) (**Table 6**). In addition, the frequency of having sexual intercourse more than twice a week is 62.8%, often or always; this rate was 23.4% in women with at least one child (p<0.001) (**Table 6**). It was observed that there was no relationship between women's having children and their attitudes towards sexual myths.

Table 6. The effect of having children on women's sexual life and sexual relationship qualities.

	Women with children		p
	-	+	
Indifference of towards sexual life of women, [n, (%)]	11/43 (25.6)	75/128 (58.6)	0.048
"always/often" having sexual intercourse more than twice a week, [n, (%)]	27/43 (62.8)	30/128 (23.4)	<0.001
"never" go without sex for a week? [n, (%)]	12/43 (27.9)	13/128 (10.2)	0.032

*p value <0.05 is considered significant.

The effect of family structure on sexual life and sexual myths was examined. All of the women with extended family structure stated that only the time allotted for sexual intercourse during making love is sufficient; 27.1% of women with nuclear family structure stated that this period was not enough (p=0.037). The family structure did not have an effect on other sexual life and sexual relationship qualities. While 34.2% of women with a nuclear family structure agree with the myth that "homosexual men act like women"; 7.6% of women with extended family structure agreed with this myth (p=0.039).

DISCUSSION

In this study, the attitudes of married women on sexual life and relationship qualities and sexual myths, for which there is a limited data in the literature, were examined. Sexuality, which is one of the important parts of a happy and permanent marriage, is seen as a taboo in our country as it is in many societies. Therefore, ignorance, misinformation and sexual myths about sexuality negatively affect the marriage process. Due to both religious and cultural differences in developing countries, very few studies are published on women's sexual life, sexual problems and sexual satisfaction (10). In this study, sexual life qualities and perspectives on sexual myths, which are considered taboo in our country, were investigated in married women.

In the few studies that analyzed the rate of believing in sexual myths among women in our country, it was reported that this rate was between 18-44% (11, 12). In our study, more than half of the respondents gave the answer "I disagree" in 22 of 28 sexual myths (78.6%). With this result, the rate of believing in sexual myths was found to be 21.4%, similar to the literature. The fact that the women included in our study are married and that they talk more freely about sexuality after marriage and obtain more accurate information can explain this rate.

Sexual dysfunction in women occurs when they have problems such as low arousal, low desire, dyspareunia and orgasm difficulties. The resulting sexual dysfunction includes physiological, medical, anatomical, social and psychological causes (13, 14, 15). Approximately 30-50% of women suffer sexual dysfunctions which are progressive, very common and increase with age (16). The frequency of having sexual intercourse decreases as the age of women increases. Estrogen and testosterone hormones, which decrease with age, are shown as the most important reason for this situation (17, 18). In our study, women had sexual intercourse more than twice a week as their age decreased; As age increases, it has been shown that he does not have sexual intercourse for more than a week. In addition, it has been reported that with increasing age in married women, women avoid sexual activity and have hypoactive sexual lives (19, 20). In this study, similar to the literature, it was seen that women never or rarely avoid having sex with their spouses as their age gets younger. There are studies reporting that the state of believing in sexual myths increases as women's age increases, and there are studies showing that as women's age increases, their life experiences increase and their belief in opposite sexual myths decreases due to the fact that they realize false information about sexuality (21). In addition, Ekmen BU et al. (22) stated that the age of the women did not have any effect on their beliefs in sexual myth. In our study, as the age of women increased, the belief in the myth of homosexuality as a disease increased; there was no relationship between other myths and the age of the women. It can be thought that the different results in the literature and in our study are due to the regional cultural differences of the women participating in the studies.

Due to the increase in the education level of women, access to healthy and accurate information about sexuality increases. As a result, it is thought that women's sexual satisfaction is also positively affected. However, it has been stated in the literature that the education level of women also affects their beliefs about sexual myths (22). It has been reported that the rate of believing in myths is higher among women with lower education levels than

those with higher education levels (22). On the other hand, Hofstadt et al. (23) reported that there was no relationship between education level and belief in sexual myths; even those with a high level of education have been shown to have sexual myths. In our study, similar to the literature, it was determined that both those with a high level of education (university and higher education) and those with a low level of education had myths. This result shows that women have sexual myths regardless of their education level, due to the characteristics of the society, culture and taboos in our country.

In our study, as a contribution to the literature, it has been shown that working in any job does not affect the belief in sexual myths and that unemployed women are more indifferent to sexual life than working women.

It has been reported that the level of belief in sexual myths is higher than women with a nuclear family structure, since women with an extended family structure are not free in their marriage life and cannot experience sexual intercourse more comfortably (22, 24). In our study, the family structure is effective on sexual life and sexual relationship qualities and sexual myths. Moreover, the fact that women had children affected both their sexual life and sexual relationship qualities and their belief in sexual myths.

Limitations of the study; (1) the fact that our study was carried out in a single-center and high-level center constitutes an important limitation in the generalization of our results; (2) insufficient number of women participating in the study; (3) that it only includes married women.

CONCLUSION

Sociocultural characteristics and taboos affect women's sexual intercourse qualities and their belief in sexual myths. With this study, it has been shown that the age, family structure, education level and employment status of married women affect their sexual lives and belief in sexual myths. In particular, increasing the education level of married women and making them more knowledgeable about sexuality can reduce the rate of believing in sexual myths.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was approved by the Demiroğlu Bilim University Clinical Researches Ethics Committee (Date: 22.02.2022, Decision No: 2022-04-03).

Informed Consent: Informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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Factors independently associated with prognosis in patients operated for pancreatic cancer: Assessing the role of various parameters including red cell distribution width, neutrophil-to-lymphocyte ratio, and platelet-to-lymphocyte ratio

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ABSTRACT

Objective: We aimed to assess whether, among other parameters, preoperative red cell distribution width (RDW), neutrophil-to-lymphocyte ratio (NLR), and platelet-to-lymphocyte ratio (PLR) values were associated with prognosis in patients operated for pancreatic cancer (PC).

Material and Method: This retrospective cohort was conducted from February 1, 2016 to February 1, 2021 at the general surgery department of a university hospital in Turkey. A total of 75 patients histologically diagnosed with PC who had undergone surgery were included in the study.

Results: The PLR values of patients with poorly differentiated and undifferentiated tumors were found to be higher than those with moderately and highly differentiated tumors. Also, there was a significant relationship between PLR values and the length of hospital stay. PLR values increased as the length of hospital stay increased. There was a statistically significant positive correlation between CA 19-9 levels and NLR and PLR. High total bilirubin level was related with increased risk of death, while adjuvant chemotherapy recipients had 4.049-fold lower risk of death than those without adjuvant chemotherapy.

Conclusion: Our results indicate that preoperative NLR, PLR and RDW cannot be used as prognostic indicators of mortality in patients with operated PC, but high PLR appears to be associated with lower level of tumor differentiation and prolonged hospital stay. We also found that high total bilirubin was a poor prognostic factor, while adjuvant chemotherapy was a good prognostic factor. Further multicenter, prospective studies with larger sample sizes will help to verify these results.

Keywords: Pancreatic cancer, NLR, PLR, RDW, CA 19-9, bilirubin

INTRODUCTION

Pancreatic cancer (PC) is one of the malignancies with the highest reported levels of short- and mid-term morbidity and mortality (1). Despite developing medical technologies, adjuvant therapies and surgical techniques, the 1-year and 5-year survival rates of the operated patients are reported to be 21% and 3%, respectively (1-3). Surgical resection and adjuvant chemotherapy remain as the primary management options in PC; however, overall survival (OS) and prognosis are still very poor due to rapid local recurrence and systemic spread of disease (4). Careful preoperative risk assessment of patients with PC can optimize patient selection for radical surgery and improve treatment outcomes and patient outcomes. Therefore, there is a fundamental need for the identification of preoperatively-measurable prognostic markers (5).

Various markers have been associated with poor prognosis in PC, such as carbohydrate antigen 19-9 (CA 19-9) and bilirubin (6,7). In addition, it is well established that inflammatory activation plays a key role in carcinogenesis and the tumor microenvironment (8). Despite the fact that information is limited regarding the mechanisms of these relationships (5), a number of inflammation markers and indices, including neutrophil-to-lymphocyte ratio (NLR),

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platelet-to-lymphocyte ratio (PLR) and lymphocyte-monocyte ratio (LMR), and red cell distribution width (RDW), have been investigated for their potential importance in PC prognosis and diagnosis (9,10). Many authors have identified pretreatment RDW (10), NLR (11) and PLR (12) values as being associated with OS in patients with PC. Meta-analyses have also supported these suggestions by showing that high PLR, NLR, and RDW were suggestive of poor prognosis in PC (1,4,10,13). Since PLR, NLR, and RDW are easily-accessible inflammatory indices, their possible association with postoperative outcomes can be valuable for the assessment of patients with PC and treatment decisions, such as identifying candidates for either surgery or neoadjuvant therapy (5).

We hypothesized that these markers could predict prognosis in patients with operated for PC. In this study, we aimed (i) to reveal whether preoperative levels of RDW, PLR, NLR and other parameters were associated with stage, morbidity and survival of PC, and (ii) to identify significant prognostic factors associated with mortality in patients with operated PC.

MATERIAL AND METHOD

Study Design

This was a retrospective single center study conducted between February 1, 2016 to February 1, 2021 at the General Surgery & Surgical Oncology Department of Osmangazi University Faculty of Medicine, Eskişehir, Turkey. The protocol of this study was approved by the Medical Ethics Committee of Osmangazi University Faculty of Medicine (Date: 15.06.2021, Decision No: 04), and all steps and procedures associated with the research were carried out in accordance with the ethical standards stated in the Declaration of Helsinki and its amendments. As the study has a retrospective nature, Osmangazi University Medical Ethics Committee did not require acquisition of written informed consent from patients. All data were recorded anonymously.

Study Population and Follow-up

A total of 75 patients histologically diagnosed with PC who had undergone surgery were included in the study. Exclusion criteria were presence of synchronous or metachronous cancer, undergoing emergency tumor surgery, having cirrhosis, autoimmune disease or hematological malignancy, and having used corticosteroids within the last 6 months. In addition, recipients of neoadjuvant treatment and subjects with stage 4 disease or incomplete data were also excluded.

The following information of each patient was acquired from hospital records: demographic characteristics, laboratory measurements, pathological outcomes (detailed later), preoperative application of endoscopic retrograde cholangiopancreatography (ERCP) and stenting, time between diagnosis and surgery (days), resectable lesion (according to preoperative computed tomography), whether adjuvant chemotherapy or adjuvant radiotherapy was applied, length of stay in hospital. Recorded surgical characteristics were: operation type, whether the spleen was preserved, pancreaticojejunostomy type, gastrojejunostomy type, whether the pylorus was preserved, and portal vein resection. Finally, all recurrences and outcomes, such as leakage, fistula, postoperative infection, recurrence and mortality data (including survival time) were recorded.

Laboratory Analysis

Blood samples were acquired from the antecubital vein for complete blood count (CBC) and other parameters including albumin, total bilirubin, direct bilirubin, and CA 19-9. Hemoglobin level, RDW value, and counts for neutrophils, lymphocytes and platelets were obtained from CBC. NLR was defined as the total number of neutrophils divided by the total number of lymphocytes. PLR was defined as the total number of platelets divided by the total number of lymphocytes. Laboratory analyses were carried out in all patients within 2 weeks prior to respective surgeries via routine devices in the Clinical Biochemistry Department of Osmangazi University Faculty of Medicine.

Pathological Analysis

All of the specimens obtained from fully resected tumors were sent to the pathology unit of the Pathology Department of Osmangazi University Faculty of Medicine for pathological examinations. Pathological diagnosis, differentiation, tumor primary and localization, tumor size (mm), number of lymph nodes, number of metastatic lymph nodes, extracapsular invasion, perineural invasion, lymphovascular invasion, resection margin, surgical margin, clinical stage (reported according to the pathological classification criteria of the 7th Edition of the American Joint Committee on Cancer guidelines for PC) were determined and reported by qualified pathologists.

Statistical Analysis

The statistical analyses of this study were performed with SPSS ver. 25.0 (SPSS Inc., Chicago, IL, USA). Histogram and Q-Q plots were used to determine whether variables are normally distributed. Data are given as mean±standard deviation or median (1st quartile-3rd quartile; interquartile range; IQR) for continuous variables according to normality of distribution, and as frequency (percentage) for categorical variables. Between-group analyses were performed with the Mann-Whitney U test. Spearman correlation coefficients were calculated to evaluate relationships between continuous variables. Survival times were calculated with the Kaplan Meier method. Between-group comparisons of survival times were performed with the Log rank test. Cox regression analysis (forward conditional method) was performed to determine

significant prognostic factors that were independently associated with mortality. Two-tailed p values of less than 0.05 were considered statistically significant.

RESULTS

Forty-six male and 29 female patients were included in our study, and the median age of the patients was 65 (IQR: 59-70) (range: 40-80) years. Summary of patients and tumor characteristics, laboratory measurements, pathological features, surgical features and all other outcomes are depicted in **Table 1** and **Table 2**.

Table 1. Summary of patients and tumor characteristics

Age	65 (59-70)
Sex	
Female	29 (38.7%)
Male	46 (61.3%)
Preoperative ERCP + Stent	19 (25.3%)
Time between diagnosis and surgery, days	22 (14-36)
Operation	
Whipple procedure	66 (88.0%)
Distal pancreatectomy	5 (6.7%)
Total pancreatectomy	4 (5.3%)
Diagnosis	
Adenocarcinoma	72 (96.0%)
Neuroendocrine tumor	0 (0.0%)
Other	3 (4.0%)
Spleen	
Not-preserving	52 (69.3%)
Preserving	23 (30.7%)
Differentiation	
Undifferentiated	2 (2.7%)
Poorly differentiated	23 (30.7%)
Moderately differentiated	42 (56.0%)
Highly differentiated	8 (10.7%)
Stage	
Stage 1A	4 (5.3%)
Stage 1B	3 (4.0%)
Stage 2A	4 (5.3%)
Stage 2B	38 (50.7%)
Stage 3	26 (34.7%)
Primary tumor	
Pancreas	75 (100.0%)
Ampulla	0 (0.0%)
Distal choledochal	0 (0.0%)
Location	
Head	69 (92.0%)
Neck	0 (0.0%)
Body	2 (2.7%)
Tail	4 (5.3%)
Uncinate process	0 (0.0%)
Tumor size, mm	31.83±13.27
Number of lymph nodes	23.79±12.91
Number of metastatic lymph nodes	2 (1-4)
Extracapsular invasion	14 (18.7%)
Resectability (preoperative CT)	
Resectable	48 (64.0%)
Borderline	27 (36.0%)
Unresectable	0 (0.0%)
Perineural invasion	62 (82.7%)
Lymphovascular invasion	52 (69.3%)

Data are given as mean±standard deviation or median (1st quartile-3rd quartile) for continuous variables according to normality of distribution and as frequency (percentage) for categorical variables

Table 2. Summary of laboratory and surgical outcomes

Resection margin	
R0	59 (78.7%)
R1	16 (21.3%)
R2	0 (0.0%)
Surgical margin	
Negative	59 (78.7%)
Pancreatic parenchyma	3 (4.0%)
Choledochal	1 (1.3%)
Retropancreatic	12 (16.0%)
Choledochal and pancreatic parenchyma	0 (0.0%)
Pancreaticojejunostomy type	
Ducto-jejunostomy	60 (80.0%)
Others	6 (8.0%)
None	9 (12.0%)
Gastrojejunostomy type	
Simple	58 (77.3%)
Roux-en-Y	11 (14.7%)
None	6 (8.0%)
Pylorus	
Preserving	0 (0.0%)
Classic	69 (92.0%)
None	6 (8.0%)
Portal vein resection	12 (16.0%)
Adjuvant chemotherapy	59 (78.7%)
Adjuvant radiotherapy	11 (14.7%)
Length of stay in hospital, days	11 (7-15)
Leakage	
Biochemical	4 (5.3%)
Macroscopic	10 (13.3%)
Fistula	
Grade A	3 (4.0%)
Grade B	3 (4.0%)
Grade C	7 (9.3%)
Postoperative infection	21 (28.0%)
Recurrence	24 (33.8%)
Albumin	4.04±0.52
Hemoglobin	12.78±1.58
Neutrophil ($\times 10^3$)	4.8 (3.8-6.4)
Lymphocyte ($\times 10^3$)	1.7 (1.4-2.11)
Platelet ($\times 10^3$)	276.56±79.43
RDW	14.9 (13.7-16.8)
Total bilirubin	3.69 (1.15-8.18)
Direct bilirubin	2.87 (0.43-6.91)
CA 19-9	122.5 (39.5-476.0)
Neutrophil / lymphocyte ratio	2.92 (1.92-3.79)
Platelet / lymphocyte ratio	157.14 (116.74-203.36)
Status	
Exitus	38 (50.7%)
Alive	37 (49.3%)

Data are given as mean±standard deviation or median (1st quartile-3rd quartile) for continuous variables according to normality of distribution and as frequency (percentage) for categorical variables

We examined the association of tumor characteristics and tumor related continuous variables with RDW, NLR, and PLR. It was observed that the PLR value decreased significantly as the degree of differentiation of

the tumor (un-differentiated and poorly differentiated versus moderately and highly differentiated) increased (p=0.030). There was a significant positive correlation between the length of stay in hospital and PLR value (r=0.262, p=0.023). We also found a significant positive correlation between CA 19-9 values and NLR (r=0.277, p=0.016) and PLR (r=0.278, p=0.016). The RDW, PLR and NLR values did not demonstrated any significant relationships with other tumor characteristics or tumor-related continuous variables, including differentiation, clinical stage, extracapsular invasion, perineural invasion, lymphovascular invasion, resection margin, tumor size, number of lymph nodes, number of metastatic lymph nodes, leakage, fistula, postoperative infection, recurrence, death status, resectability, length of stay in hospital and CA 19-9 (**Table 3** and **Table 4**).

Table 4. Relationships between RDW, PLR, NLR and tumor related continuous variables

		RDW	NLR	PLR
Tumor size	r	-0.143	0.155	0.060
	p	0.221	0.185	0.612
Number of lymph nodes	r	0.117	-0.071	-0.039
	p	0.316	0.547	0.740
Number of metastatic lymph nodes	r	0.005	-0.022	0.002
	p	0.968	0.854	0.986
Length of stay in hospital	r	0.068	0.152	0.262
	p	0.561	0.192	0.023
CA 19-9	r	0.093	0.277	0.278
	p	0.427	0.016	0.016

r: Spearman correlation coefficient

Table 3. Summary of RDW, NLR and PLR with regard to tumor characteristics

	RDW	p	NLR	p	PLR	p
Differentiation		0.800		0.067		0.030
Un-differentiated & poorly differentiated	14.5 (13.5-17.2)		3.14 (2.43-5.63)		174.00 (138.57-220.00)	
Moderately & highly differentiated	15.0 (14.1-16.1)		2.75 (1.74-3.70)		147.45 (111.76-181.82)	
Stage		0.978		0.956		0.533
Stage 1 & 2	14.8 (13.7-16.5)		2.92 (1.93-3.79)		156.80 (115.64-188.89)	
Stage 3	15.15 (13.8-17.0)		2.89 (1.92-3.78)		164.67 (126.95-203.77)	
Extracapsular invasion		0.678		0.523		0.138
No	14.9 (13.7-16.7)		2.92 (1.92-3.78)		151.58 (114.55-195.07)	
Yes	14.9 (14.5-17.0)		2.90 (2.43-4.86)		169.17 (137.70-253.57)	
Resectability (preoperative CT)		0.187		0.877		0.453
Resectable	15.2 (13.7-17.6)		2.99 (1.83-3.80)		164.35 (126.34-203.65)	
Borderline	14.5 (13.7-15.9)		2.82 (1.93-3.54)		141.73 (111.76-195.65)	
Perineural invasion		0.700		1.000		0.944
No	15.2 (13.5-15.9)		2.36 (1.95-3.60)		164.36 (114.55-188.89)	
Yes	14.8 (13.8-17.0)		2.94 (1.75-3.79)		154.19 (117.14-203.36)	
Lymphovascular invasion		0.304		0.654		0.679
No	15.7 (13.5-18.4)		3.03 (1.61-4.06)		158.50 (105.88-195.07)	
Yes	14.7 (13.9-16.15)		2.92 (1.95-3.74)		156.97 (122.87-203.57)	
Resection margin		0.591		0.393		0.130
R0	14.7 (13.7-16.8)		2.92 (1.83-3.79)		151.58 (114.55-188.89)	
R1	15.25 (13.8-16.7)		3.09 (2.24-4.66)		176.65 (128.75-230.18)	
Leakage		0.812		0.765		0.978
No	14.9 (13.8-16.7)		3.03 (1.83-3.81)		156.80 (115.64-203.36)	
Yes	14.95 (13.6-17.2)		2.87 (2.22-3.43)		161.42 (125.73-168.10)	
Fistula		0.732		0.425		0.644
No	14.85 (13.8-16.7)		3.04 (1.83-3.96)		156.97 (115.64-203.77)	
Yes	15.2 (13.6-17.2)		2.82 (2.22-3.03)		158.50 (125.73-166.00)	
Postoperative infection		0.781		0.962		0.243
No	14.85 (13.7-16.8)		2.99 (1.83-3.81)		146.32 (110.96-195.65)	
Yes	15.2 (14.0-16.4)		2.92 (2.22-3.60)		164.35 (144.00-203.95)	
Recurrence		0.711		0.488		0.402
No	14.9 (13.5-17.0)		2.82 (1.92-3.78)		161.82 (116.74-206.92)	
Yes	14.55 (13.8-16.3)		3.17 (1.84-4.25)		151.50 (121.29-179.60)	
Status		0.311		0.147		0.275
Exitus	15.2 (14.2-16.8)		3.10 (2.15-4.29)		165.29 (126.95-210.00)	
Alive	14.6 (13.4-16.5)		2.49 (1.83-3.50)		144.00 (116.74-181.82)	

Data are given as median (1st quartile-3rd quartile) according to normality of distribution

In addition, we evaluated patient-related, tumor-related and surgical characteristics, presence/absence of chemotherapy or radiotherapy after surgery, and complications during follow-up with respect to their possible association with survival time, 2-year survival rate and mortality rates using the Kaplan Meier method with Log rank test (**Table 5**). There was a significant positive correlation between receiving adjuvant chemotherapy and survival time and survival percentage ($p < 0.001$). No significant correlation was found between survival and any of the other variables (**Table 5**, **Figure 1**, **Figure 2**).

Table 5. Survival times (months) with Kaplan Meier method and comparisons of groups with Log rank test

	n	Exitus	Mean (95% CI)	Median (95% CI)	2-year survival rate (%)	p
Overall survival	75	38	25.17 (18.51-31.84)	15 (10.36-19.64)	29.6±7.2	N/A
Sex						0.095
Female	29	12	34.05 (22.18-45.93)	23 (7.8-38.2)	42.2±12.2	
Male	46	26	17.89 (12.97-22.81)	15 (10.89-19.11)	21.8±8.6	
Operation						0.216
Whipple procedure	66	35	20.14 (15.44-24.83)	15 (11.96-18.04)	26.1±7.5	
Distal & Total pancreatectomy	9	3	40.75 (20.26-61.24)	*	75.0±15.3	
Spleen						0.893
Not-preserving	52	20	30.45 (20.85-40.05)	14 (8.1-19.9)	38.3±9.1	
Preserving	23	18	21.65 (15.04-28.27)	19 (11.17-26.83)	30.4±9.6	
Differentiation						0.102
Un-differentiated & poorly differentiated	25	13	14.56 (9.01-20.12)	10 (5.55-14.45)	11.5±9.9	
Moderately & highly differentiated	50	25	27.96 (19.77-36.15)	19 (10.61-27.4)	36.1±9.0	
Stage						0.736
Stage 1 & 2	49	25	25.64 (17.09-34.18)	15 (8.9-21.1)	31.1±8.8	
Stage 3	26	13	20.04 (14.48-25.60)	16 (7.51-24.49)	27.6±12.3	
Extracapsular invasion						0.209
No	61	29	27.06 (19.27-34.84)	19 (12.87-25.13)	34.8±8.5	
Yes	14	9	13.90 (9.16-18.64)	13 (8.58-17.43)	10.4±9.9	
Resectability (preoperative CT)						0.791
Resectable	48	26	25.13 (17.27-32.99)	16 (9.77-22.23)	26.7±8.5	
Borderline	27	12	19.90 (12.83-26.96)	14 (8.22-19.78)	38.2±12.4	
Perineural invasion						0.091
No	13	5	39.07 (22.56-55.58)	*	67.3±13.6	
Yes	62	33	18.88 (14.24-23.51)	14 (10.12-17.88)	19.7±7.2	
Lymphovascular invasion						0.684
No	23	11	29.86 (16.60-43.13)	19 (1.07-36.93)	46.3±12.7	
Yes	52	27	20.12 (15.03-25.20)	15 (12.37-17.63)	22.1±8.0	
Resection margin						0.162
R0	59	30	27.63 (20.01-35.26)	16 (10.44-21.56)	36.2±8.0	
R1	16	8	13.96 (8.65-19.28)	10 (4.61-15.39)	0.0±0.0	
Portal vein resection						0.142
No	63	35	22.88 (16.15-29.60)	14 (11.53-16.47)	22.7±7.3	
Yes	12	3	31.00 (21.72-40.28)	*	83.3±10.8	
Adjuvant chemotherapy						<0.001
No	16	12	7.85 (4.91-10.80)	9 (1.11-16.89)	11.5±10.0	
Yes	59	26	29.07 (21.35-36.79)	20 (12.03-27.97)	35.7±8.6	
Adjuvant radiotherapy						0.133
No	64	33	21.37 (14.52-28.22)	15 (12.07-17.93)	19.8±7.9	
Yes	11	5	29.65 (17.92-41.38)	*	58.3±16.1	
Leakage						0.536
No	61	29	25.30 (17.83-32.77)	16 (10.57-21.43)	30.7±8.4	
Yes	14	9	19.68 (9.36-29.99)	14 (10.9-17.1)	25.4±13.9	
Fistula						0.640
No	62	30	24.98 (17.67-32.28)	16 (11.22-20.78)	29.9±8.2	
Yes	13	8	20.24 (9.10-31.37)	15 (4.38-25.62)	27.7±15.0	
Postoperative infection						0.739
No	54	26	21.15 (15.92-26.39)	16 (9.9-22.1)	28.4±8.7	
Yes	21	12	26.45 (13.56-39.33)	14 (11.57-16.44)	30.8±12.6	
Recurrence						0.251
No	47	17	32.90 (22.44-43.35)	19 (1.06-36.94)	47.1±10.3	
Yes	24	17	17.27 (13.45-21.08)	15 (12.8-17.2)	15.5±9.5	

SE: Standard error, CI: Confidence interval, * Can not be calculated due to low number of exitus cases

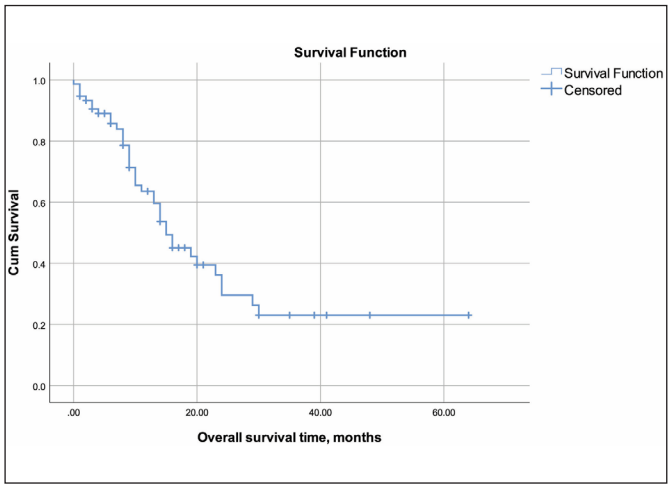

Figure 1. Overall survival plot

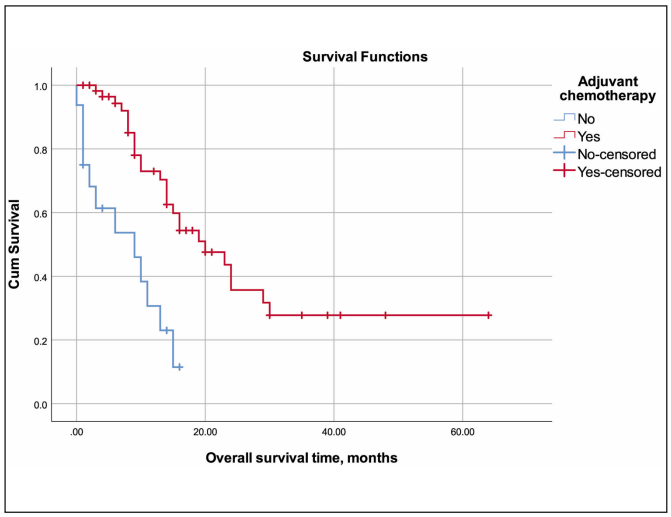

Figure 2. Overall survival plot with regard to adjuvant chemotherapy

We performed Cox regression analysis to determine significant prognostic factors associated with mortality. High total bilirubin level was a poor prognostic factor and adjuvant chemotherapy was a good prognostic factor. High total bilirubin level was associated with increased risk of death (p=0.045). Patients who received adjuvant chemotherapy had 4.049-fold lower risk of death than those without adjuvant chemotherapy (HR: 0.247, 95% CI: 0.118-0.519, p < 0.001). Other variables included in the model, age (p=0.621), sex (p=0.299), time between diagnosis and surgery (p=0.130), tumor size (p=0.084), number of metastatic lymph nodes (p=0.915), albumin (p=0.557), hemoglobin (p=0.093), RDW (p=0.143), CA 19-9 (p=0.253), NLR (p=0.971) and PLR (p=0.601) were found to be non-significant (**Table 6**).

DISCUSSION

Currently, there is no suitable preoperative blood test or analysis method enabling the prediction of morbidity and mortality in patients with PC (14). In this study, we aimed to evaluate many factors, with particular focus on PLR, NLR and RDW, to ascertain whether they were associated with the various characteristics of patients with PC and its prognosis. According to our results, PLR values of patients with poorly-differentiated and undifferentiated tumors were significantly higher than those with moderately and highly differentiated tumors. In addition, we observed that the PLR values increased significantly as the length of hospital stay was prolonged. There was a significant positive correlation between CA 19-9 levels and NLR and PLR. Regarding the effects of factors on survival, we observed that high bilirubin (poor prognosis) and receiving adjuvant chemotherapy (good prognosis) were the only factors independently associated with mortality.

Many researchers have investigated the role of inflammation and inflammatory markers/indices in predicting the postoperative prognosis of various tumors, including PC (1,5,8). The ability of platelets to protect cancer cells from the immune response and facilitate their attachment to the endothelium and the antitumor role of lymphocytes support the hypothesis that PLR may be valuable in predicting prognosis in cancer patients (10,15). The mechanism underlying the prognostic value of PLR in PC remains unclear. Platelets can also promote tumor growth, angiogenesis, metastasis, and cancer-associated thrombosis (2). Some researchers focusing on PC have found results showing that patients with high PLR may have shorter OS compared to patients with low PLR (16,17). In contemporary studies, results have shown that PLR value is significantly associated with OS in patients with PC, similar to the research outcomes concerning other cancers (2,4). In a multivariable analysis, greater PLR was found to be an independent risk factor for OS, along with important prognostic factors such as larger tumor diameter, positive microscopic surgical margin, and moderate or poor differentiation (16). A meta-analysis including 14 studies with 2743 patients showed that low preoperative PLR values were associated with better OS in patients with PC. Furthermore, in the aforementioned review, four studies with a total of 1062 patients reported results pertaining to disease-free survival (DFS). They demonstrated that low PLR appeared to be prognostic for better DFS, whereas high preoperative PLR had no

Table 6. Significant prognostic factors of the mortality, Cox regression analysis

	β coefficient	Standard Error	p	Exp(β)	95.0% CI for Exp(β)	
Adjuvant chemotherapy	-1.399	0.379	<0.001	0.247	0.118	0.519
Total bilirubin	0.053	0.027	0.045	1.055	1.001	1.111

CI: Confidence Interval

impact on OS (5). In another study, an optimal threshold value of 150 for PLR was associated with survival time in patients with resectable PC, while in another study, the same threshold value did not show prognostic value in patients with unresectable tumors (16,18). Another meta-analysis (including three studies) aiming to investigate the prognostic value of PLR found that PLR was not associated with OS in PC (19). Similar to the results of this study Kishi et al. (20) examined 65 patients with PC and concluded that PLR was not associated with the prognosis of these patients. We found a significant association between the presence of undifferentiated or poorly-differentiated PC and the level of PLR. We also observed that there was a significant relationship between CA 19-9 and PLR. While there was a statistically significant relationship between PLR and hospital stay, we did not find a significant association between PLR and survival or other morbidities. Although low PLR levels have the potential to be used as a predictor of better OS in patients with PC, current evidence does not support a role for PLR as a reliable prognostic factor for OS.

Similar to PLR, NLR is an inexpensive, easily available and widely used marker and has been found to be an important prognostic marker for many malignant tumors (3). Several studies have demonstrated the potential prognostic impact of NLR values in patients with PC (21,22). For instance, low NLR has been shown to be significantly associated with improved OS (22). On the other hand, interestingly, high NLR was reported as a surrogate marker in patients with resectable pancreatic adenocarcinoma (23). In another study conducted in China, preoperative NLR values were found to be an independent prognostic factor for OS in patients with early-stage PC (22). Similarly, preoperative serum NLR value in patients with operable pancreatic head cancer was found to provide prognostic information related to OS (24). In a meta-analysis of 34 studies involving 7105 patients with PC, NLR was shown to constitute new prognostic markers for predicting the prognosis of patients with PC, and high NLR value correlated with poor OS. In the same study, it was emphasized that NLR is a better predictor of the prognosis of patients with PC than PLR (1). A recent study investigating the prognostic predictive power of many important factors also found NLR to be an independent prognostic factor in PC, together with factors whose prognostic importance has been proven in many studies, such as CA 19-9, adjuvant chemotherapy, lymph node metastasis, and number of distant organ metastases (25). Hasegawa et al. (26) investigated the link between pretreatment NLR and response to neoadjuvant chemotherapy in PC patients and concluded that pretreatment NLR levels were independently associated with pathological response to treatment. In a study evaluating NLR and PLR together, patients with normal values for both NLR and PLR were found to have significantly better OS compared to those with elevated PLR or NLR values. Additionally, the authors suggested that NLR was superior to PLR as a prognostic marker in patients with PC (27). Finally, high NLR at preoperative assessment has also been found to be associated with poor survival in patients with metastatic PC (21,28,29). As a result of our study, in contrast to all these studies, we did not find a significant relationship between NLR value and any other tumor-related, patient-related, prognostic and survival-related factor, except for CA 19-9.

The RDW reflects the heterogeneity of the volume and size of circulating red blood cells and is a parameter readily available from routine blood tests (10). Previous studies have demonstrated that RDW may have diagnostic and prognostic value for various tumor types (14,15). In a study on PC, it was concluded that high RDW levels in patients with pancreatic masses may indicate malignancy (13). Studies have also reported that RDW could be used as an independent prognostic factor in patients with PC undergoing radical surgery. It was concluded that patients with high RDW may be at a more advanced stage of the disease and that the timing of surgery and the duration of neoadjuvant therapy can be determined by looking at the preoperative values of RDW (10), while other research has revealed that high RDW was associated with disease progress and longer postoperative hospital stay (30). In the present study, we did not find a prognostic value for RDW in operated PC patients.

CA 19-9 is a type of modified Lewis(a) blood group antigen and is recommended by the National Comprehensive Cancer Network guidelines for the diagnosis and follow-up of PC (31). Several reports have noted the usefulness of CA 19-9 in the monitoring of patients with PC.(32,33) Several studies have shown independent relationships between high levels of CA 19-9 and poor OS in advanced PC (7,25). However, in approximately 20% of patients with PC, CA 19-9 level may be normal before surgery (31) –which must be taken into account when evaluating patients in this respect. In our study, a significant correlation was found between NLR and PLR values and the level of CA 19-9; however, the possible relationships between these seemingly unassociated parameters require further research.

This study reports neoadjuvant chemotherapy and bilirubin levels as the only parameters independently associated with mortality in patients who underwent surgery for PC. It is understood that PC development leads to early systemic spread, and the majority of patients relapse soon after therapeutic surgery. Since the poor prognosis of PC is primarily determined by systemic

rather than local failure, it becomes clear that adjuvant treatment strategies need to be developed (34). Available data from randomized trials (35) and meta-analyses (34) showed that although receiving adjuvant chemotherapy significantly prolongs survival in patients with PC, no clear information is available for 5-year survival rate (34). Nonetheless, a recent study stated that receiving adjuvant chemotherapy was an independent good prognostic factor for PC (25), consistent with our findings.

Bilirubin is an endogenous antioxidant that plays a role in many physiological and pathological processes and also exhibits anti-carcinogenic effects (6). Increased bilirubin accumulation in the liver may be associated with PC and this condition can damage liver cells, causing liver dysfunction and even liver failure. These pathological processes may negatively affect survival in patients with advanced PC, resulting in poor prognosis (6). Nakata et al. (36) showed that, in patients with pancreatic head cancer, presence of obstructive jaundice at the time of diagnosis could predict unfavorable survival. Obstructive jaundice is a very common picture in patients with PC (37). Current discussions regarding the need for preoperative biliary drainage in these patients is another issue that should be taken into account (37). Previously, total bilirubin level has been identified as an independent prognostic factor in pancreatic body/tail cancer but not in pancreatic head cancer (25). A 10-year study of 5460 men and 4843 women revealed that high serum bilirubin (in the normal range) was associated with lower likelihood of cancer-related death (7). Supporting most of the literature, we found that high bilirubin was associated with poor prognosis in our group of patients with PC.

Study Limitations

Our study has some limitations. First, as this was retrospective single-center study, these results must be confirmed through unbiased prospective studies. Second, the number of investigated inflammatory markers was low, and the great range of variations in these parameters from patient to patient must be considered before attempting to generalize our findings. Third, the small number of patients may have affected the reliability of statistical evaluations. Finally, prognostic assessment in this study was directly based on mortality, and therefore, possible conclusions regarding associations with morbidity or quality of life cannot be drawn from our data.

CONCLUSION

Preoperative levels of NLR, PLR and RDW do not appear to have value as prognostic indicators for mortality in patients with PC. Nonetheless, our results demonstrate that the PLR elevation in patients with PC could be indicative of the presence of poorly differentiated & undifferentiated tumors. We also observed that greater PLR values were associated with prolonged hospital stay, indicating that PLR could be valuable in estimating hospital stay. Although CA 19-9 levels showed significant correlations with NLR and PLR, the correlation coefficients were too low to suggest a direct relationship between this tumor marker and inflammation. Most importantly, high bilirubin level was found to be associated with increased likelihood of death, while having received adjuvant chemotherapy reduced the likelihood of death. Large-scale prospective studies with greater patient numbers are needed to adequately evaluate the possible role of inflammatory markers in predicting prognosis in PC.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of the Ethics Committee of Osmangazi University Faculty of Medicine (Date: 15.06.2021, Decision No: 04).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Post-COVID syndrome? COVID-19 survivors suffer from cognitive difficulties, somatic complaints and anxiety

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ABSTRACT

Aim: Although primarily known as a respiratory system pathology, COVID-19 may cause various systems and cause serious complications including neuropsychiatric problems. These complications may be formulized as post-COVID syndrome. The current study aims to investigate prolonged cognitive, somatic and psychiatric effects of COVID-19.

Material and Method: A total number of 120 COVID-19 survivors were compared with 120 health controls in means of three measures, which are Cognitive Failures Questionnaire (CFQ), Body Sensations Questionnaire (BSQ), Hospital Anxiety and Depression Scale (HADS) to assess cognitive difficulties, body perceptions and anxiety/depression.

Results: Our findings show that COVID-19 survivors have reported significantly more cognitive difficulties, increased body sensations and higher levels of anxiety. The groups did not differ in means of depression scores. Further, the measures were significantly correlated with each other.

Conclusion: This study reveal that COVID-19 survivors suffer from significant cognitive deficits in everyday activities, are significantly more sensitive to various body sensations and have increased anxiety levels. In discordance with the current literature, our findings showed that COVID-19 patients are not more depressed than healthy subjects. In summary, the current study showed that various neuropsychiatric complications may be an important part of prolonged effects of COVID-19.

Keywords: COVID-19, neuropsychiatry, post-COVID syndrome, cognitive deficits

INTRODUCTION

In December 2019 a new severe acute respiratory syndrome, named as SARS-CoV2, has been reported from Wuhan Region of China. Further studies on this new type of viral infection resulted to rename it as Coronavirus Disease 2019 (COVID-19) by World Health Organization (WHO) (1). Further, WHO defined this new infection as a pandemic in March 2020, as the infection spread worldwide in a very short period of time (2). Various studies reported that main route of transmission is virus containing droplets and close contact with an infected person (3).

Although COVID-19 is mostly known as viral infection effecting respiratory system, almost all body systems or organs can be affected by it. Most common symptoms are fever, non-productive cough, dyspnea, headaches, dizziness, fatigue, diarrhea and vomiting (4). Apart from the serious respiratory signs like respiratory distress or even respiratory failure, COVID-19 can cause various complications. Several studies report myocarditis, cardiac arrythmias or acute coroner syndrome related to COVID-19 (5). Further, neurological complications of COVID-19 such as vertigo, headache, altered consciousness, acute ischemic stroke or intracranial hemorrhages has been also reported (6).

Beside the abovementioned complications, some of the recent viral pandemics are also known for their negative neurocognitive consequences. Several studies reported neuropsychiatric complications associated with viral infections like Influenza A (H1N1), SARS or Middle East Respiratory Syndrome (7). For example survivors of SARS has been reported to suffer from both cognitive difficulties like concentration difficulties, memory impairments, insomnia and symptoms of anxiety and depression (8).

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Coronaviruses are widely known as neuro-invasive, neurotropic and neurovirulent type viruses (9). It is known that infection caused by these types of viruses may cause various psychiatric problems in acute or chronic phases (10). Additionally, it has been reported that existence of somatic symptoms like fever, cough, fatigue and gastrointestinal disturbances may exacerbate psychological complications (11)

In case of SARS-Cov-2 viruses, it has been postulated that viruses use ACE receptors a binding site to invade various cell types (12) . More specifically ACE2 receptors are mostly involved in this process. Studies show that apart from respiratory system, ACE2 receptors are widely distributed in central nervous system and SARS-Cov-2 type viruses can reach to the various nervous system cells like neurons, astrocytes and oligodendrocytes through these receptors (8). Another pathophysiologic mechanisms have been also proposed to understand SARS-Cov-2 viruses and their neuropsychiatric complications. One of these mechanisms is knowns as "cytokine storm" theory. According to this theory after binding to ACE-2 receptors, Coronaviruses trigger a cytokine storm characterized by significant increases in Interleukin-1, Interleukin-2 and Tumor Necrosis Factor (13). These increased cytokine levels cause significant cell damage and consequently results with increased vascular permeability and widespread inflammation. These changes may also be evident in Blood Brain Barrier (BBB), as cytokine storm can damage BBB and therefore cause some neuropsychiatric complications like memory impairments and working memory difficulties (14).

Apart from the abovementioned biologic factors, numerous psychological factors were also proposed to understand psychiatric complications of COVID-19. Traumatic experiences like natural catastrophes or diseases may affect the feelings of security or act as a remainder of death (15). In case of COVID-19, factors like uncertainties regarding the course of the pandemic, lack of cure for the disease, overload of information, restricted social and physical activities may be significant factors which may serve as precipitating or causative factors for neuropsychiatric complications (16). In this context, several studies report that both COVID-19 survivors and general population show increased levels of anxiety, depression, fear responses and sleep disturbances (17,18). Relatedly, studies show that almost half of the COVID-19 survivors report emotional disturbances after acute phase of the infection and there is an increased risk to be referred for psychiatric treatment (19). In this regard, several studies also show that both severe and mild COVID-19 infections are associated with neurocognitive deficits (20). For example Gennaro et al. (21) showed that neurocognitive deficits and depression in COVID-19 patients are related to prolonged effect of inflammatory hyperactivation.

In summary, several studies on COVID-19 show that both acute and post-acute period of this disease are associated with various complications and it has been proposed that a possible post-COVID Syndrome is already definable (22). Further, it has also been shown that neurocognitive deficits and psychiatric symptoms are one of the most common symptom clusters associated with post-COVID syndrome (23).

In this current study it was aimed to search the effect of COVID-19 infection in means of cognitive difficulties, perception of body sensations and depression and anxiety by comparing COVID-19 survivors with healthy subjects.

MATERIAL AND METHOD

This case-controlled study was carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. Ethics approval was applied and obtained from the Kafkas University Ethics Committee (Date: 11.03.2021, Decision No: 80576354-050-99/26).

An online data form including informed consent, sociodemographic info and three questionnaires was generated in Google Docs and participants were asked to fill this form online. A written informed consent was obtained from all participants, both patient group and healthy controls. This study was conducted from March 2021 to May 2021 with a total number of 240 participants, 120 COVID-19 survivors and 120 healthy controls. All participants were over the age of 18. Participants were reached and recruited from patient pool of an University Hospital in Türkiye. Exclusion criteria for patient group were any history of psychiatric or neurological disorders, being illiterate, acute phase of COVID-19 ,ongoing treatment for COVID-19 or receiving treatment in the intensive care unit due to COVID-19. Healthy controls were recruited among the college students and social media platforms. Exclusion criteria for control group were any chronic medical conditions including psychiatric and neurological disorders, being infected with COVID-19 or being vaccinated with any type of COVID-19 vaccines and being illiterate.

Measures

Cognitive Failures Questionnaire (CFQ): Developed by Broadbendt et al. (24), CFQ is a self-report measure to evaluate everyday cognitive functions. CFQ consists of 25 items to measure three dimensions of cognition: perception, memory and motor functions. It has a five-point Likert style and every item can be scored between 0 to 4. Total scores range between 0 – 100 and higher scores indicate greater cognitive difficulties in daily activities. A Turkish validation study of CFQ has been conducted by Ekici et al. (25) and it has been found to be a reliable and valid measure to use in Turkish.

Body sensations questionnaire: Body Sensations Questionnaire (BSQ) is a self-reported measure and consists of 17 items (26). Items of BSQ consist of various bodily sensations and subjects are asked to choose a score to assign how fearful each sensation is. Items can be scored between 1 and 5 points and the total score can be reported. Higher scores on BSQ indicates more fear from somatic sensations. Turkish validation study of BSQ was conducted by Kart et al. (27) and it was reported as a valid and reliable scale in Turkish population.

Hospital anxiety and depression scale (HADS): HADS was developed in 1983 and it is widely used in different clinical or non-clinical settings to evaluate depression and anxiety in somatic disorders (28). It is a self-report measure and consists of 14 items, 7 for anxiety and 7 for depression. It has a four-point Likert structure and each item can be scored between 0 – 3 points. Higher scores indicate higher levels of anxiety and depression. Turkish validation study of HADS was conducted in 1997 and it was reported as a valid and reliable measure (29).

Statistical Analysis

The G* Power software version 3.01 (Franz Faul, Kiel, Germany) was implemented for power analysis to estimate sample size. At a=0.05 significance level, to reveal a medium effect size (d=0.5) with 80% power, participants in each study arm (totally 244 participants) were found to be necessary. The distribution analysis of data was evaluated by Kolmogorov-Smirnov and Shapiro Wilks test. Independent samples t-test was used for normally distributed continuous variables. Mann-Whitney-U test was used as nonparametric test for continuous variables. Categorical variables were analyzed with the Chi-Squared Test. Pearson correlation analysis was used to determine the correlation between the continuous variables. Standard Multiple Regression technique was used to determine the effects of various parameters on cognitive effects of COVID-19. IBM SPSS Statistics 24 (SPSS IBM, Chicago) program was used to analyze. Statistically significance was accepted as p-values below 0.05.

RESULTS

The patient group and health controls did not differ significantly from each other in means of age, sex and years of education as shown in **Table 1**.

The distribution of time period after COVID-19 infection in patient group was as following; 8 (6.7%) patients between 15 days to 1 month, 37 (30.8%) patients between 1-3 month, 64 (53.3%) patients between 3-6 months and 11 (9.2%) patients more than 6 months.

14 (11.7%) of 120 COVID-19 patients were admitted to hospital and 106 (88.3%) of 120 patients were participants who were treated as outpatient.

Table 1. Sociodemographic variables of participants

Parental Measures	Patient Group	Control Group	p value
n	120	120	
Sex [n males/females]	38/82	44/76	0.414 (χ2:0.667)[1]
Age [M (SD)]	33.62 (±11.39)	31.38 (±9.24)	0.962[2]
Years of Education: [M (SD)]	15.45 (±2.67)	15.10 (±3.46)	0.393[2]

1: Chi-square test, 2: independent samples t-test

69 (57.5%) participants form patient group subjectively responded that they suffer from ongoing somatic effects of COVID-19. Similarly, 40 (33.3%) patients have reported that they have still negative psychological effects of COVID-19.

The mean scores of measures from both groups have been found as following; The CFQ scores were 40.29 (±15.48) in the patient group and 36.00 (±15.58) in the control group, BSQ scores were 33.06 (±13.17) in the patient group and 28.01 (±10.09) in the control group HADS-A scores were 9.00 (±4.31) in the patient group and 7.39 (±4.22) in the control group. HADS-D scores were 6.92 (±4.37) in the patient group and 6.45 (±4.41) in the control group. HADS-T scores were 15.93 (±7.76) in the patient group and 13.84 (±7.65) in the control group.

The patient group had significantly higher scores on CFQ (p:0,034), BSQ (p:0,001), HADS-A (p:0,004) and HADS-T (p:0,037), but the groups did not differ in means of depression scores on HADS-D (p:0,404) as shown in **Table 2**. Similarly, a significant positive correlation relationship between scores of measures has been found as shown in **Table 3**.

Table 2. Comparison of measures between parents of patients and healthy controls

Measures	Patient Group	Control Group	p value[1]
n	120	120	
CFQ[M (SD)]	40.29 (±15.48)	36.00 (±15.58)	**0.034**
BSQ[M (SD)]	33.06 (±13.17)	28.01 (±10.09)	**0.001**
HADS-A[M (SD)]	9.00 (±4.31)	7.39 (±4.22)	**0.004**
HADS-D[M (SD)]	6.92 (±4.37)	6.45 (±4.41)	0.404
HADS-T[M (SD)]	15.93 (±7.76)	13.84 (±7.65)	**0.037**

1: independent samples t-test CFQ: Cognitive Failures Questionnaire, BSQ: Body Sensations Questionnaire, HADS-A: Hospital Anxiety and Depression Scale – Anxiety, HADS-D: HADS Depression, HADS-T: HADS Total Score

Table 3. Correlation analyses of measures in patient and control groups and total sample

Total Sample (n:240)					
Measure	**CFQ**	**BSQ**	**HADS-A**	**HADS-D**	**HADS-T**
1. CFQ	1	0.471**	0.533**	0.482**	0.570**
2. BSQ			0.481**	0.362**	0.474**
3. HADSA				0.581**	0.888**
4. HADSD					0.891**
5. HADST					1

CFQ: Cognitive Failures Questionnaire, BSQ: Body Sensations Questionnaire, HADS-A: Hospital Anxiety and Depression Scale – Anxiety, HADS-D: HADS Depression, HADS-T: HADS Total Score * p < .05 (two-tailed). ** p < .01 (two-tailed).

Finally the regression analysis showed that perception of body perceptions, anxiety and depression scores significantly contribute to cognitive impairment, as shown in **Table 4**.

Table 4. Predictive values of BSQ, anxiety and depression scores on CFQ

Number of Subjects: 240			
Predictor	**Beta**	**p**	**r**
BSQ	0.251	**0.000**	0.217
HADS-A	0.275	**0.000**	0.207
HADS-D	0.230	**0.000**	0.185
History of COVID	0.020	0.704	0.020
Fit for model R2=0.378, Adjusted R2=0.368, p <0.00			

CFQ: Cognitive Failures Questionnaire, BSQ: Body Sensations Questionnaire, HADS-A: Hospital Anxiety and Depression Scale – Anxiety, HADS-D: HADS Depression

DISCUSSION

The findings from the current study show that survivors of COVID-19 suffer from cognitive difficulties during everyday practices as measured by CFQ, increased sensitivity to various body sensations as measured by BSQ and increased anxiety as measured by HADS. Moreover, these impairments seem to be correlated to each other.

The neurocognitive deterioration after COVID-19 is a well-known and replicated finding from numerous studies and some authors formulize this situation as "cognitive COVID" (30). Studies show that these cognitive impairments are mostly related to general inflammatory processes and the long-term outcomes of these impairments are largely unknown. Nevertheless, some studies conclude that high frequency of cognitive impairments in COVID-19 survivors deserve special attention and early interventions targeting these impairments should be considered (31). In this regard our findings that cognitive dysfunction was found to be significantly higher in the patient group also replicate this findings, as 33% of our patient sample subjectively reported that, they suffer from psychological consequences of COVID-19, apart from their scores on measures used in the current study. Also the CFQ scores from the current study indicates that COVID-19 survivors suffer from cognitive impairments. This finding should be discussed in light of high anxiety levels of patient group in the current study. Our results show that there is a high correlation between cognitive impairments and anxiety levels and it may be suggested that anxiety can also be a precipitating factor in cognitive difficulties in COVID-19 patients. Moreover our regression analysis model also shows that although BSQ scores, anxiety and depression have separately significant effects on cognitive difficulties, most significant factor among these parameters was anxiety. Further this regression analysis showed that there is no significant effect of COVID-19 itself on cognitive dysfunctions in patients. Therefore it can be postulated that cognitive difficulties seen in COVID-19 survivors are probably through increased anxiety, fear from somatic sensation and depression rather than COVID-19 itself. This relationship is already evident in different medical conditions, as studies show that anxiety levels are correlated with cognitive dysfunctions in inflammatory disorders (32). This finding is also evident in COVID-19, as studies show high anxiety levels in COVID-19 survivors and its relationship between cognitive dysfunction (33,34).

In contrast to increased anxiety levels in COVID-19 survivors compared to healthy controls, it this study these two group did not differ in means of depression scores. This finding is seems to be unusual in the literature, as almost all studies conclude an increased risk of depression in COVID-19 patients or survivors compared to healthy subjects (35). This finding may be caused from different factors like age, sex and disease severity. In contrast to other studies our sample seems to be younger in age and has more females compared to males (22). Furthermore the rate of admitted patients to the hospital due to COVID-19, a strong predictive factor of symptom severity, seems be lower in the current study compared to the literature (22). Therefore it can be interpretated that our patient group might have lower symptom severity compared to similar studies. Another reason of similar depression scores between groups may be the exclusion criteria of the current study, as patients with current psychiatric disorders including mood disorders were excluded from data analysis to neutralize possible confounding effect of depression on cognitive functions. This exclusion may be important, as studies point that history of any psychiatric disorder seems to be associated with worser post-COVID psychological functioning (22).

Another important finding from the current study is the increased BSQ scores in COVID-19 survivors compared to healthy subjects. This finding should be discussed with the another finding from current study which shows that more than half of the COVID-19 survivors subjectively think that they still suffer from one or more somatic symptoms. BSQ is mostly used to predict the fear from bodily perceptions associated with anxiety, somatoform disorders and non-organic pain related disorders (36). Although there has been no other study using BSQ in COVID-19 can be found in the literature, some studies showing increased levels of somatization and health anxiety related to COVID-19 (37,38).

Limitations

One of the main limitations of the current study is the sampling method, as both patient and control groups are reached through online platforms and asked to fill questionnaires online. This may limit the generalizability of our results. Another limitation was the information gathering method. All our questionnaires were self-report

measures, and this type of measures may not be ideally objective. This problem can be more crucial in case of cognitive assessment, and we suggest more objective measures to assess neurocognitive functions for further studies. There was one person over 65 years of age in the patient group and two people in the control group. Although all patients were healthy in terms of dementia on examination, advanced age may have affected our results. Having an additional chronic disease was determined as an exclusion criterion in the healthy control group but not in patient group because of the risk of sampling bias in COVID-19 group. However, not questioning an additional chronic disease which may impair cognitive functions in the patient group may be considers as an another limitations of the current study. Similarly the lack of information on the medications of patients, which can also effect cognitive functions, might have also limit the generalizability of the results.

CONCLUSION

In summary, apart from depression levels, our results seem to be in line with the current literature on neuropsychiatric outcomes of COVID-19. Based on findings from the current study, it may be suggested that COVID-19 survivors suffer from high anxiety, cognitive impairments and more fear from body sensations. Therefore it may be important to screen COVID-19 patients in long term in means of preventive measures and interventions regarding cognitive abilities and psychological functioning.

ETHICAL DECLARATIONS

Ethics Committee Approval: Ethics approval was applied and obtained from the Kafkas University Ethics Committee (Date: 11.03.2021, Decision No: 80576354-050-99/26).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

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Evaluation of the impact of platelet-rich plasma in women with reduced ovarian reserve

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ABSTRACT

Introduction: Infertility is the most critical factor disrupting the marital relationship, which imposes high financial and psychological costs on couples. Despite vast advances, the problem of infertility has not yet been entirely resolved. The new method of injecting platelet-rich plasma (PRP) has been promising for couples. This study investigated the effect of PRP injection on the fertility of infertile women.

Material and Method: In this study, 40 women with a history of infertility with a mean age of 37.75 were included in the study. Prior to the demographic information intervention, laboratory findings, including serum anti-mullerian hormone (AMH) levels and ultrasound for the number of antral follicles count (AFC) were performed. Autologous PRP was then prepared for each patient, and an intraovarian injection was performed. Two months after injection, serum levels of AMH and AFC levels were re-evaluated.

Results: The mean AMH levels before and after the injection were 0.07±0.05 and 0.13±0.06, respectively ($p<0.001$). The mean follicle-stimulating hormone (FSH) levels before and after the injection were 33.95±12.59 and 22.10±3.29, respectively ($p<0.001$). The amount of AFC increase one number after the injection. Linear regression showed that patient and partner age, body mass index (BMI) and infertility years had no effect on PRP injection response. PRP is associated with a significant increase in AMH levels, decrease in FSH levels and a significant increase in the number of AFCs. Patient and partner age, BMI and infertility years have no effect on response to PRP injection.

Conclusion: This study showed that the PRP injection improving ovarian reserve markers as measured by AMH and FSH serum levels, and AFC. However, further studies should be done to assess the effect of PRP on pregnancy outcomes.

Keywords: Infertility, platelet-rich plasma, follicle-stimulating hormone, antral follicle count , anti mullerian hormone

INTRODUCTION

Infertility has become a prevalent issue, which about 15% of couples have faced. There are several possible causes, 40% of which are related to women, 40% to men, and 20% to both couples (1). Disorder of the ovulation process is one of the most common causes of infertility in women (2). Particularly, the referral of women with poor ovarian reserves (POR) to infertility centers has increased in recent years due to a change of priorities in young girls and their marriage at older ages (3). Female age is largely the main factor limiting the success of spontaneous fertilization and assisted reproductive technology (ART), which is mainly due to the loss of ovarian follicle reserve and ovule quality as women age increases, especially after the age of 37 (4-6). Approximately 12.7- 35.5 % of infertility cases in women are related to ovulation disorders (7). In cases it is the only cause of infertility, there is a highly satisfactory response to adjuvant treatment. These individuals' successful treatment is dependent on careful examination and discovery of the main cause of anovulation. Ovulation stimulation is a pharmacotherapy used in women without regular ovulation with ovulation disorders, or polycystic ovary syndrome (PCOS). These pharmacotherapies are performed to increase the number and quality of ovules to increase the chance of fertility and the success rate of ART (8). The drugs used for this purpose are called ovulation-stimulating drugs, which can be used to increase the number and quality of ovules. To use ART, one of the

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measures which are first taken is ovulation stimulation to obtain the proper number of the high-quality oocyte. Although the natural cycle can also be used in women with normal ovarian function to prepare oocytes, since these treatments require time and money, controlled ovarian stimulation should be used to increase the chance of fertility. To stimulate ovulation in ART treatments, it is better to avoid ovarian overstimulation as far as possible to prevent potential risks while obtaining a suitable number of the oocyte. Therefore, the person should be carefully examined before any treatment (9).

One of the new treatments for the controlled ovarian stimulation for the treatment of women with reduced ovarian reserve is the platelet-rich plasma (PRP) injection. PRP has been widely used and successfully in different fields such as orthopedics, wound healing, hair growth promotion, ophthalmology, and wound regeneration, and its application is increasing (10). PRP has now become a field of interest in reproductive medicine with higher focus on infertility. PRP is used in research fields such as poor ovarian reserve, menopause, premature ovarian failure, and thin endometrium (11-13). PRP increases local delivery of cytokines, growth factors ,lysosomes and chemokines and also,modifies the inflammatory responses,cell proliferation and cell differentiation (11).

In this study, the effect of PRP injection on ovarian function has been investigated. Mean serum level of anti-müllerian hormone (AMH) and follicle-stimulating hormone (FSH) levels before and after PRP injection and the number of antral follicle count (AFC) in this study have been measured and ovarian function was measured on its basis. The main aim is to evaluate the success rate of the PRP injection method and to evaluate the factors affecting the success rate.

MATERIAL AND METHOD

The study was carried out with the permission of the Clinical Research Ethics Committee of Gümüşhane University. (Date: 09/06/2021, Decision No: 2021/4) All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. Fourty participants in this study visited Medical Park Samsun Hospital and BAU Göztepe Medical Park Hospital for infertility treatment from January 2020 to January 2021. Participants ranged in age from 23 to 40 years.

Inclusion and Exclusion Criteria

The inclusion criteria were the age of women between 25-45 years with POR, and the duration of infertility was more than one-year. POR diagnosis was made by using POSEIDON criteria. The exclusion criteria were malignancy history, previous abdominal surgeries, and anticoagulant use.

Laboratory Parameters

PRP is taken from a venous blood sample and injected into the ovary after preparation. PRP activates factors such as VEGE and TGF, which are effective in VEGE migration and extracellular matrix accumulation.

PRP preparation

We prepared PRP using a T-lab autologous platelet-rich plasma kit (T-Biotechnology Laboratory, Bursa, Turkey). The preparation process was based on the manufacturer's instructions (24). Under sterile conditions, 20 ml of blood samples were taken from each patient, and then the samples were centrifuged in 830 g tubes for eight minutes. A 5 ml syringe with a 16G needle was used to receive PRP. About 2 to 4 cc of PRP from the first tube and about 4 to 8 cc of PRP from the second tube were collected without removing the growth factor-rich blood clot. After PRP collection, the resulting solution was transferred to a separate tube and then gently shaken for thirty to sixty seconds.

Intraovarian Injection

Two hours after PRP preparation and under conscious sedation, the intraovarian injection was performed in the operating room. We used a 17 G 35 cm single lumen needle (Cook, USA) and ultrasound guidance to inject PRP transvaginally into at least one ovary. Older patients with POR usually have small, fibrotic ovaries; In these cases, new planes in the ovary were created by distention and injection at various sites, and PRP injection was performed in this way. After the operation, the patients were transferred to the recovery room, and after 40 minutes of monitoring, they were discharged.

Statistical Analysis

Data were analyzed, tabulated, and subjected to using the SPSS (version 26). The continuous data were displayed as mean±SD. At the same time, categorical data were illustrated as percentages and numbers. The Kolmogorov-Smirnov test of normality was utilized to test the normality hypothesis. Based on the test results, proper parametric (Paired-samples t-test) and nonparametric tests (Wilcoxon signed-rank test) were used. A p-value of <0.05 was regarded as statistically significant.

RESULTS

A total of 40 women (mean age±SD: 37.75±4.46) with the diagnosis of POR were included in the study. The participants' BMI and partner age were 23.72±2.51, and 39.10±3.76, respectively. The mean duration of infertility is 6.17±2.08 years. **Table 1** shows the explanatory information of the variables. Descriptive characteristics of other variables omit for brevity.

Table 1. Explanatory information of the variables

Variable	N	Minimum	Maximum	Mean	SD
Patient age (yr)	40	25.00	45.00	37.75	4.46
Partner age(yr)	40	33.00	45.00	39.10	3.76
Body mass index	40	21.00	30.00	23.72	2.51
Infertility duration (yr)	40	3.00	12.00	6.17	2.08

A paired-samples t-test was conducted to compare AMH scores in an infertile woman before and after PRP injection. There was a significant difference in the scores for Pre-AMH (M=0.07, SD=0.05) and Post-AMH (M=0.13, SD=0.06) conditions; t(39)=-7.804, p=0.000.

A Wilcoxon signed-rank test showed that PRP injection did a statistically significant change in FSH scores in infertile woman (Z=-5.481, p=0.000). There was a significant difference in the scores for Pre-FSH (M=33.95, SD=12.59) and Post-FSH (M=22.10, SD=3.29).

A Wilcoxon signed-rank test was conducted to compare the quantity of AFC in an infertile woman before and after PRP injection. There was a significant difference in the quantity for Pre-AFC (M=1) and Post-AFC (M=2). **Table 2** shows the significant difference in variables before and after PRP injection **Figure 1** shows PRP treatment resulted in lower serum FSH, higher serum AMH, and higher AFC. Linear regression showed that patient and partner age, BMI and infertility years had no effect on PRP injection response in terms of serum level of AMH. **Table 3** shows linear regression analysis predicting serum AMH difference.

Table 2. The significant difference in variables before and after PRP injection

Variables	Pre PRP Injection		Post PRP Injection		t(39)/Z	p
	Mean	SD	Mean	SD		
FSH	33.95	12.59	22.10	3.29	-5.481	0.000*
AMH	0.07	0.05	0.13	0.06	-7.804	0.000**
AFC	1	0	2	0	-6.325	0.000*

*Wilcoxon signed-rank test, **Paired-samples t-test

Table 3. Linear regression analysis predicting serum AMH difference

Variables	B	SE	Beta (β)	t	p
Patient age	-0.003	0.002	-0.301	-1.226	0.228
Partner age	0.001	0.003	0.058	0.233	0.817
Body mass index	0.000	0.003	-0.022	-0.132	0.896
Duration of infertility (years)	0.005	0.004	0.236	1.392	0.173

Note: Constant=0.121, F(4,40=1.206), p<.001, RP=.121

DISCUSSION

This study dealt with the effect of PRP injection on FSH, AMH, and AFC. Our findings showed improved ovarian reserve markers such as FSH, AMH, and AFC with a 2-month treatment course with PRP. In addition, there was an association between the use of PRP and significantly increased biochemical variables such as AMH. Our findings showed that none of the factors such as age, BMI, and infertility duration affect the effect of the PRP method on improving ovarian function.

The first study was performed on the effect of PRP on ovarian reserve with Sills et al. (14) in 2018 with four patients. After PRP injection, an improvement in ovarian reserve was observed. All four patients showed decreased

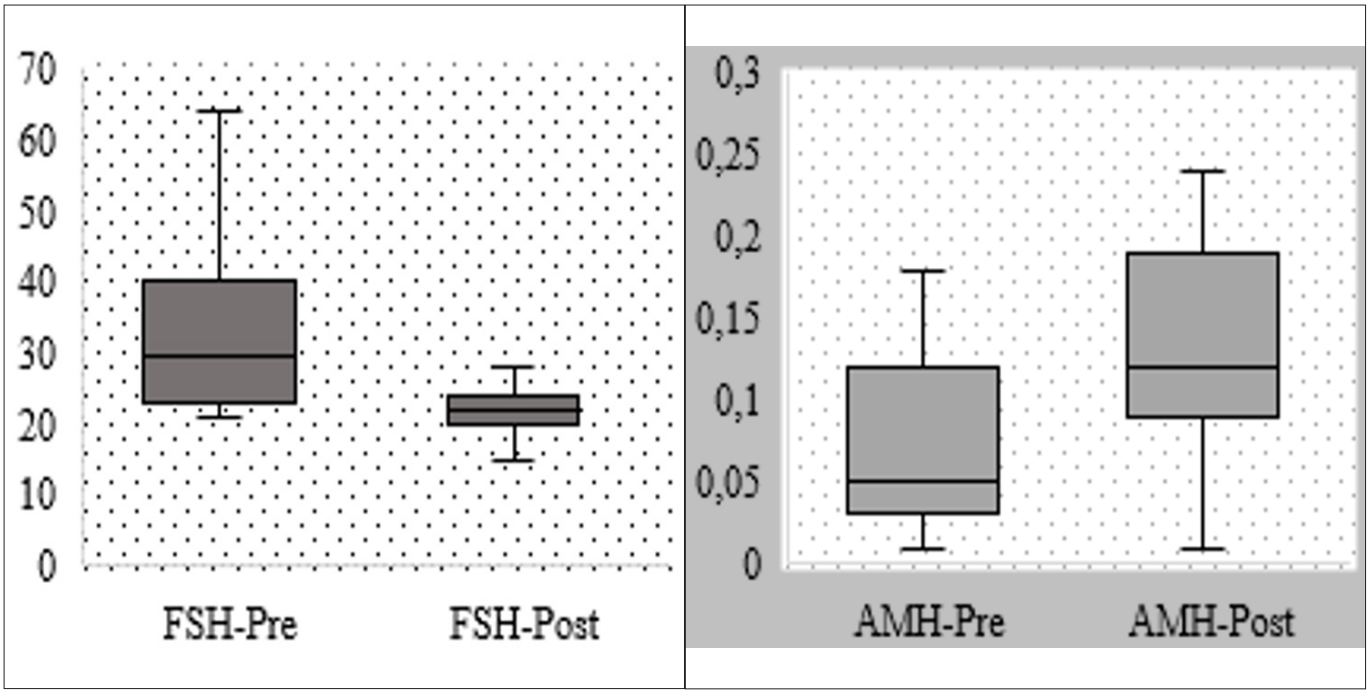

Figure 1. AMH and FSH levels before and after PRP injection.

FSH. Three of the four patients showed an increase in AMH. In 2020, Sills measured the previous research question in another study, expanding the number of samples to 182. Measurement of FSH and AMH levels in two-week intervals found that PRP injection had a significant effect on improving ovarian function. The most frequent positive effect has been reported after 4 weeks (15).

Sfakianoudis et al. (16) reported improved ovarian function in women with POR after PRP injection. Three studied patients reported an increase in AMH and a decrease in FSH after three months of PRP injection. Sfakianoudis et al. (17) in another study reported reduced cycle cancellation rates after PRP treatment.

In a study, Pacu et al. (18) measured the effect of PRP on 20 women with POR. Based on findings, the PRP injection decreased the FSH, luteinizing hormone (LH), and estradiol (E2) values and increased the AMH. The number of canceled cycles has also decreased significantly. Six months after PRP injection, the ovarian function has returned to the pre-injection period.

In a study with 510 women with POR, Cakiroglu et al. (19) focused on improving ovarian function after PRP injections. This study is of special value among similar studies due to its large sample size. Based on the findings, the PRP injection significantly increases AMH and significantly decreases FSH.

Melo et al. (20) reported an improved ovarian function in the case group after PRP injection comparing ovarian function in the case and control groups. In 46 women with POR in the case group, a significant improvement was reported compared to the control group with 37 women in ovarian function, including increased AMH and decreased FSH .

Other studies performed with three patients and with one patient emphasized the significant effect of PRP injection on the ovarian function of the studied patients (21,22).

The effect of PRP injection on the fertility rate has also attracted the attention of researchers. In the conducted studies, the PRP injection has been reported on fertility rates in spontaneous and ART methods (19-20,23,24). However, the effect of PRP injection on increasing fertility and live birth rates requires further studies with larger sample size.

Improved ovarian function is not affected by factors such as female age, partner's age, infertility duration, and BMI due to the effect of PRP injection. Based on our study findings, female age does not affect the probability of success of the PRP injection method as a determining success factor of the ART. Infertility duration, partner's age, and BMI also affect improved ovarian function.

CONCLUSION

This study showed that the PRP injection is secure. This improves ovarian reserve markers measured by FSH and AMH serum levels and antral follicle count. However, further studies should be done to assess the effect of PRP on outcomes of pregnancy among women undergoing ART.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Clinical Research Ethics Committee of Gümüşhane University (Date: 09/06/2021, Decision No: 2021/4)
Informed Consent: All patients signed the free and informed consent form.
Referee Evaluation Process: Externally peer-reviewed.
Conflict of Interest Statement: The author has no conflicts of interest to declare.
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Author Contributions: The author declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Characteristics of patients with transudative efusion followed in an university hospital

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ABSTRACT

Aim: To determine the etiological causes, radiological and laboratory features of transudative pleural effusions and to observe the clinical course after therapeutic thoracentesis.

Material and Method: The files of patients with transudative effusion who underwent therapeutic thoracentesis by the Interventional Radiology Department between 01.01.2012 and 30.11.2012 were retrospectively reviewed. Pleural effusion (PE) anatomical features were evaluated with Postero anterior (PA) chest X-ray and Thorax Ultrasonography (USG). Demographic and clinical features, pleural effusion analysis results, presence and rates of complications were analyzed.

Results: As a result of pleural fluid analysis, our study group consisted of 60 transudative pleural effusion cases, 36 (60%) women. The mean age was 71.23±2.36 years. Patients using diuretic therapy in cases with pleural effusion were statistically significantly higher than patients who did not ($p<0.05$). The most common etiologic causes were Congestive heart failure (CHF) and the accompanying disease hypertension (HT). Fifty (83.3%) of the pleural effusions were unilateral and 39 (65%) of them were right-sided ($p<0.05$). Diagnostic and therapeutic thoracentesis of our cases was performed by the radiologist under the guidance of thorax USG, and pneumothorax was observed in only one case (1.7%). In our 2-month clinical follow-up, the presence of recurrent pleural effusion was not detected in any of the cases.

Conclusion: In cases with persistent transudative pleural effusion, therapeutic thoracentesis can be considered in cases where fluid resorption is not at the desired level despite effective treatment.

Keywords: Pleural effusion, thorax USG, thoracentesis, transuda

INTRODUCTION

Pleural diseases are common medical problems with more than 50 known causes, including diseases confined to the pleura or lungs, systemic diseases, organ dysfunctions and drugs (1).

Two basic mechanisms play a role in the formation of pleural effusion (PE); increased fluid formation and/or decreased fluid resorption. Pathological pleural fluids constitute 4% of patients admitted to internal medicine clinics (2).

While the main cause of exudative fluids may be malignancy, infections, or iatrogenic or trauma, transudative fluids are usually caused by congestive heart failure (CHF), chronic renal failure (CRF) or hypoalbuminemia (3).

Light criteria have been used since 1970 in the separation of pleural fluids. Puncture of the fluid in the pleural space consisting of visceral and pariaetal leaves can be done under the guidance of thorax ultrasonography (USG) (3).

While thoracentesis may not be required for effusions in the pleural space, which are usually bilateral, etiologically clear, and which we expect to regress with the treatment of the underlying disease, therapeutic thoracentesis may be considered in cases where we cannot obtain the desired results with unilateral effective treatment (4).

In transudative effusion, there is no pleural disease or pleural involvement of a disease. Problems leading to increased pulmonary or systemic hydrostatic pressure or decreased plasma oncotic pressure lead to transudative pleurisy. Transudative fluids are often the result of systemic problems. For this reason, in the case of detecting a transudate fluid, further investigation may not be necessary in terms of lung disease (5,6).

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There are conflicting results about which effusions to intervene and what the results are in these cases of transudative PE (7,8).

Our aim in this article; to determine the etiological causes, radiological and laboratory features of persistent transudative pleural effusions, which can cause difficulties for clinicians, and to observe the clinical course in their follow-up after therapeutic thoracentesis.

MATERIAL AND METHOD

This retrospective study was approved by the Baskent University Clinical Researches institional review board and produced from thesis (Date: 28.02.2012, Decision No:12/32). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Our study included 169 patients who underwent therapeutic thoracentesis by the Interventional Radiology Department of a University Medical Faculty Hospital between 01.01.2012 and 30.11.2012. Patients with exudate as a result of the fluid analysis performed according to Light's criteria and who only underwent sampling thoracentesis were excluded from the study, 60 patients with transudate were included in the study. File contents of patients with transudative pleural effusion were reviewed retrospectively. The amount of pleural effusion was determined by direct chest radiographs. Patients with transudative pleural effusion who were referred from different departments of our hospital and underwent therapeutic thoracentesis by the Department of Interventional Radiology were followed up in terms of their two-month course following the thoracentesis procedure. As clinical follow-up parameters, pleural fluid analysis results of the patients, additional diseases, medical treatments they use regularly, symptoms at admission, the radiological imaging used to determine the pleural fluid, the localization of the fluid, the number of times thoracentesis was performed, the amount of fluid drained, the presence of complications and their rates were evaluated.

The Light criteria are used to differentiate transudate-exudate in pleural fluids (3). Accordingly, if the ratio of pleural fluid protein to serum protein is greater than 0.5, the LDH (lactate dehydrogenase) ratio is greater than 0.6, or the pleural fluid LDH value is greater than 200 IU, or the pleural fluid < LDH value is 2/3 of the upper limit of serum LDH. Presence of one of the criteria of being higher than ≥ indicates that the liquid is exudate. Transudates do not have any of these criteria. With the Light criteria, it is possible to distinguish between transuda and exudate in approximately 99% of cases (9).

Statistics: Categorical Data were analyzed with one-way Chi-Square test and two independent ratio z-tests. Numerical data are expressed as mean±standard deviation and minimum and maximum values. The $p<0.05$ level was considered statistically significant. The data were evaluated in the SPSS statistical package program (Version 17, Chicago IL, USA).

RESULTS

Sixty cases who underwent therapeutic thoracentesis between 01.01.2012 and 30.11.2012 and were evaluated as transudate as a result of pleural effusion were included in our study. It was seen that 28 of these patients were consulted to Chest Diseases. The demographic characteristics of the patients, their diuretic use histories and their symptoms at presentation are given in **Table 1**. The mean age of the study group was 71.23±2.36 years. There was no significant difference between the patients in terms of having or not smoking history. In cases with pleural effusion, patients using diuretic therapy were statistically significantly higher than patients not using it. ($p<0.05$). There was also a significant difference in the comparison of the patients in terms of hemodialysis application ($p<0.05$). In patients with chronic renal failure (CRF) with pleural effusion, the number of patients undergoing hemodialysis was less ($p<0.05$). There were no patients on peritoneal dialysis. **Table 2** shows the underlying diseases in transudative PE cases.

Although there were patients with more than one etiological cause of pleural effusion, the most common etiological cause was CHF. According to **Table 3**, hypertension (HT) was the most common comorbidity. The least common chronic lung diseases; asthma and chronic obstructive pulmonary disease (COPD). There were also cases with more than one comorbid disease among the patients.

Table 1. General features

Characteristics	Patients , n (%)	p value
Gender		
Female	36 (60)	P<0.05
Male	24 (40)	
Smoking history		
Yes	31 (51.7)	P=0.79
No	29 (48.3)	
Diuretic use		
Yes	39 (65)	P<0.05
No	21 (35)	
Hemodialysis		
Applied	8 (13.3)	P<0.05
Not applied	52 (86.7)	
Presenting symptoms		
Asymtomatic	1 (1.7)	
Dyspnea	46 (76.7)	
Cough	2 (3.3)	P<0.05
Fever	2 (3.3)	
Cough and dyspnea	8 (13.3)	
Chest pain	1 (1.7)	

One-way Chi-Square test used and $p<0.05$ level was considered statistically significant.

Table 2. Underlying diseases		
Characteristics	**Patients , n (%)**	**p value**
Congestive heart failure		P<0.05
Yes	39 (65)	
No	21 (35)	
Chronic renal failure		P<0.05
Yes	14 (23.3)	
No	46 (76.7)	
Chronic liver failure		P<0.05
Yes	4 (6.7)	
No	56 (93.3)	
Malignancy		P<0.05
Yes	14 (23.3)	
No	46 (76.7)	

One-way Chi-Square test used and p<0.05 level was considered statistically significant.

Table 3. Concominant diseases	
Characteristics	**Patients , n (%)**
Chronic obstructive pulmonary disease	
Yes	12 (20)
No	48 (80)
Asthma	
Yes	4 (6.7)
No	56 (93.3)
Atrial fibrillation	
Yes	19 (31.7)
No	41 (68.3)
Hypertension	
Yes	39 (65)
No	21 (35)
Diabetes mellitus	
Yes	23 (38.3)
No	37 (61.7)

As for pleural fluid localization, 50 (83.3%) cases were unilateral and 10 (16.7%) cases were bilateral (p<0.05). 39 (65%) of 50 unilateral pleural effusion cases were right-sided (p<0.05). Pleural effusion was detected in 98.3% of the patients by taking posteroanterior (PA) X-ray. Statistically significant moderate amount of pleural effusion was more common in the patients.

It was observed that 36.7% of the patients had repeated thoracentesis during the two-month follow-up. A minimum of 150 ml and a maximum of 3500 ml of fluid were drained in the thoracentesis procedures performed once or more in patients. It was observed that a maximum of 1500 ml of fluid was drained during a single thoracentesis procedure performed under USG guidance by interventional radiology in all patients. After the thoracentesis procedure, 59 (98.3%) of the patients had a control PA chest X-ray. Complications were seen in only 1 (1.7%) patient. This complication was minimal pneumothorax, which did not require a chest tube in the treatment.

Biochemical measurement results in all pleural fluids were transudate according to the Light Criteria. A total of 57 patients pleural fluid was sent to microbiology and no growth was observed in any of them. The fluid of the remaining 3 cases was not evaluated microbiologically. Pleural fluids were sent for cytological evaluation in 44 of 60 patients, and cytological examination was not performed in 16. As a result of the 44 pleural fluid cytology sent, mesothelial cells and inflammatory cells were detected in 21 (51.2%) of the cases. Malignant cells were detected in the pleural fluid cytology of 2 cases (3.3%).

Table 4. Biochemical results for transudate criteria				
Characteristics	**minimum**	**maximum**	**mean**	**Standart deviasion**
Pleura protein (gr/dl)	0.8	6.3	2.12	0.14
Serum protein (gr/dl)	0.19	8.2	6.05	0.19
Pleura / serum protein	0.06	0.6	0.32	0.01
Pleura LDH (U/L)	48	204	102.6	5.95
Serum LDH (U/L)	140	688	282.5	17.82
Pleura / serum LDH	0.14	1.2	0.38	0.02
Pleura albumin (gr/dl)	0.04	2.7	1.14	0.07
Serum albumin (gr/dl)	2	4.3	3.11	0.07
Albumin gradiyent	0.31	3.06	1.92	0.07

DISCUSSION

In our study, we evaluated 60 cases who underwent therapeutic thoracentesis under USG guidance by the interventional radiology clinic of our hospital between 01.01.2012 and 30.11.2012 and whose pleural fluid result was determined as transudate according to light criteria. We found the complication rate after the procedure to be significantly low (1.7%) in these cases, and we found that pleural effusion did not develop again in any of them with thorax USG control performed after 2 months of clinical follow-up.

The mean age of the patients included in our study was 71.2±2.3. Transudative pleural effusions are usually the problems of the older age group, as in our cases. When other demographic characteristics were examined, our findings were in line with the literature in terms of gender distribution in 36 female cases (60%) (10).

According to the data of "Global Adult Tobacco Survey 2016"; 19.2 million people (31.6%) in Turkey still use tobacco products. The prevalence of tobacco use is higher in men (44.1%) than in women (19.2%) (11). In our study, we found the rate of smoking to be 51.7% higher in our patient group with intertwined comorbidities. Smoking rates in hospitalized patients are generally reported at higher rates in parallel with our findings (12,13). Smoking is among the important risk factors in the etiology of the underlying diseases of our patients.

65% of our cases with transudative pleural effusion were receiving diuretic therapy. This suggested that pleural fluid may occur despite the use of diuretic therapy in

CHF. Consistent with the literature, CHF was the most common cause of transudative pleural effusion in our cases (14). While peripheral edema is resolved in a shorter time with diuretic treatment, fluids in third body cavities such as pleura and peritoneum may persist. In this case, an unloading process is required for palliation. Our study also supports this observation.

In our daily practice, we may also encounter "difficult transudates" that acquire the character of exudate despite having a transudate, that we have difficulty in treating or that we cannot explain (15). Under diuretic therapy, the pleural fluid feature may shift to exudate. The fluid in these cases is interpreted as "awaited transudate". In their study investigating the effect of diuretics on protein concentrations in the transudative pleural fluids of patients with CHF, Romero et al. (16) concluded that the use of protein and albumin serum-fluid gradient is more beneficial in the separation of pleural fluid transudate-exudate. Since all of our cases were indisputably transudate, there was no need to evaluate these parameters in our study.

Hemodialysis was applied to 8 of 14 patients with CRF diagnosis. Dialysis indication has not yet been established in other CRF patients. Although our hospital is a reference center in solid organ transplantation, CRF was ranked second in our series in the etiology of transudate. The low rate of this can be explained by the effective fluid withdrawal in patients' dialysis programs. An effective hemodialysis program in CRF can significantly reduce the volume load in the body and the likelihood of developing pleural effusion.

One of the most common symptoms in pleural effusion is dyspnea (17). Cough and chest pain are also common symptoms. Dyspnea was the most common presenting symptom in 76.7% of our cases. Dyspnea is often the main reason for the need for therapeutic thoracentesis. Therapeutic thoracentesis is recommended for symptom palliation even if the amount of pleural fluid is small. Our study also supports this view.

There was a diagnosis of malignant disease in 23.3% of our cases. However, in such cases, there is usually more than one underlying cause of pleural effusion, and it should not be attributed to malignant disease alone. There are many transudate causes such as malnutrition and hypoalbuminemia, hypervolemia and heart failure, immobility and atelectasis secondary to malignancy (18). This can also be explained by our detection of malignancy as the cause of transudative pleural effusion in approximately one fourth of our cases.

One of the most important findings of our study was that approximately half of the cases who underwent thoracentesis were referred to the radiology department without evaluation by our department. The fact that patients were consulted to our department at a lower rate (47%) before the thoracentesis procedure can be explained by the fact that the diagnoses of COPD and asthma, which are the accompanying chronic lung diseases, are quite low (26.7% in total). When the pleural effusion problem is attributed to extrapulmonary causes according to the clinical findings of the patient, it is usually managed by the relevant departments. We think that the number of recurrent thoracentesis can be reduced by applying a multidisciplinary approach, including chest diseases from the beginning. It is an important result of our study that 36.7% of the cases are performed more than once in thoracentesis.

Of our cases, 83.3% of pleural effusions were unilateral, of which 76% were on the right side. In the reviews published by Light and Sahn (19), who are important authors in pleural diseases in the world, it is argued that if the fluid is bilateral and/or in small amounts in a patient with CHF, the treatment response for failure should be followed without trying torecentesis. However, although the majority of our cases were CHF patients, the pleural fluid was highly unilateral (83.3%). This situation has led to the widening of thoracentesis indication.

98.3% of our patients had a PA chest X-ray and therapeutic thoracentesis was performed in all of our patients with thoracic USG in interventional radiology. USG guidance increases the success rate of pleural aspirations. Many studies have shown that in 88% of patients in whom clinical and plain radiography-guided interventions fail, fluid can be successfully removed using USG (20). The very low incidence of complications detected after thoracentesis in our cases was also associated with performing the procedure under USG guidance.

98.3% of our patients, whose thoracentesis procedures were all performed under USG, had a control PA chest X-ray after the procedure. Complications were seen only at a rate of 1.7% (in 1 patient). This showed that it is safe to perform the procedure with USG and that it is necessary to take a PA chest X-ray after the procedure. Barnes et al. (21) analyzed the records of 450 patients who underwent diagnostic thoracentesis for the first time in their centers. They found a decrease in pneumothorax and tube thoracostomy rates with the routine use of USG during diagnostic thoracentesis. Grogan et al. (22) also showed that the complication rate was independent of the amount of effusion in their study by randomized 52 patients. USG can also be a preliminary idea in the evaluation of pleural fluid in terms of transudate and exudate. The presence of septations brings the clinician closer to the exudate, while the appearance of free and

homogeneous fluid suggests the possibility of transudate. Since the diagnostic and therapeutic procedures were applied simultaneously in our cases, such a preliminary evaluation could not be made.

Considering the amount of pleural effusion in our patients, only 15% were massive. Massive pleural effusions are most commonly associated with malignancy (23). In our cases, the diagnosis of malignancy was 23.3%. Kıral et al. (24). retrospectively analyzed 159 cases with effusion on PA chest X-ray. They found the etiology as malignancy in 46.9% of massive effusions. The low number of our cases prevents us from obtaining sufficient data in the evaluation of this relationship.

In 57 of our cases, pleural fluid samples were sent to culture and there was no growth observed in any of them. Growth in culture in transudate fluids is not an expected result. Microbiological processes increase the cost unnecessarily. Another self-criticism of this study is that we found that almost all of the pleural fluids resulting from transudate were sent to the microbiology laboratory.

Fluid samples were sent to cytology in 44 (73.3%) of our patients. When we look at the cell contents according to the cytology results, the majority of them were mesothelial cells. This was an expected result for transudative fluids. The incidence of malignant cells at a rate of 3.3% suggested that cytological examination would be significant in this group. This situation reveals the necessity of sending the fluid to cytology in all patients.

Gonlugür et al. (25) retrospectively reviewed all cases diagnosed as malignant or paramalignant pleural effusion in a tertiary hospital over a 10-year period. 67 patients with malignant mesothelioma, 45 patients with metastatic disease and 36 patients with paramalignant effusion were included in the study. 1.5% of malignant mesotheliomas, 6.8% of metastatic diseases and 11.1% of paramalignant effusions were classified as transudate. In conclusion, they thought that the examination of pleural fluid in patients with unexplained transudative effusion was essential in excluding malignant processes.

Our limitations in this article can be considered as; being a single center and having relatively low number of patients.

CONCLUSION

We checked our cases for effusion with thoracic USG 2 months after the pleural effusion was drained and observed that the transudative fluids did not reoccur. All patients were also receiving treatment for their effective primary disease after therapeutic thoracentesis. Until recently, it was accepted that there was no need for additional examination and treatment effort in transudate fluids. We determined that there is a need for detailed evaluation regarding the necessity of therapeutic thoracentesis procedure in transudative fluids. We are of the opinion that there is a need for series consisting of more cases with longer follow-ups on the course of transudative fluids.

ETHICAL DECLARATIONS

Ethics Committee Approval: This retrospective study was approved by the Baskent University Clinical Researches institional review board (Date: 28.02.2012, Decision No:12/32,).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Malignant bone tumors around the knee: A single-center experience

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ABSTRACT

Aim: This study aimed to determine the frequency of malignant bone tumors (primary and metastatic) seen around the knee in our region, the patients' type and demographic characteristics, and the treatments' outcomes.

Material and Method: A retrospective analysis of the patients who were diagnosed and treated with histopathologically malignant tumors in the knee region in our hospital between 2004-2021 was performed from the hospital database. Patients' complaints, demographic information, and diagnostic and imaging findings were examined. In addition, tumor types, tumor localization, and treatments applied were analyzed.

Results: Malignant bone tumor was detected in 88 (35.7%) of 246 patients included in the study. The patients were 48 women and 40 men, with a mean age of 39.72±21.8 (6-76 years). A total of 88 patients were divided into the pediatric group (<18 years; n=39) and the adult group (≥18 years; n=49). The most common tumors were osteosarcoma in 54 (61.3%) and metastatic tumors in 22 (25%) patients. The most common localization of tumors was the distal femur with a rate of 75%. Metastasis was detected in 12 (18.2%) of 66 patients treated and followed up for primary malignant bone tumors. Limb sparing surgery was performed in 70 (79.5%), and various levels of amputation were performed in 14 (15.9%). Palliative radiotherapy was applied to two patients with metastatic lesions, while two patients who were in the neoadjuvant chemotherapy period died. The 5-year overall survival was 63.7%. Pediatric and adult age groups did not differ significantly in terms of survival (p=0.74), gender (p=0.585), and metastasis development (p=0.53).

Conclusion: The knee is a region that requires attention regarding bone tumors around it. As malignant bone tumors are rarely seen around the knee, a misdiagnosis may be made, and appropriate treatment may be delayed. Although the first diagnosis to come to mind for patients presenting with knee pain is trauma and growing pains, it must not be forgotten that a tumor could be the cause.

Keywords: bone tumors, malign, knee, surgery

INTRODUCTION

The knee is the most common location for malignant bone tumors as well as many soft tissue sarcomas. The clinical presentation of these lesions usually includes pain and swelling associated with a palpable mass. In most primary bone tumors, the patient attributes their symptoms to traumatic events in the first place. This may lead to misdiagnoses and errors in the selection of the therapeutic approach. A treatment strategy in malignant bone tumors provided by a multidisciplinary team is the key factor for optimal management of such patients. In this study, cases of malignant bone tumors localized in the knee region, diagnosed and treated in our center, were reviewed in terms of various factors and treatment results.

Primary bone tumors are one of the most uncommon groups of oncological diseases. Of all tumors, approximately 1% are seen in the bones, and the majority of these are benign (1). According to the American Cancer Society data, 1,898,160 new cancer patients were diagnosed in 2021, and of these, a primary malignant bone tumor was determined in only 6310 (2).

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The reason for presentation to the hospital is generally painful or painless swelling. Patients with pain generally associate the pain with traumatic events. In particular, in the elderly patient group, symptoms associated with degenerative knee disorders can mask a bone tumor around the knee.

Diagnosis can be easily overlooked as the frequency is low in the general population, and therefore, the onset of treatment can be delayed. Starting treatment in the early stage is extremely important regarding tumor recurrence, metastasis, and survival (3-5).

This study aimed to determine the frequency of malignant bone tumors (primary and metastatic) seen around the knee in our region, the patients' type and demographic characteristics, and the treatments' outcomes.

MATERIAL AND METHOD

A retrospective examination was made of patients with a bone tumor around the knee who presented at our hospital between 2004 and 2021. The study was carried out with the permission of Ondokuz Mayıs University/Training and Research Hospital, Noninvasive Clinical Ethics Committee (Decision No: 2022/178). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

The study included malignant bone lesions determined in the distal femur (the region 8 cm proximal from the joint line), the proximal tibia (8 cm distal from the joint line), the proximal fibula, and the patella. Soft tissue masses determined in the same region, lesions caused by reasons other than the tumor, synovial origin lesions, and benign bone tumors were excluded from the study. A record was made for each patient of the complaints on presentation, demographic information, diagnosis, radiographic imaging findings, and laboratory test results. The tumors of all the patients included in the study were diagnosed radiologically and/or pathologically. Then the tumor types, treatment applied, localization of the tumor, and demographic information was examined. The study flowchart is shown in **Figure 1**. Patients were divided into pediatric and adult age groups to investigate the effect of age groups on oncological outcomes. Analysis was performed in terms of factors of survival, gender, and metastasis development between these groups. The study flowchart is shown in **Figure 1.**

Statistical Analysis

Data obtained in the study were analyzed statistically using SPSS for Windows vn. 21.0 software (SPSS Inc., Chicago, IL, USA). Statistical values were stated as mean±standard deviation (SD), or median values for continuous variables, and as frequency (n) and percentage (%) for categorical variables. The effect of prognostic factors on survival was analyzed using Kaplan- Meier analysis and the Log Rank (Mantel-Cox) technique. A value of $p<0.05$ was considered statistically significant.

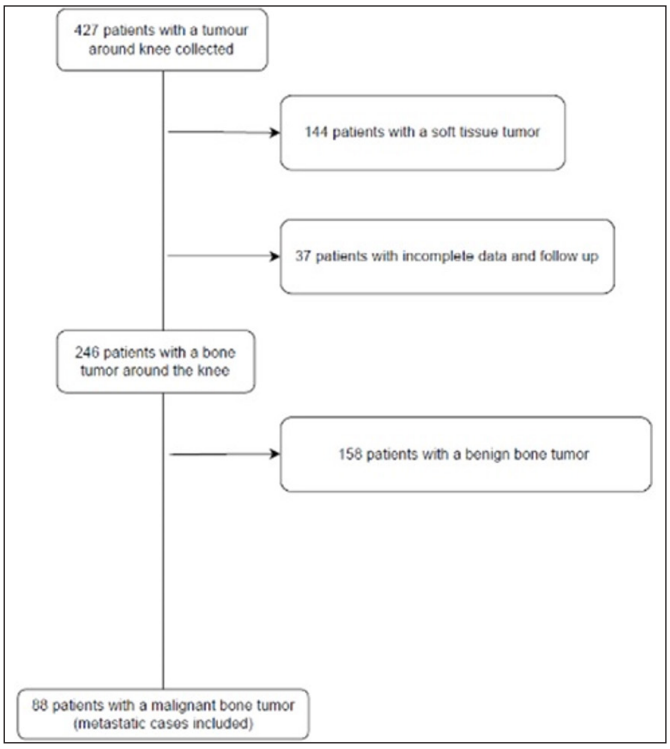

Figure 1. Study flowchart. The number of patients included in the study and demonstration of excluded patients.

RESULTS

Of the 246 patients included in the study, 88 (35.7%) determined a malignant bone tumor The patients comprised 40 (45.4%) males and 48 (54.5%) females with a mean age of 39.72±21.8 years, and a mean follow-up period of 51.6 (3-156) months.

Osteosarcoma was determined in 54 (61.3%) patients, followed by metastatic tumors in 22 (25%). Ewing sarcoma was observed in 6 (7.2%) patients and chondrosarcoma in 6 (7.2%). The tumor was localized in the distal femur in 66 (75%) patients, in the proximal tibia in 18 (20.4%), in the proximal fibula in 3 (3.4%), and in the patella in 1 (1.2%) (**Table 1**).

Osteosarcoma was determined in 54 (61.3%) patients, localized in the distal femur in 42 (64.6%), and in the proximal tibia in 12 (18.75%). Of the six patients with Ewing sarcoma, localization was in the distal femur in 5 (83.3%) and the proximal fibula in 1 (16.6%). Surgical treatment was applied to 52 patients with osteosarcoma, as extremity-preserving surgery for 49 and amputation for 3. The 5-year survival rate was determined as 63.7%. Chondrosarcoma was determined in 6 patients, localized in the distal femur in 5 (83.3%) and the proximal tibia in 1 (16.6%).

In 12 (18.2%) of the 66 patients treated and followed up for primary malignant bone tumor, metastasis was determined. Of these, metastasis was to the lungs only in 8 patients and to the lungs together with multiple metastases in 4.

Of the 88 malignant bone tumors determined, 22 (25%) were metastatic lesions, localized in the distal femur in 14 (63.6%), the proximal tibia in 5 (22.7%), the proximal fibula in 2 (9%), and in the patella in 1 (4.5%) (**Table 1**).

Table 1. Demographic characteristics

Parameters	n	%
Follow-up (months)	51.6 (3-156)	
Age (mean)	39.7±21.8	
<18	39	44.3
≥18	49	67.1
Sex		
Male	40	45.4
Female	48	54.5
Tumor types		
Osteosarcoma	54	61.3
Metastatic tumors	22	25
Ewing sarcoma	6	7.2
Chondrosarcoma	6	7.2
Localization		
Distal femur	66	75
Proximal tibia	18	20.4
Proximal fibula	3	3.4
Patella	1	1.2
Complaints on presentation		
Pain	67	76.1
Swelling	9	10.2
Pathologic fracture	12	13.6
Metastasis		
Yes	12	18.2
No	54	81.8

Of the 88 patients who presented with malignant bone tumors, extremity-preserving surgery was applied to 70 (79.5%), and amputation at various levels was performed on 14 (15.9%). Palliative radiotherapy was applied to two patients with metastatic lesions. Two (2.2%) patients were lost to mortality before surgical treatment could be applied.

The 5-year overall survival was 63.7%. The 5-year overall survival rates were 70.7% in the pediatric group and 57.9% in the adults (p=0.74). The 5-year survival rates of pediatric patients were 68.8% in males and 69.1% in females. In the adult group, it was found to be 46.6% and 61.2%, respectively(p=0.585). There was no statistical effect of metastasis development on overall survival in age groups(p=0.53) (**Table 2**).

Table 2. Evaluation of patient-related factors in terms of survival

Parameters	5-year overall survival	p value
Age group	63.7%	
Pediatric/adult	70.7% vs 57.9%	0.740
Sex		
Male (pediatric/adult)	68.8% vs 46.6%	
Female (pediatric/adult)	69.1% vs 61.2%,	0.585
Patients with metastases		
Pediatric/adult	67.1% vs 62.7%	0.531
Total Patient	63.7%	

DISCUSSION

Bone tumors are frequently seen in the knee region. In a study of 1925 patients in our clinic in 2014, of 687 bone tumors determined, 174 (25.3%) were in the knee region (6). In another study conducted in Turkey, Öztürk et al. (7) reported that of 1139 bone tumors determined, 246 (21.2%) were localized in the distal femur. The area of most involvement around the knee was the distal femur in the current study, consistent with the literature. In our study of 246 tumors, 162 (65.8%) were localized in the distal femur, 67 (27.2%) in the proximal tibia, 13 in the proximal fibula (5.2%), and 4 (1.6%) in the patella.

Pain is the most common reason for patients to present at the hospital. Swelling around the knee and restricted movement are frequently seen symptoms. Especially in patients of poor socioeconomic status, the reason for the first presentation at the hospital may be a pathological fracture. The incidence of pathological fracture development during diagnosis or while receiving chemotherapy has been reported to be 5-10% in the literature (6). Of the 88 patients in the current study, 67 (76.1%) presented with pain at the hospital. The complaint on presentation was swelling alone in 9 (10.2%) patients. The pathological fracture was seen in 12 (13.6%) patients. Of the 54 patients diagnosed with osteosarcoma, the pathological fracture was seen in 3 (5.5%), during diagnosis in 2, and while receiving neoadjuvant chemotherapy in 1. The incidence of pathological fracture was higher in patients with metastatic lesions. Of the 22 patients diagnosed with metastasis, nine presented at the hospital because of pathological fracture.

Osteosarcoma is the most frequently determined primary malignant bone tumor of all bone tumors(8). In a study by Bielack et al. (9) of 1702 patients with osteosarcoma, it was reported that 43% were localized in the distal femur and 23% in the proximal tibia. In the current study, osteosarcoma was the most common malignant tumor around the knee, determined in 54 (21.9%) of 246 patients. Consistent with findings in the literature, the most common site of localization in the current study was the distal femur (64.6%) (**Figure 2**).

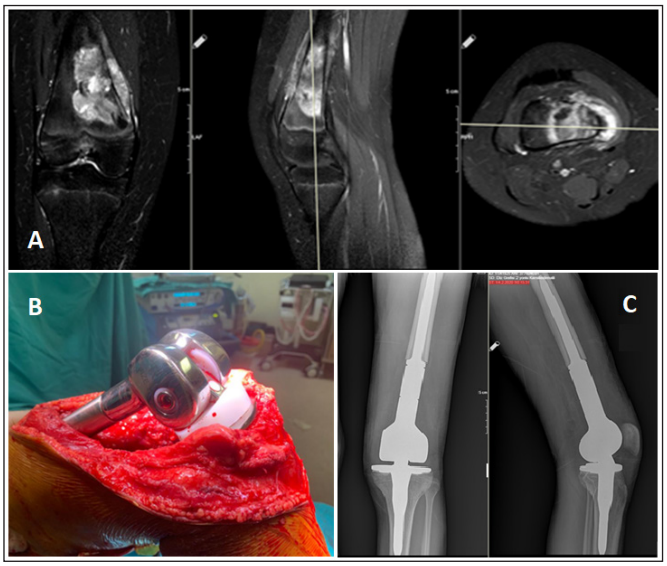

Figure 2. A) Images of osteosarcoma localized in the distal femur in different sections in MR T2 sequence B) Endoprosthetic prosthesis application after resection C) Postoperative AP and lateral radiographs

In a study by Marko et al. (10) of 9595 cases, metastasis was determined during the diagnosis of 18% of the patients with osteosarcoma. In the current study, there was seen to be metastasis in 4 (7.4%) patients during diagnosis, and of these, three were isolated lung metastasis. In the past, amputation was the gold standard in treating osteosarcoma. Still, in recent years, extremity-preserving surgery has come to the fore due to developments in chemotherapy, radiotherapy, and surgical techniques (11, 12). Simon et al. (13) evaluated 227 patients with sarcoma who were applied with extremity-preserving surgery or amputation and reported no difference in terms of survival of the patients. Two patients were lost to mortality in the current study while neoadjuvant chemotherapy was ongoing. The remaining 52 patients with osteosarcoma were treated surgically, with extremity-preserving surgery applied to 49 and amputation to 3 patients. In literature, the 5-year survival rate for patients aged <40 years with osteosarcoma has been reported in the range of 53%-71%(14, 15). In the current study, the 5-year survival rate was 71.4% in the pediatric age group and 62.5% in the adult group. Yao et al. (16) reported this rate as 57.66% in the pediatric age group, while Tina et al. (17) reported it as 86%.

Ewing sarcoma is rarely seen around the knee. It is determined more in the diaphysis of long bones and the metaphysic-diaphyseal junction (18, 19). It has been reported that 10% of Ewing sarcomas could be localized around the knee (20). Consistent with the literature, involvement around the knee was determined in 6 (10.9%) of the 55 patients determined with Ewing sarcoma in our clinic between 2004 and 2021. The localization was determined in the distal femur in 5 patients and the proximal fibula in 1.

In the current study, the 5-year survival rate was found to be 50%. In the literature, no study was found in terms of survival in Ewing sarcoma cases localized around the knee. This may be because this rare tumor often has diaphyseal involvement. However, since it is mostly seen in the pediatric age group, no comparison can be made with the adult group in terms of survival.

The worst prognosis criterion in Ewing sarcoma is metastasis at diagnosis. In a previous study, the 2-year disease-free survival rate was 32% in 39 patients with isolated lung metastasis and 20% in patients with widespread metastasis (21, 22). In the current study, there was metastasis during diagnosis in 2 patients with tumor localization in the distal femur. High femoral amputation was performed in 1 of these patients, followed by chemotherapy and radiotherapy.

The other primary malignant bone tumor localized around the knee that was determined in the current study was chondrosarcoma, localized in the distal femur in 5 patients and the proximal tibia in 1. There was seen to be lung metastasis in 1 patient during diagnosis. In 1 patient, there was involvement in the ipsilateral trochanter major together with lung metastasis. Hip disarticulation was applied to that patient and femoral amputation to the other five patients. In the current study, the 5-year survival rate was 33% in adult patients and 100% in the pediatric group. In the literature, we could not find any study in terms of the survival of chondrosarcoma localized around the knee.

After the liver and lungs, the bones are the 3rd most common region where metastasis is determined (23). Metastising tumors are usually lung, breast, prostate, kidney, and thyroid tumors (24). Of the bone lesions determined in the current study, 22 (25%) were seen to be metastatic lesions, of which 19 were metastasis from organs, and three were determined to be malignant mesenchymal tumor metastasis in adjacent tissue. There was metastasis in the distal femur in 14 patients, the proximal tibia in 5, the proximal fibula in 2, and the patella in 1 patient. A total of 9 patients presented at the hospital because of pathological fracture, and the most common reason for presentation in the other patients was pain.

Metastasis related to lung cancer was determined most often. Of the 9 cases of lung metastasis, involvement was in the distal femur in 7 and the proximal tibia in 2. Two patients presented at the hospital because of pathological fractures. Metastasis related to breast cancer was determined in 8 patients. Of these, involvement was in the distal femur in 7 and the patella in 1 and 3 patients presented with pathological fracture. Renal cell carcinoma metastasis was determined in 4 patients, all of which had a lesion in the distal femur, and all presented

at the hospital with a pathological fracture. Fracture fixation was applied to 3 patients, and in 1 patient, there was involvement in the ipsilateral femoral trochanteric region, so hip disarticulation was applied. Metastasis related to prostate cancer was determined in the distal femur of 1 patient. Surgical treatment was not applied to patients with multiple metastases.

It is known that the development of metastases has a negative effect on survival rates in malignant bone tumors. In our study, no statistical effect of metastasis development on overall survival was found in age groups ($p<0.05$). We think that this result may have been caused by the heterogeneity between the patient groups.

There were some limitations to this study, primarily the retrospective design. The data used were patients discussed in the tumor council, so the rates determined do not reflect the frequency of tumors seen in the general population. As this study was specific to a certain body area, some tumors such as chondrosarcoma and Ewing sarcoma were determined at low numbers. However, the fact that the groups are not homogeneous and the treatment methods are not standardized is an important limitation of the study.

CONCLUSION

The knee is a region that requires attention regarding bone tumors around it. As malignant bone tumors are rarely seen around the knee, a misdiagnosis may be made, and appropriate treatment may be delayed. The tumor grade is critical in the treatment and survival of patients. Early diagnosis and treatment can be life-saving. Tumors may be localized around the knee in all age groups, but primary malignant tumors, in particular, are seen more often in the first two decades of life. Patients' active and healthy appearance in this age group may be misleading for clinicians. Although the first diagnosis to come to mind for patients presenting with knee pain is trauma and growing pains, it must not be forgotten that a tumor could be the cause.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Ondokuz Mayıs University/ Training and Research Hospital, Noninvasive Clinical Ethics Committee (Decision No: 2022/178).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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Original Article

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The effect of Trendelenburg position on outcomes of retrograde intrarenal surgery for medium sized renal pelvis stones

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ABSTRACT

Aim: To compare safety and efficiency between Trendelenburg position retrograde intrarenal surgery (tRIRS) and conventional position retrograde intrarenal surgery (cRIRS) in the management of renal pelvis stones 10-20 mm in size.

Material and Method: From September 2018 to September 2019, the patients undergoing RIRS for single renal stones between 10-20 mm were included in the study prospectively. Patients were divided into two groups randomly. First group of patients were positioned completely parallel to the ground (cRIRS), second group were positioned with Trendelenburg (tRIRS). Success was evaluated at end of 3rd months by non-contrast enhanced tomography. Stones that smaller than 4 mm were accepted as clinical insignificant residual fragment. Complications was classified according to Clavien, class 2 or more complications were recorded.

Results: Totally 100 patients were included to final analyze. Patients' age, gender, stone side and mean stone surface area were similar between groups. Success rate was higher in tRIRS group (90% vs 72% p=0.022). Mean operation time was lower (41.8 vs 58.2 min. p<0.001), and mean session number for each patient was lower in tRIRS group (1.17 vs 1.4 p=0.024). Class 2 or higher complications were occurred in six patients; five was in cRIRS, and one in tRIRS group and rate was similar (p= 0.09).

Conclusions: Inclined Trendelenburg position improves success rate and decrease mean session number and operation time on patients whom performed RIRS for renal pelvis stones. Trendelenburg position has similar complication rate compared to conventional position.

Keywords: Retrograde intrarenal surgery, flexible ureteroscopy, Trendelenburg position, renal pelvis stone

INTRODUCTION

Urinary stone disease is a common world health problem causing significant patient morbidity with serious socioeconomic consequences (1). Over last few decades, progress in biomedical technology have enabled urologists to better treat urolithiasis with few complications. Treatment of renal stones has undergone changes during this period with replacement of open surgery by minimally invasive interventions such as shock wave lithotripsy (SWL), percutaneous nephrolithotomy and retrograde intrarenal surgery (RIRS) which are widely accepted as standard treatment modalities for kidney stones less than 2 cm in diameter (2). The decision among these modalities for 1-2 cm renal stones depends on patient and/or urologist. The definitive treatment is chosen according to patient's compliance and comorbidities, treatment costs, available equipment, complications of the treatment, stone clearance time, and need of auxiliary procedures (3). RIRS, also popular as flexible ureterorenoscopy (FURS), is a less invasive modality with fewer complications. FURS has shown its superiority to SWL in the management of renal stones smaller than 2 cm in kidney and even in patients with complex renal anatomy or using anticoagulants with better stone clearance (4). Stone-free rates (SFRs) up to 90% are provided by RIRS carried out by FURS (5). Investigators tried to increase SFRs in renal and ureteral stones with some auxiliary maneuvers, such as inverted position during SWL session or ureterorenoscopic lithotripsy (6-8). The previous information suggested that, inclining patients in Trendelenburg position could theoretically promote the migration of stone into the

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upper collecting system, or at least away from the lower pole calices, which provides FURS with convenience to treat the fragments remaining in the upper collecting system (9). Such an approach may potentially decrease the operative time and increase SFRs. To the best of our knowledge, no comparative study between RIRS in Trendelenburg and RIRS in plain lithotomy positions for renal pelvis calculi has been published. Herein, we present a prospective, randomized study comparing the safety and clinical value of cRIRS and tRIRS in the treatment of single 1-2 cm renal pelvis stone.

MATERIAL AND METHOD

The study was carried out with the permission of Sancaktepe Prof. Dr. İlhan Varank Training and Research Hospital Scientific Researches Ethics Committee (Date: 10.02.2021, Decision No: 2021-98-24.02.2021). All patients were informed about the objectives of the study in detail and gave written informed consent. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki.

From September 2018 to September 2019, patients with a single stone between 10-20 mm in renal pelvis and planned for RIRS at our institution were enrolled in the study. Those with stones other than renal pelvis, undergoing prior stone surgery, or extracorporeal shock-wave lithotripsy (SWL), retrograde intrarenal surgery (RIRS) for significant residual stone, children, patients with comorbidities such as diabetes, hypertension, and ischemic heart disease and patients on anticoagulants, with prior nephrostomy or double-j stent due to infection, serum creatinine level >1.5 mg/ dL or coexisting ipsilateral upper urinary tract pathologies were excluded from the study. The eligible patients were randomized into conventional (cRIRS) group and Trendelenburg (tRIRS) group. Random numbers were generated by computer and assigned to consecutive patients. Age, gender, stone side and surface area, operation time, number of sessions, success and complications were recorded.

Surgical Technique

Urine culture was taken in addition to routine laboratory examinations, the operation was performed when urine culture became negative. Preoperative 1 g ceftriaxone was administered intravenously. Patients in tRIRS arm were inclined to Trendelenburg lithotomy position with head down 300 under general anesthesia. All patients were attempted to insert access sheath. Access was attempted through the guide wire with reusable FURS (Flex X2™,Karl Storz®,Germany) even if the access sheath could not be placed. Zebra guide wire™ (Boston Scientific®, USA) was inserted into ureter with FURS, and intramural ureter was dilated with FURS up to the mid ureter, then the access sheath was tried over the guide wire. Access sheath of Flexor-Regular (R; 9.5/11.5F, Cook Medical, USA) were used in reusable FURS. The Quanta LithoTM holmium laser was applied as an energy source set at 0.8–1.0 J and a rate of 6–10 Hz. DJ stent was inserted to all patients at the end of first procedure. All operations were carried out by a single urologist experienced in flexible ureteroscopy.

Evaluation of Outcomes and Complications

Each RIRS operation for same patient was considered as a separate session; removal of DJ stents was not counted as a session. Operation time involved the duration from insertion of FURS through urethral meatus up to DJ stent placement. All operations were carried out by a single urologist experienced in flexible ureteroscopy. Complications were classified according to Clavien-Dindo system, grade 2 and above were recorded. Complete SFR status or clinically insignificant stones (≤ 4 mm) in non-contrast computed tomography three months after the last operation was considered successful.

Statistical Analysis

Chi-square and Mann Whitney U tests were used for statistical analysis and p value< 0.05 was considered significant. Continuous variables with normal distribution were expressed as the mean±SD and compared with Student's t test. All analyses were performed using the Statistical Package for the Social Sciences (SPSS for Windows, version 17.0., IBM Inc., Chicago, USA).

RESULTS

One hundred and ten patients complied with the inclusion criteria of this study, 55 cases were assigned to cRIRS group, whereas 55 cases were assigned to tRIRS group. In 10 patients (5 in cRIRS group and 5 in tRIRS group), a primary insertion of FURS was failed. Thus, hundred patients (50 in cRIRS group and 50 in tRIRS group) were finally analyzed in this study. The demographic data and the clinical features of the patients were listed in **Table 1**.

Table 1. Patients' characteristics in cRIRS(conventional retrograde intrarenal surgery) group and tRIRS (Trendelenburg position retrograde intrarenal surgery) group.

	cRIRS	tRIRS	P value
Patient number (n)	50	50	
Mean age (year)±sd	49.1±13.3	48.3±12.6	0.764
Gender (m/f)	31/19	20/20	0.838
Side (R/L)	26/24	27/23	0.841
Mean stone surface area (mm2)±sd	134±65.3	130±59	0.739

No statistical difference was found in the patient's characteristics between the two groups, in terms of age, gender, as well as stone side and stone surface area (**Table 1**). The clinical outcomes of the two groups were compared in **Table 2**.

Table 2. Operative and clinical outcomes in cRIRS group and tRIRS group

	cRIRS	tRIRS	P value
Access sheath inserted (%)	41 (82%)	39 (78%)	0.617
Mean session number±sd	1.4±0.64	1.17±0.37	0.024
Mean operation time (min)±sd	58.2±15.6	41.8±11.9	<0.001
SFR (%)	36 (72%)	45 (90%)	0.022
Complication (%)	5 (10%)	1 (2%)	0.090

The mean operative time was significantly prolonged in cRIRS group than in tRIRS group (58.2±15.6 min., 41.8±11.9 min., p<0.001), while the SFR at 4 weeks was significantly higher in tRIRS group than in cRIRS group (93.2 vs. 98.3%, p< 0.001). The mean operative session number was significantly higher in cRIRS group than in tRIRS group (1.4±0.64 vs1.17±0.37, p=0.024) SFRs in cRIRS and tRIRS were 36 (72%) and 45 (90%) respectively (p=0.022). Class 2 or higher complications occurred in 5 patients in cRIRS, and one in tRIRS group requiring termination of the operation which were ureteral injury in four patients, and bleeding in two. Two groups are similar in terms of complication seen rate (p= 0.09). These six patients had no additional intervention except for DJ stent placement and the stones were removed in the second session.

DISCUSSION

The reported SFRs of RIRS in the literature varies between 54%-96% for renal stones smaller than 2 cm after one session regardless of their location (10). Our overall stone-free rate was %81 and was similar to literature. Location of the stone in renal collecting has been reported to be have impact on RIRS outcomes. Lower pole stones were reported to be a predictive factor for SFR at RIRS (11). In the series of Lim et al. (12) SFRs of RIRS in stones in the lower calyx was 73.3% which was lower than SFR of stones in upper and middle calyx or renal pelvis (94.4%) . Even with the most up-to-date flexible ureteroscope, the initial SFR was found to be 64.9 %, the retreatment rate was 16.2 % and the auxiliary procedure rate 21.6 % for the lower calyceal stones (13). Hence, before considering RIRS for renal pelvis stones, it is important to prevent the calculus or fragments from migrating to the lower calices than other part of the renal collecting system. During RIRS, gravity force tends to drive stone fragments in the lower calyx having a reverse infundibulopelvic angle. Previous studies investigating inclined positioning of patients during treatment with SWL and semirigid ureterorenoscopy suggested better outcomes for renal stones. Leong et al. (14) reported that simultaneous Trendelenburg position in SWL assured 1.28 times improvement in SFR with no or minimal additional costs. In the study of Pan et al. (7) authors claimed that semirigid URS in Trendelenburg position (tURS) rendered higher SFR and less operative time compared to conventional position URS (cURS) in upper ureteral stones. In case of retropulsion, surgeons were able to follow the stones or fragments by semirigid URS up to the renal collecting system, and then, a lithotripsy was performed in renal pelvis, middle calices or even in upper calices (7). Moreover, the requirement of FURS was mostly for lower calyceal stones which could potentially reduce the medical cost. Based on these results, we placed the patients in a Trendelenburg position during RIRS in order to promote stone fragments proximally, away from the lower calices, which might decrease the total operative time and improve the SFRs. Stone fragments migrating in the upper calices or remaining in renal pelvis would be certainly easier to treat by a FURS. In our study, we found that mean operation time (58.2±15.6 min., 41.8±11.9 min., p<0.001), and mean session number (1.4±0.64 vs1.17±0.37, p=0.024) were lower in tRIRS group compared to cRIRS group. This might be related to more fragments migration into lower calices. We postulate that fragments escaping to lower calyces create unfavorable conditions on SFR outcomes in three ways; First, limited maneuverability of FURS with laser fiber or basket catheter in its working channel at downward hyperflexion, second, poor visibility caused both by blurred fluid by stone dust and hemorrhage hardly circulated through infundibulum of lower calyx and obliterated FURS working channel and thirdly, fragments and debris remaining in the upper calyces, middle calyces and renal pelvis after any lithotripsy method may spontaneously pass down through ureter, which is improbable for those in lower calyces. In our study, SFR status was defined as absence of stone or clinically insignificant stones (≤ 4 mm) in non-contrast computed tomography (NCCT) three months after the last operation in contrast to most studies where imaging is performed to define clearance at postoperative 4-6 weeks (15, 16). Only a few studies suggested the timing of control imaging at 60-90 days. However, the precise timing of postoperative imaging for SFR status control is not established yet, according to recent data, early control imaging is useful for assessment of complications such as hydronephrosis, hematoma or pyelonephritis but may show some residual fragments that could be cleared spontaneously within three months following RIRS (17,18). Therefore, early control imaging risks to display lower SFR and mislead physicians to overtreatment. In our study, we performed control scans three months after of the procedure.

Our study has also some limitations. First, it is based on a limited number of patients, secondly, it is an unblinded study, the surgeons were aware of patients' position at operation theater. Thirdly, factors like hydronephrosis, infundibular angle, the stone composition or density which might influence the duration of RIRS and SFRs were not evaluated.

CONCLUSION

As suggested in various upper urinary lithotripsy series, the Trendelenburg position can improve the SFRs and may be considered as an auxiliary method in the treatment of renal stones with RIRS as well. The present comparative study showed that tRIRS was safe and efficient for the management of medium sized renal pelvis stones, with lower complication rates, it rendered higher SFRs and less operative time compared with cRIRS. Moreover, avoiding secondary RIRS and/or SWL in tRIRS could potentially reduce the patient burden and medical cost.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Sancaktepe Prof. Dr. İlhan Varank Training and Research Hospital Scientific Researches Ethics Committee (Date: 10.02.2021, Decision No: 2021-98-24.02.2021).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

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Author Contributions: The author declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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Assessment of posterior tilting of the hyoid bone in relation to carotid atherosclerosis: A CBCT study

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ABSTRACT

Aim: The present study aimed to investigate whether the presence and areal and volumetric measurements of the unilateral extra-cranial carotid artery calcifications (ECACs) are associated with posterior tilting of the hyoid bone.

Material and Method: A total of 658 cone-beam computed tomography (CBCT) scans were screened for the presence of ECACs. The calcifications were categorized as unilateral (right or left) or bilateral. Study group was consisted of cases with unilateral ECACs. A control group without ECACs matching with study group by age and gender was created. Volumetric and areal measurements in the ECAC group were done by using Mimics Medical software. Posterior tilting of the hyoid bone in relation to mid-sagittal plane and the dimension of posterior inclination through the greater horns were measured on i-Cat Vision software.

Results: In total, 71 (10.8%) ECACs (30 bilateral and 41 unilateral) were detected. Study group consisted of 41 (6.2%) unilateral ECAC cases [25 (61%) females and 16 (39%) males]. Gender and age distributions were similar between ECAC and control groups. No significant difference between two groups was found considering the prevalence of posterior tilting of the hyoid bone (63.4% vs. 43.9%, p=0.240). Similarly, there was no significant difference in the mean dimension of posterior inclination between groups (2.48±2.12 mm. vs. 2.24±1.47 mm, p=0.646). The volume and areal measurements of calcifications were not correlated with the dimension of posterior inclination of the hyoid bone.

Conclusion: Posterior tilting of the hyoid bone may be a frequent finding in cases of unilateral ECAC. However, the present findings suggest that no significant relationship exists between the presence of unilateral ECACs and posterior tilting of the hyoid bone.

Keywords: Carotid artery, cone-beam computed tomography, hyoid bone, vascular calcification

INTRODUCTION

The calcified carotid artery atheroma is of great importance as it represents an increased risk of stroke (cerebrovascular accident - CVA) which is a major global cause of morbidity and mortality (1). Regarding the evaluation of the patients with carotid artery calcifications (CACs), there are a number imaging modalities including ultrasonography, magnetic resonance imaging, computed tomography (CT), and angiography (2).

Calcifications in the cervical region are named as extra-cranial CACs (ECACs). In dentistry, there has been a growing interest in detecting calcifications in this region on maxillofacial images since the visualization of the lesions on panoramic radiographs firstly reported in the literature in 1981 (3). More recently, the ability of cone-beam CT (CBCT) imaging to show the presence, course, and the severity of the CACs have been described in various studies (4-8).

Cerebrovascular accidents correlated with mechanical interference between cervical structures and carotid arteries have been reported in the literature (9-11). There also have been some papers describing the impingement or compression of the internal carotid arteries by hyoid bone and trauma caused by the greater horn of the hyoid bone resulting in stenosis or traumatic pseudoaneurysm of the carotid arteries (12-14). In a recent report, a case of hyoid bone compressing the carotid artery with its right greater horn tilted posteriorly has been described (15).

Based on previous findings, it was suggested that anomalous position of the hyoid bone can provoke the formation of local calcification in the cervical region (9, 15) and may be a frequent finding in cases of ECACs. The present study aimed to investigate whether the presence and areal and volumetric measurements of the unilateral ECACs are associated with posterior tilting of the hyoid bone.

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MATERIAL AND METHOD

This retrospective study was approved by the Research Ethics Board of Hacettepe University in Ankara, Turkey (Date: 15/10/2019, Decision No: 2019/24-28). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

CBCT images obtained between January and June 2019 were retrieved from the imaging database of Department of Dentomaxillofacial Radiology. All examinations were belonged to patients 40 years of age or older (4, 7) and requested for different diagnostic tasks such as evaluation of trauma, complications of dental and maxillofacial pathologies, and implant treatment planning. The initial sample consisted of 1264 images. The scans including C2 to C4 vertebral levels were screened (16). Following the exclusion of the scans with inadequate field of view (n=570) and motion or metal artefacts (n=36), the final sample of 658 images were obtained and selected for evaluation.

CBCT examinations were obtained with an i-Cat Next Generation device (Imaging Sciences International, Hatfield, PA, USA). The imaging protocol was: 120 kVp, 3–8 mA, 16x13 cm field-of-view, 0.20-mm voxel size, and 26.9-s scan time. The scans were imported into i-CAT Vision software and were viewed on a 24-inch LCD monitor.

All examinations were conducted by an oral and maxillofacial radiologist (N.K.) with at least 7 years of experience in CBCT imaging. Each CBCT data set was evaluated regarding the presence of any calcification. The examiner was blinded to the patient's medical and dental history and other clinical and radiographic findings. However, the age and gender of the patient was accessible.

The image analysis was performed by evaluating the axial, coronal, and sagittal multiplanar reconstructions (MPR) and the ECACs were identified as described previously in the literature (4,17,18): in axial sections, calcifications located lateral to the anterior tubercle of the transverse processes; in coronal sections, lateral to the anterior tubercle of the cervical vertebrae; in sagittal sections, medial and inferior to the angle of the mandible. The slice thickness was set at 0.2 mm. The presence of calcification was confirmed when it was detected in at least three sequential slices. The calcifications were categorized as unilateral (right or left) or bilateral.

A total of 71 (10.8%) ECACs (30 bilateral and 41 unilateral) were detected. Of these unilateral ECAC cases (n=41, 6.2%) were included in the study group. A control group (n=41) was created by matching records without ECACs by age and gender, which was selected from the final sample of 658 images.

CBCT data were exported as DICOM files for unilateral ECAC samples. The volumetric and cross-sectional area (CSA) measurements were conducted by using Mimics Medical (Materialise, Leuven, Belgium) which is a commercial software capable of segmentation process for volumetric measurements. The total volume (in cubic mm) and CSA (in square mm) measurements of the calcification were automatically calculated by the software (**Figure 1**).

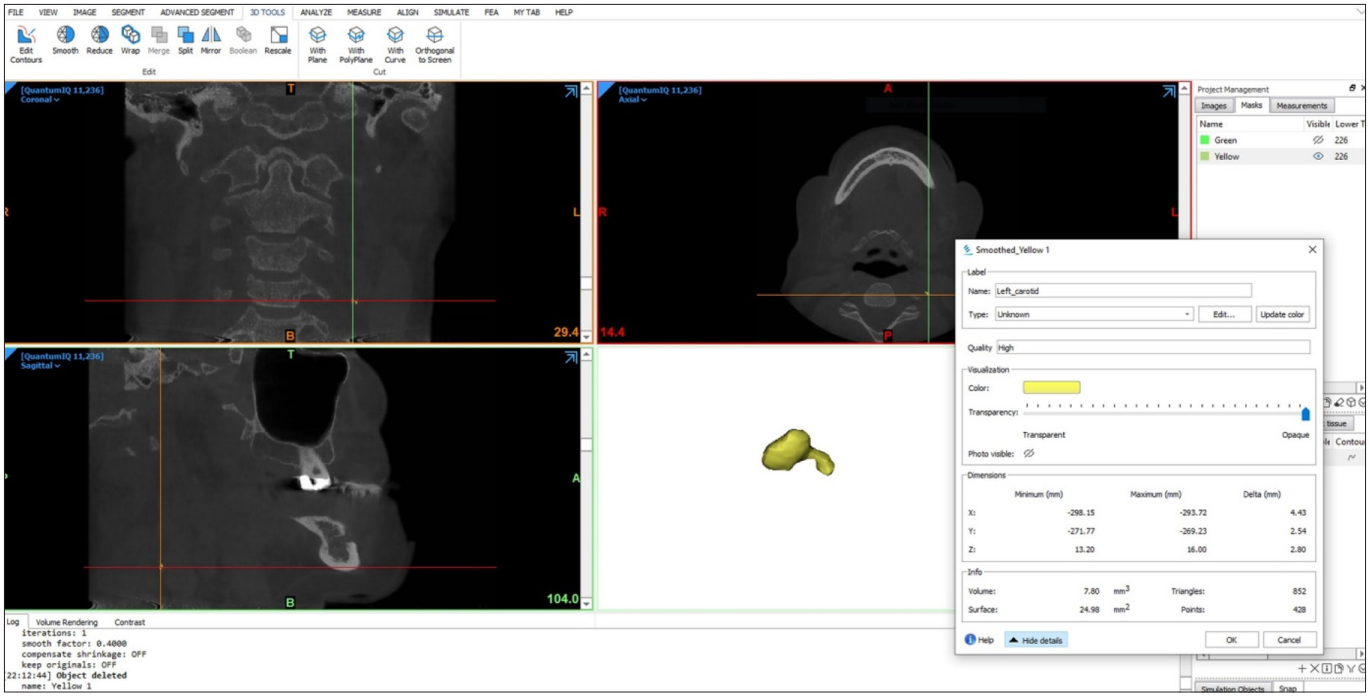

Figure 1. An example of volumetric measurement of the left extra-cranial carotid artery calcification on the Mimics software.

Measurements related to the posterior tilting of the hyoid bone were done on MPR sections of the i-Cat Vision software for unilateral ECAC group and control group. The reference points and planes were defined as follows:

- Mid-sagittal plane: Sagittal plane crossing the tip of anterior nasal spine and the posterior midpoint of the vertebral spine (**Figure 2a**) (19).
- Mid-hyoid bone: The median part of the hyoid body (**Figure 2b**).
- HLp: The most posterior point of the hyoid bone left greater horn (**Figure 2c**).
- HRp: The most posterior point of the hyoid bone right greater horn (**Figure 2c**).
- C2sp: The most superior and posterior point on the body of the second cervical vertebra (C2).
- C4ip: The most inferior and posterior point on the body of the fourth cervical vertebra (C4).
- C2sp – C4ip: Post-vertebral line (Pvl) (20, 21) (**Figure 2d**).
- HLp – Pvl: Perpendicular distance in mm between HLp to Pvl.
- HRp – Pvl: Perpendicular distance in mm between HRp to Pvl (**Figure 2e**).

The angulation between reference mid-sagittal plane and a line intersecting cervical vertabra body and the most anterior aspect of the mid-hyoid bone was measured on axial CBCT sections (**Figure 2b**). A positive degree of angulation was defined as posterior tilting of the hyoid bone on the left side. A negative degree of angulation was defined as posterior tilting on the right side. The difference between HLp – Pvl and HRp – Pvl expressed in millimeters was defined as the degree dimension of posterior tilting inclination of hyoid bone through its greater horns.

Before the analysis, head orientation was corrected on MPR sections (22): the hard palate was aligned with the horizontal plane on the mid-sagittal view, lower border of the left and right orbital ridges were aligned on the coronal view, and the left and right zygomatic arches were evaluated on the axial view.

Prior to all analysis, the examiner was calibrated with the use of 30 CBCT scans from the sample of bilateral ECACs. All measurements were done twice within an interval of 15 days.

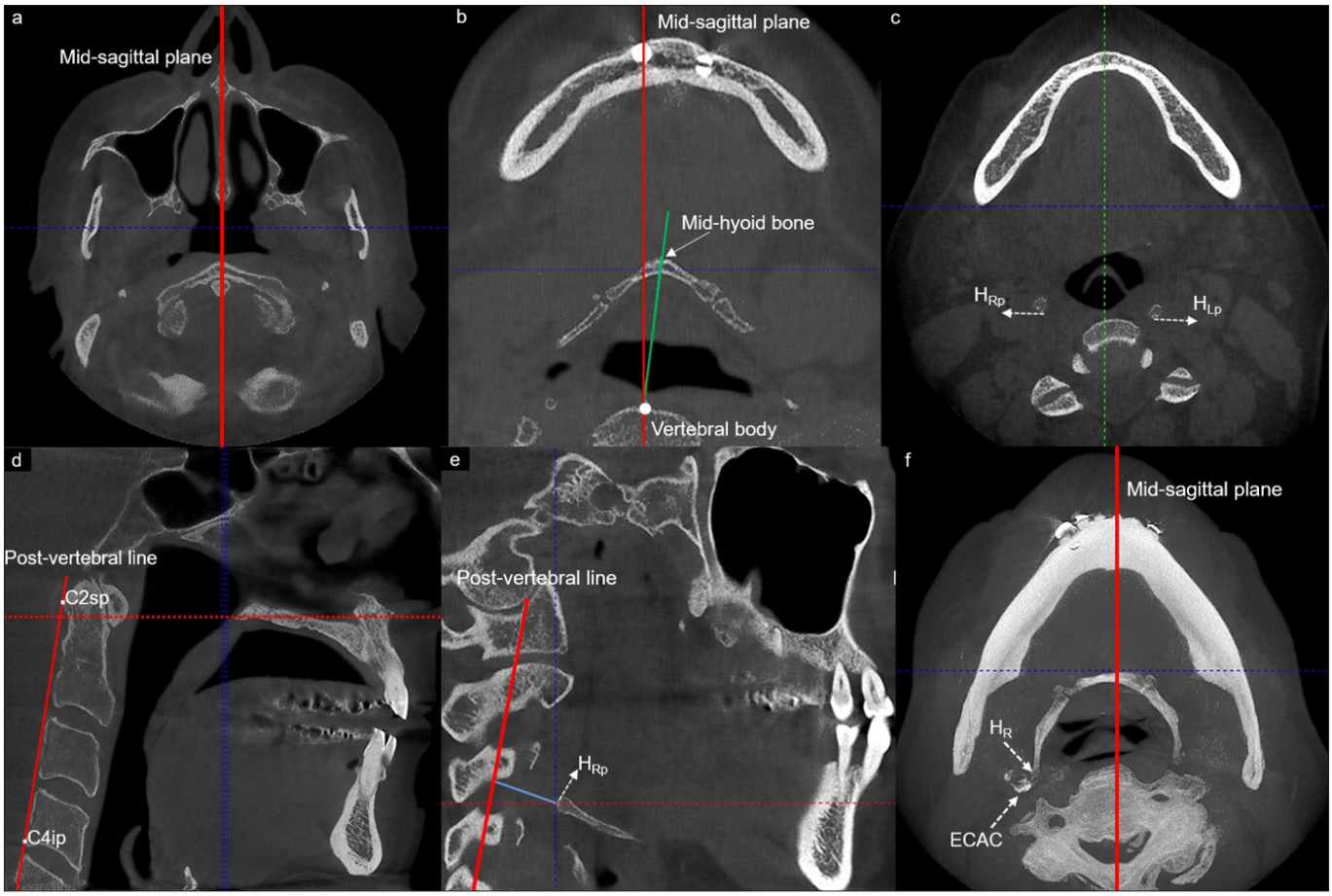

Figure 2. Axial cone-beam computed tomography (CBCT) images depicting mid-sagittal plane (a), assessment of posterior tilting of hyoid bone (b), and most posterior points of the hyoid bone left greater horn (HLp) and the right greater horn (HRp). Sagittal CBCT images showing post-vertebral line (C2sp – C4ip) (d) and the perpendicular distance from the most posterior point of the hyoid bone right greater horn (HRp) to the post-vertebral line (e). Maximum Intensity Projection (MIP) axial view (f) showing extra-cranial carotid artery calcification (ECAC) (17), hyoid bone right greater horn (HR), and the posterior tilting of the hyoid bone on right side in reference to mid-sagittal plane.

Statistical Analysis

Statistical analysis was performed using MedCalc Statistical Software version 20.018 (MedCalc Software Ltd, Ostend, Belgium). Group differences were analyzed using Mann–Whitney U test or t-test. Pearson's Chi-Square test was used to examine relationships between categorical variables. The Spearman's correlation coefficient was used to evaluate the relationship between the dimension of posterior inclination of hyoid bone and areal and volumetric measurements. Intraclass correlation coefficient (ICC) was used to evaluate the intra-observer reliability. A value of $p<0.05$ was considered statistically significant.

RESULTS

The ICC was above 0.75 for all measurements. The study group was consisted of 41 (6.2%) patients with unilateral ECACs, 25 (61%) were females and 16 (39%) were males. The control group (n=41) was matched by gender distribution. There was no significant difference between the mean ages of the ECAC group (62.97±8.72 years) and the control group (65.14±6.48), ranging from 46 to 83 years (p=0.205).

Among ECAC group, the overall mean volume was 10.52±13.91 mm³ (ranging between 0.18 and 51.65 mm³), while the overall mean CSA was 29.45±30.67 mm² (ranging between 1.90 and 117.32 mm²).

Posterior tilting of the hyoid bone on calcification side (**Figure 2f**) was detected in 26 cases (63.4%) in ECAC group and in 18 cases (43.9%) in the control group. In 15 cases (36.6%) among the ECAC group, either tilting of the hyoid bone towards the unaffected side or no posterior tilting was detected. The number of cases without posterior tilting of the hyoid bone was 23 (56.1%) in the control group. There was no significant difference between the groups regarding the prevalence of posterior tilting of the hyoid bone (**Table 1**, p=0.240).

The mean dimension of posterior inclination of the hyoid bone was 2.48±2.12 mm in ECAC group, whereas that was 2.24±1.47 mm in the control group (p=0.646).

Table 1. Distribution (right, left, and absent) and frequency of posterior tilting of hyoid bone according to case and control groups.

Control Group	Case Group			Total n (%)	P value
	Right n (%)	Left n (%)	Absent n (%)		
Right	6 (14.6)	4 (9.8)	3 (7.3)	13 (31.7)	0.240*
Left	1 (2.4)	0 (0.0)	4 (9.8)	5 (12.2)	
Absent	8 (19.5)	7 (17.1)	8 (19.5)	23 (56.1)	
Total	15 (36.5)	11 (26.9)	15 (36.6)	41 (100.0)	

*Pearson's Chi-Square test

The Spearman's correlation coefficient showed no significant correlation between dimension of posterior inclination of the hyoid bone and volume and CSA measurements of the ECACs (**Table 2**).

Table 2. Spearman's correlation coefficient for dimension of posterior inclination of hyoid bone and volume and cross-sectional area measurements of the extra-cranial carotid artery calcifications

	Dimension of posterior inclination of hyoid bone	
	r	p value
Calcification volume (mm³)	0.13	0.51
Cross-sectional area (mm²)	0.10	0.60

DISCUSSION

The present study was designed to determine a possible association between the occurrence of ECACs and anomalous position of the hyoid bone by means of posterior tilting of the greater horns. Such association could be established based on the previous findings which suggest adjacent bony structures in the cervical region may interfere with carotid arteries (12-15).

The hyoid bone with its body and greater and lesser horns, is often an overlooked small anatomical structure, although it gives attachment to several muscles and ligaments of the neck and plays a vital role in the craniomandibular functions (23). Hyoid bone position has been frequently studied in relation to oropharyngeal space dimensions (24), facial growth patterns (25), craniofacial anomalies (20), obstructive sleep apnea syndrome (26), myofascial pain (27), atypical deglutition (28), and temporomandibular joint disc displacement (29). The hyoid position in relation to carotid artery diseases as its proximity to the carotid bifurcation level is a rarely studied phenomenon, although there are several case reports describing CVAs due to unilateral compression of the hyoid bone through its greater horn (9,11,12,23,30). Of these, a recent case report has also demonstrated a dynamic evidence for the compression of internal carotid artery (ICA) by the greater horn of the hyoid bone during swallowing (31). A common conclusion based on these reports suggests that a protruding hyoid greater horn may cause repetitive traumatic forces that induce changes in the artery wall. As a result of this, disturbed blood flow and high wall shear stress may lead to the formation of unilateral atherosclerosis (9). The present study is motivated by these studies and particularly focused on the unilateral CACs. To the best of the author's knowledge, this study is first to evaluate proximity of the hyoid bone greater horns to the cervical region by considering the posterior tilting of hyoid bone in terms of its divergence to mid-sagittal plane. Posterior tilting of the hyoid bone was observed

with more than half cases (63.4%) in ECAC group. The prevalence of posterior tilting of hyoid bone in control group was lower (43.9%) than study group. However, the difference was not statistically significant.

The reference points used for the evaluation of dimension of posterior inclination of hyoid bone in the present study were the most posterior point of the greater horn of the hyoid bone and a line connecting the most posterior points of the C2–C4 vertebra bodies (Post-vertebral line-Pvl). The difference between measured dimensions of each greater horn to Pvl indicated a degree of proximity of hyoid bone to cervical region on related side. The mean dimension of posterior inclination of hyoid bone in the present study was similar in ECAC and control groups. Moreover, the volume and CSA measurements of the calcifications were not correlated with posterior tilting of the hyoid bone, which means neither the volume or dimension of the calcification was increased with the dimension of posterior inclination of hyoid bone. The present findings suggest that there is no significant relationship between the presence of unilateral ECACs and the prevalence of posterior tilting of hyoid bone. This is also consistent with a recent study by Siegler et al. (32), which investigated the proximity of the hyoid bone greater horn to the ICA on CT angiography.

The prevalence of unilateral and bilateral ECACs in this study (n=71, 10.8%) was consistent with the reported range (2.8-17.56%) in CBCT studies (33-36) in the literature but was lower than in some previous studies (4,7), which reported a considerably higher prevalence rate up to 42.88%. This might be attributed to sample selection criteria and population characteristics.

This study has some limitations. The first one is that the sample size was relatively small, which might not be sufficient to establish significant associations. Secondly, a longitudinal cohort may determine if there is a causal relationship between hyoid tilting and ECACs. Third, other adjacent structures, such as styloid processes were not taken into consideration. Fourthly, anatomic variations in the carotid vessels, the course of the carotid artery, and the degree of stenosis could not be evaluated on CBCT. Finally, inter-observer agreement for measurements were not calculated.

In conclusion, the present study is the first to investigate the relationship between the presence of unilateral ECACs and the prevalence of posterior tilting of the hyoid bone and suggests no significant relationship exists between these two entities, although posterior tilting of the hyoid bone may be a frequent finding in cases of unilateral ECACs.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was approved by the Non-Interventional Clinical Research Ethics Board of the Hacettepe University (Date: 15/10/2019, Decision No: 2019/24-28).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

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Author Contributions: The author declares that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version

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The relationship between pulmonary artery obstruction index and troponin in thorax computed tomography in pulmonary embolism

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ABSTRACT

Aim: To study the relation between troponin and pulmonary artery obstruction index in thoracic computerized tomography in patients diagnosed with acute pulmonary embolism.

Material and Method: Data obtained from patients hospitalized in the ward and intensive care units with a pulmonary embolism diagnosis between January 2016 and February 2022 were scanned retrospectively. The full blood count, D-dimer, C-reactive protein, procalcitonin, troponin I, thoracic computerized tomography (CT), angiography, and bilateral lower extremity venous Doppler ultrasonography data were extracted. Patients with left heart failure, renal failure, gastrointestinal hemorrhage, sepsis, respiratory system disease, burns, ischemic stroke, or subarachnoid hemorrhage were excluded. The obstruction indices were calculated according to storage defects in the main, right, left, lobar, and segmental pulmonary artery branches in CT angiography.

Results: While 57.0% of the 69 patients included in the study were female, 42.1% were male. The obstruction index in the high troponin-I group was significantly higher than that in the normal troponin-I group (p=0.006). In addition, the obstruction index was significantly higher in patients with bilateral pulmonary embolism than in those with unilateral pulmonary embolism (<0.001). While there was a positive correlation between obstruction index and D-dimer (r=0.310 p=0.041), a significant negative correlation was found with the CRP value (r=-0.268 p=0.042). When the clinical and laboratory data of the patients with normal and high level troponin were examined, the ratios of patients in the ICU and with bilateral embolism were significantly higher in the high troponin group and the CRP value was significantly lower (p=0.004, 0.027, 0.003, respectively).

Conclusion: The pulmonary artery obstruction index shows thrombus load and is a parameter related to troponin.

Keywords: Pulmonary embolism, obstruction index, troponin I

INTRODUCTION

Pulmonary embolism is a disease that occurs as a result of occlusion of the pulmonary artery and/or its branches by fragments of thrombi formed in the deep veins of the legs. It is a disease of advanced age, observed on average among patients aged 50 years and over. With the placement of thrombi in the pulmonary arteries, a series of pathophysiological events occur and clinical findings emerge as a result. The patient's cardiopulmonary reserve, the diameter and number of occluded vessels, the size of the thrombus, reflex vasoconstriction due to pulmonary artery dilatation, and released inflammatory mediators affect these pathophysiological events (1). Pulmonary embolism is of significance for being a disease with high mortality and morbidity that can reappear, but that can be prevented (2).

Cardiac troponin I is an enzyme specific to the cardiac muscles. In cases of acute right heart failure in pulmonary embolism, the need for oxygen is increased due to right ventricle dilatation. Due to the decrease in right coronary artery circulation, microinfarctions could occur and troponin release from this region increases (3). High troponin is known as a bad prognostic marker in pulmonary embolism (4).

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There are studies in which it is asserted that the benefits of thoracic computerized tomography (CT) angiography in pulmonary embolism are far greater than supposed. One helpful tool in this regard is the pulmonary artery obstruction index (PAOI), which is used to measure the degree of obstruction of the pulmonary arteries in thoracic CT angiography (5). The PAOI is a simple and reproducible index, and the number of studies conducted on it has increased in recent years. The aim of our study was to investigate whether there is a correlation between pulmonary artery obstruction index (PAOI) in acute pulmonary embolism and troponin.

MATERIAL AND METHOD

This study was approved by the Non-Interventional Clinical Research Ethics Committee of Adıyaman University (Date: 19.11.2019, Decision No: 2019/8-18). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

The first admission data obtained from patients hospitalized in the ward and intensive care units of Adıyaman University Training and Research Hospital with a pulmonary embolism diagnosis between January 2016 and February 2022 were scanned retrospectively. The full blood count, D-dimer, C-reactive protein (CRP), procalcitonin, troponin I, thoracic CT, angiography, and bilateral lower extremity venous Doppler ultrasonography data were extracted. Normal values were accepted as 0-0.5 for CRP (mg/dL), <0.12 for procalcitonin (ng/mL), <0.001 for troponin (µg/L), and 80-560 for D-dimer (µg/L). High values were accepted as >0.5 for CRP, >0.12 for procalcitonin, >0.001 for troponin, and >560 for D-dimer. Patients with left-sided heart failure, kidney failure, gastrointestinal bleeding, sepsis, respiratory tract disease, burns, ischemic stroke, or subarachnoid hemorrhage of the relevant branch diagnosed by the physician, as both an additional comorbidity in the history of the patients and as a new diagnosis at the time of embolism, were excluded from the study. Patients who died within 120 days (3 months) of the diagnosis of acute pulmonary embolism were considered "nonsurvivors" cases. The Simplified Pulmonary Embolism Severity Index (sPESI) was considered as 0 points=low risk and ≥1 point=high risk with application of the following values: age >80 years=1 point, cancer history=1 point, history of heart failure and/or chronic lung disease=1 point, pulse >110/minute=1 point, systolic blood pressure <100 mmHg=1 point, arterial O_2 saturation <90%=1 point (1).

While complete blood counts were evaluated with the CELL-DYN Ruby System, procalcitonin, D-dimer, troponin I, and CRP values were measured with a Radiometer AQT90 Flex device.

CT Procedure and Image Interpretation

For imaging of all patients, images with 3-mm section thickness were obtained with the patients in supine position using a Toshiba Aquilion 64 CT scanner (Toshiba Medical, Tokyo, Japan). The following parameters were used during the imaging: 80-120 kV tube voltage, 60-120 mAs, and 16 × 0.75 mm beam collimation. The field of view used was approximately 40-50 cm (from lung apex to lung base). The 350 mg/ml contrast material was administered at 4-5 ml/sec via the antecubital vein, and the imaging started when the pulmonary artery exhibited storage with optimal contrast according to the contrast time curve. For pulmonary CT angiography, the patients were administered 80 to 120 ml of contrast material depending on their weight, and imaging was performed enabling the interpretation of images by storing optimal contrast in the pulmonary arteries and their branches. The institutional database system (Oracle database V1.10.43.134) was used in the interpretation of all images. All images were evaluated by an experienced radiologist with eleven years of expertise in the field. With the method used by Quanadli et al. (6), the main, right, left, lobar, and segmental pulmonary artery branches were evaluated in the mediastinal window (WW: 300-500 HU, WL: 50 HU) . On CT, a total of 20 lung segment arteries were given 2 points if the lumen of those with filling defects was fully occluded and 1 point if it was partially occluded. Those without filling defects were given 0 points. The PAOI was obtained by scoring between 1 and 40 while evaluating all CT images in three planes (axial, coronal, and sagittal).

Statistical Analysis

The statistical analyses were conducted using IBM SPSS Statistics 23.0 (IBM Corp., Armonk, NY, USA). The descriptive statistics were expressed as mean±standard deviation (minimum-maximum) and number (percentage). The chi-square test was conducted to compare the categorical data. For comparison of the normally distributed continuous data the independent Student's t-test was used, and the data were expressed as mean±standard deviation. The Mann–Whitney U test was used for comparison of the non-normally distributed data and the data were shown as median (minimum-maximum). Pearson's correlation test was used to determine correlation. P<0.05 values were considered statistically significant.

RESULTS

While 57.9% of the 69 patients included in the study were female, 42.1% were male. There was no statistically significant relation between PAOI and gender (p=0.088). PAOI was significantly higher in the high troponin-I group compared to the normal troponin-I group (p=0.006). In addition, PAOI was significantly higher in the group with bilateral pulmonary embolism than in the group with unilateral pulmonary embolism (<0.001). There were no significant differences in PAOI values between survivors and nonsurvivors cases, presence and absence of relapse, unilateral and bilateral DVT, normal and high CRP values, and normal and high procalcitonin values (p=0.258, 0.966, 0.233, 0.754, and 0.454, respectively). Patients with sPESI scores of 0 had a mean PAOI of 17.90±10.72, and patients with sPESI scores of ≥1 had a mean PAOI of 20.18±11.00 (p=0.433) (**Table 1**).

In Pearson's correlation test, while there was a positive correlation between PAOI and D-dimer (r=0.310 p=0.041), a significant negative correlation was found with CRP value (r= -0.268 p=0.042). No significant correlation was found between PAOI and age, troponin levels, platelet count or procalcitonin or sPESI score (p=0.404, 0.376, 0.429, 0.754, and 0.334, respectively) (**Table 2**).

When the clinical and laboratory data for the patients with normal and high levels of troponin were compared, the ratios of patients in the ICU and with bilateral pulmonary embolism were higher and CRP was significantly lower in the high troponin group (p=0.004, 0.027, 0.003, respectively) (**Table 3**).

Table 1. Obstruction index according to patient demographics

	Obstruction index	p	%95 CI (confidence interval)
Female (n:40) Male (n:29)	21.43±10.42 16.90±11.16	0.088 ¥	-0.697 to +9.754
Survivors (n=4) Nonsurvivors (n=65)	13.50±8.27 19.89±10.97	0.258 ¥	-4.781 to +17.565
Recurrence (+) (n=4) Recurrence (-) (n=65)	19.75±16.52 19.51±10.64	0.966 ¥	-11.524 to +11.039
DVT (+) (n=25) DVT (-) (n=44)	22.44 +8.88 17.86+11.65	0.094 ¥	-9.946 to +0.793
Troponin (µg/L) (High)(n=46) Troponin (µg/L) (normal) (n=23)	22 (2-37) 14 (2-40)	0.006*	Z: -2.740
CRP(mg/dL) (High) (n=50) CRP(mg/dL) (normal) (n=8)	18.90±10.98 20.25±12.93	0.754 ¥	-7.222 to +9.922
Procalcitonin (ng/mL) (High) (n=38) Procalcitonin (ng/mL) (normal) (n=22)	20.34±10.65 18.09±11.98	0.454 ¥	-8.228 to +3.726
Pulmonary embolism (Unilateral) (n=15) Pulmonary embolism (Bilateral) (n=54)	6 (2-28) 22 (2-40)	<0.001*	Z: -3.575
sPESI (Low risk)(n=20) sPESI (High risk)(n=49)	17.90±10.72 20.18±11.00	0.433	-8.068 to +3.500

¥: Independent student t test, mean±SD, *P<0.05; Mann-Whitney U test, median (minimum-maksimum),

Table 2. Obstruction index, age, CRP, troponin, D.Dimer, procalcitonin correlation table

	Age	CRP	Troponin	Platelet	D-dimer	Procalcitonin	sPESI
Obstruction index	r: 0.102 p: 0.404	r: -0.268 p: 0.042	r: -0.108 p: 0.376	r: -0.097 p: 0.429	r: 0.310 p: 0.041	r: 0.027 p: 0.839	r: 0.118 p: 0.334

(Pearson correlation test)

Table 3. Comparison of clinical and laboratory data of patients with normal and high troponin levels

	Troponin (Normal) (n=23)	Troponin (High) (n=46)	p
DVT[a]	10	15	0.432
CRP (mg/dL)[c]	9.40 (0.40-35.80)	2.30 (0.30-21.60)	0.003*
D-dimer (µg/L)[b]	7558±6861	12008±11312	0.196
Thorax CT angio (Unilateral filling defect /Bilateral filling defect)[a]	9/14	6/40	0.027*
Number of patients in the intensive care unit [a]	6 (26.1%)	30 (65.2%)	0.004*
Length of stay in intensive care unit (days)[b]	4.5±2.3	4.5±3.3	0.297
Mortality [a]	1 (4.3%)	3 (6.5%)	0.716
Time to Ex (days)[c]	0 (0-30)	0 (0-120)	0.467
Recurrence [a]	1 (4.3%)	3 (6.5%)	0.709

*P<0.05; a, chi-square test (n,%);b, Independent student t test (mean±SD); c, Mann-Whitney U test (median, minimum-maximum)

DISCUSSION

In our study, the PAOI was significantly higher in patients with high troponin levels and in patients with bilateral pulmonary embolism. Furthermore, there was a significant positive correlation between D-dimer and PAOI. In Shokoohi et al. (7), a correlation was found between troponin and PAOI in patients diagnosed with pulmonary embolism in the emergency department, and a significant correlation was found between high troponin-I values and pulmonary artery involvement. It is asserted that elevated troponin level could predict main pulmonary artery embolism with 53.8% (95% CI, 37.6-66) sensitivity and 92.3% (95% CI, 87-96.4) specificity. We found PAOI levels significantly higher in patients with high troponin. However, we found no significant correlation between troponin and PAOI. We think that the difference in the correlation results in our study compared to the study of Shokoohi et al. may be due to the differences in the characteristics of massive/submassive/nonmassive pulmonary embolism in the patient population.

In Gul et al. (8), troponin levels were correlated with both RV/LV ratio and PAOI. However, PAOI did not predict 30-day mortality. In our study, no difference was found in PAOI between survivors and nonsurvivors cases. There was a significant relationship between PAOI and troponin, but no correlation was found.

In Langroudi et al. (9), PAOI was significantly higher in patients with acute pulmonary embolism who died than in those who did not die (23.6 and 10.4, respectively) ($p<0.001$). In addition, they found that a PAOI value greater than 21.5 predicted hospital mortality with high accuracy. The reason for the lack of a significant difference between the survivors and nonsurvivors cases in our study could be the lower number of nonsurvivors cases. This may be due to the low number of nonsurvivors cases and the 120-day period considered for mortality in our study.

Apfaltrer et al. (10) conducted a prospective study in 50 patients diagnosed with acute pulmonary embolism and they evaluated three different pulmonary artery obstruction scores, called Mastora, Qanadli, and Mastora central. In none of these scores was a significant difference found between patients with and without adverse clinical findings. The authors concluded that the pulmonary artery obstruction scores were not correlated with adverse clinical findings. We found higher PAOI scores in patients with higher troponin values. In addition, we found a positive correlation between PAOI and D-dimer. We think that this correlation could be related to thrombus load. We found a negative correlation between PAOI and CRP. We attribute this finding to the high CRP values in patients with subsegmental embolism, especially in those with infarcted areas in the parenchyma (10), as in central-main pulmonary embolism the CRP values did not elevate significantly, and in contrast PAOI values increased in embolisms in the central-main arteries.

It has been proven that elevated troponin levels in pulmonary embolism are related to decreased survival in the long term, and it is regarded as an estimator of increased mortality (12). In our study as well, the ratios of patients in the ICU and with thrombus in the bilateral pulmonary arteries were significantly higher in the high troponin group. In addition, even though the mortality percentage was higher, it was not statistically significant. The reason for this could have been the smaller number of cases.

In the study of Soares et al. (13), no significant relationship was found between the vascular obstruction index and mortality, which is consistent with our study. In addition, there was no significant relationship between the vascular obstruction index and sPESI. We think that the results related to mortality and PAOI values are different due to differences in methodologies and the characteristics of the patient populations in these studies.

The most important limitation of our study is that it was a retrospective study. Moreover, the population of our study was relatively small. The patients could not be grouped according to massive/submassive/nonmassive pulmonary embolism due to lack of data.

CONCLUSION

The PAOI shows thrombus load and is a parameter related to troponin. D-dimer, another indicator of thrombus load, is positively correlated with the PAOI. In cases where the troponin value is not checked, PAOI can be used, at no additional cost.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was approved by the Non-Interventional Clinical Research Ethics Committee of Adıyaman University (Date: 19.11.2019, Decision No: 2019/8-18).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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Results of Y-stent-assisted-coiling with a low-profile Neuroform Atlas stent in complex bifurcation aneurysms

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ABSTRACT

Aim: Neuroform Atlas is a low-profile stent with an open-cell structure that can be deployed via a low-profile microcatheter. This study analyzed the safety, durability, and efficiency of Y-stent-assisted coiling (Y-SAC) with two Neuroform Atlas stents for treating unruptured wide-neck complex bifurcation aneurysms.

Material and method: We retrospectively reviewed patients who were treated for intracranial bifurcation aneurysms using the Y-SAC technique with two Neuroform Atlas stents. A total of 94 consecutive patients were included in the study. Clinical and angiographic results and complications were evaluated before and after the procedure.

Results: Y-SAC was successfully performed (100%) without any technical complications in any case. The mean angiographic follow-up period was 14.6±6.6 months. Follow-up DSA was performed on 93.6% of patients. The last follow-up angiograms demonstrated complete occlusion (RROC I) in 92%, and near-complete occlusion (RROC II) in 7% of the aneurysms. There was no mortality in this study. A procedure-related complication occurred in 4.2% of patients and caused permanent morbidity in 1% of patients.

Conclusion: In the endovascular treatment of wide-neck complex bifurcation aneurysms, the Y-SAC method with two Neuroform Atlas stents is safe and effective with high aneurysm occlusion rates and a low risk of procedural complications.

Keywords: cerebral aneurysm, treatment, embolization

INTRODUCTION

Stent-assisted coiling (SAC) technique for endovascular treatment of wide-neck aneurysms (dome-to-neck ratio of <2 or a neck diameter of >4 mm) prevents coil protrusion into the parental artery, improves aneurysm occlusion, and reduces recurrence (1). Because of wide-neck complex bifurcation aneurysms involving one or more side branches, SAC may be required with two stents in various configurations (2, 3). Y-stent assisted coiling (Y-SAC) has been widely used to treat complex bifurcation aneurysms (4). Previously, Y-SAC could be performed with a large-profile micro-catheter, causing more complications. However, recently, low-profile stents, such as the Neuroform Atlas (Stryker) have been developed that can be loaded onto coil catheters (5–7).

In the current literature, the results of Y-stenting performed with a low-profile stent combination of closed-cell or braided cell with an open-cell stent are reported mostly. The number of Y-SAC series using two Neuroform Atlas stents (open cell-open cell Y-configuration) is a few in the literature (5, 8–9).

In this study, we analyzed the outcomes of unruptured complex bifurcation aneurysms treated with two Neuroform Atlas stent-assisted Y-SAC.

MATERIAL AND METHOD

Study Design and Data Collection

This retrospective study was approved by the Adıyaman University Clinical Researches Ethics Committee (Date 6.01.2021, Decision No: 1358). Informed consent was not obtained as the study was retrospective. All procedures were performed under the ethical standards of the institutional and/or national research committee and the 1964 Helsinki declaration or comparable ethical standards. Y-SAC patients who were treated with two Neuroform Atlas stents were reviewed between Jan

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2017 and Dec 2020. Single Neuroform Atlas, double Neuroform Atlas but non-Y configuration, or Y stents other than Atlas-Atlas configuration were excluded from the study. A total of 94 consecutive patients were included in the study. Patient demographics, aneurysm characteristics, procedural details, complications, and follow-up outcomes (recanalization or retreatment ratio, parent artery patency) were evaluated. Complications were classified as intraprocedural, early (within 30 days), and late complications (after 30 days). Neurological complications were assessed according to the modified Rankin Scale (mRS) as major (death, major stroke resulting mRS >2) or minor (resulting mRS ≤ 2).

Neuroform Atlas Stent

The Neuroform Atlas stent is a laser-cut, nitinol, self-expandable stent that consists of open and closed cells together. This unique hybrid design permits microcatheter re-crossing and facilitates Y stent configuration. Better wall apposition, reduced shortening ratio, high navigability, and lower complication rates are advantages of the Neuroform Atlas stent. Available stent diameter sizes are 3.0, 4.0, and 4.5 mm and length sizes are 15, 21, 24, and 30 mm. The Neuroform Atlas stent is suitable for use in 2–4.5 mm-sized arteries.

Neuroform Atlas stent could be deployed through the low-profile microcatheters. A low-profile microcatheter helps catheterization of the sharply-angled second branch. The open cells at the mid-portion of the Neuroform Atlas provide an advantage over braided stents, while the microcatheter passes through the open-cell stent struts to create Y-SAC. The second stent can be opened better at the intersection of the stents and complication risks of the parent artery are reduced (5).

Procedure: Y-SAC Technique

The treatment decisions are taken by an endovascular team consisting of neurosurgeons and interventional neuroradiologists depending on the aneurysm's morphometric characteristics in 2D and 3D angiograms. Y-SAC was performed if primary coiling or single stent deployment could not prevent coil protrusion because of the wide neck and complex morphology.

All patients were pretreated with a loading dose of clopidogrel (300–450 mg) or prasugrel (30–60 mg). Intravenous (iv) tirofiban infusion (25 mcg/kg) was administered postoperatively as needed. All procedures were performed under general anesthesia with systemic heparinization (50 to 70 units per kilogram bolus followed by infusion). First, a long introducer placed sheath via the femoral artery route with micropuncture technique. Femoral ultrasonography was performed to guide puncture in case of difficulty to achieve the vascular access. Then through the long sheath, we reached internal carotid artery or distal V2 segment of the vertebral artery using a Fargomini distal access catheter (Balt, France) with an Excelsior SL-10 (Styker, USA) 0.0165" microcatheter over a Synchro (Stryker, USA) 0.014" microguidewire. The sharply angled and difficult-to-access branch was catheterized the first and a Neuroform Atlas stent was deployed into the first branch. Then, the microcatheter was passed through the first stent struts to the second branch and the second low-profile stent was deployed, creating a Y-stent configuration. A coil catheter was placed into the aneurysm with the jailing technique or trans-strut technique. Coiling was performed using bare platinum coils until the aneurysm sac was filled. Aneurysm occlusion and stent patency were evaluated with immediate angiograms according to the Ray Raymond Occlusion Classification (RROC) (10). Dual antiplatelet treatment was maintained for six months with 75 mg clopidogrel or 10 mg prasugrel and acetylsalicylic acid (ASA) 100 mg daily, then continued with ASA.

Follow-up

Postoperative computed tomography (CT) was performed within 24 h to exclude hemorrhagic complications. Angiographic follow-up with digital subtraction angiography (DSA) and/or magnetic resonance angiography (MRA) was performed at 1, 6, and 12 months. Patients were evaluated with MRA to check for asymptomatic ischemic findings in the first month. Follow-up DSA was performed for 9–12 months to assess aneurysm occlusion according to RROC grading (10). Two operators independently evaluated the angiograms. Aneurysm filling was evaluated as RROC I (complete occlusion), RROC II (residual neck filling), and RROC III (residual dome filling). Some patients had been followed up with only MRA because of the patient's reluctance to DSA. The primary efficacy outcome is accepted as the 12 months angiographic complete (100%) aneurysm occlusion rate or RROC I, without retreatment and parent artery stenosis (>50%) at the stent localization. If there was no significant reduction of aneurysm opacification after a one-year follow-up, retreatment was advised.

Neurological evaluation of patients was performed using the modified Rankin scale (mRS) before the procedure, at discharge, at 30 days and 90 days after the procedure.

Statistical Analysis

Descriptive statistical analysis was performed using SPSS statistics (IBM, Armonk, New York).

RESULTS

Baseline Population and Aneurysm Features

A total of 94 unruptured aneurysms in 94 patients (59 female) were included in the study. The median age was 59.2±11.7 years old, with a range of 32 to 88 years. Thirteen (13.8%) aneurysms (4 primary coils and 9 clipped) were treated previously, but the residual filling was observed in the radiologic follow-up, they were re-operated with Y-SAC.

All patients had wide-necked complex aneurysms. The locations of aneurysms were as follows; 43 (45%) middle cerebral artery bifurcation (MCA bifurcation), 27 (28%) anterior communicating artery (Acom), 14 (14%) basilar tip, 3 (3%) internal carotid artery bifurcation (ICA bifurcation), 3 (3%) early cortical branch of MCA (MCA-ECB), 3 (3%) A2-3 segment of the anterior cerebral artery (ACA-A2-3), one aneurysm (1%) the superior cerebellar artery (SSA). The majority (84%) of aneurysms were located in the anterior circulation. According to the diameter, aneurysms were classified as; 47 (50%) aneurysms <7 mm, 27 (29%) aneurysms 7–10 mm, 16 (17%) aneurysms 10-15 mm, and 4 aneurysms (4%) 15-20 mm. The mean dome size was 6.8±2.3 mm (range 4–20 mm), the mean neck width was 4.2±1.3 mm and the mean dome-to-neck ratio was 1.5±0.3 for the aneurysms.

Demographics, presentation, and aneurysm characteristics were summarized in **Table 1**.

Table 1. Demographics, presentation, and aneurysm characteristics

Mean age	59.2±11.7 yr (range 32–88 yr)
Sex	
Female	59 (61%)
Male	37 (39%)
Clinical presentation	
Incidental	36 (38%)
Headache	44 (47%)
SAH	13 (14%)
Recurrence	1 (1%)
Aneurysm location	
MCA bif	43 (46%)
Acom	27 (29%)
Basilar tip	14 (15%)
ICA tip	3 (3%)
M1-ECB	3 (3%)
ACA A2-3	3 (3%)
SSA	1 (1%)
Aneurysm size	
< 7 mm	47 (50%)
7–10 mm	27 (29%)
10–15 mm	16 (17%)
15–20 mm	4 (4%)
Mean dome size	6.8±2.3 mm
Mean neck width	4.2±1.3 mm
Mean dome/neck ratio	1.5±0.3

Procedural Details

In all procedures, Y-SAC was successfully performed without any technical complications in all cases (100%). Immediate post-procedural angiography revealed total aneurysm occlusion (RROC I) in 81 (86%), neck filling (RROC II) in 12 (13%) patients, and sac filling in one patient.

Complications

No mortality occurred in this study group. A procedure-related complication developed in 4 patients (4.2%). Acute stent thrombosis developed in 3 patients during the procedure. A tirofiban infusion was started immediately, but the thrombus did not completely dissolve and the blood flow continued to slow, so a third stent was placed in this segment. They were discharged from the hospital without permanent morbidity (mRS 0–1). In one patient with preprocedural mRS 2, an ischemic infarct developed on the tenth day, early postoperatively. This patient has been followed up with mRS 4. We didn't observe any late complications (>30 days). All 4 patients were administered 450 mg of clopidogrel preoperatively more than two hours before the stent implantation.

Follow-up Results

The mean follow-up time was 14.6±6.6 months (range, 6–34 months). The follow-up mRS was ≤2 in 91 of 94 cases and mRS>2 in the remaining 3 cases. One patient was discharged as mRS 4 because of a thromboembolic event during the early postoperative period. The other two patients had mRS 2 at their presentation. There was no change after surgery.

Follow-up DSA was performed on 88/94 (93.6%) of patients. The last follow-up angiograms demonstrated complete occlusion (RROC I) in 81/88 (92%) and near-complete occlusion (RROC II) in 6 (7%) of the aneurysms. In one patient, the first-year follow-up angiogram revealed persistent aneurysmal sac filling (RROC III), so a second coiling procedure was performed. We did not detect recanalization or in-stent stenosis in the follow-up angiograms. The residual filling was not observed in 6 patients who were followed up with MRA. **Figure 1** shows the preoperative, intraoperative, and follow-up angiograms of a 67-year-old female patient with an aneurysm in the basilar apex.

Follow-up aneurysm occlusion rates (RROC) are summarized in **Table 2**.

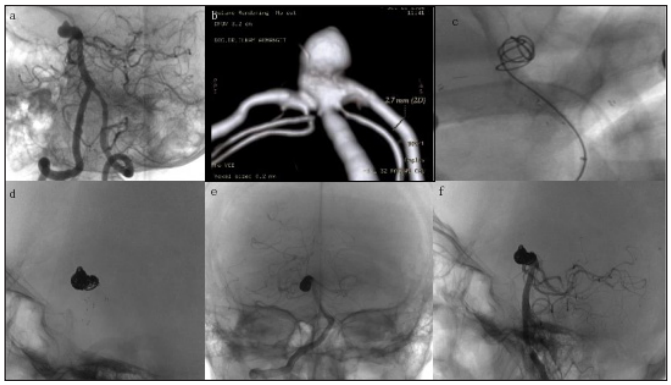

Figure 1. (a) Preoperative DSA of a 67-year-old female patient demonstrating an aneurysm in the basilar apex (b) 3D reconstruction imaging demonstrated aneurysm sizes, PCA and SCA branches clearly (c) Intraoperative lateral angiogram showing how Y configuration stenting was performed by passing from the basilar artery to the right and left PCA and then coiling (d) Postoperative lateral view shows two Neuroform Atlas deployed in Y-configuration and aneurysm sac coiled (e, f) Follow-up AP and lateral angiograms (12th month) demonstrate no residual filling (RROC 1)

Table 2. Followed-up Aneurysm Occlusion Rates (RROC)

RROC	Immediate DSA	Last follow-up DSA
RROC I	81 (86%)	81 (92%)
RROC II	12 (13%)	6 (7%)
RROC III	1 (1%)	1 (1%)

DISCUSSION

Endovascular treatment of distal complex bifurcation aneurysms is a challenging issue. The recently introduced WEB embolization devices may not be suitable for non-spheric amorphous-shaped bifurcation aneurysms incorporating more than one side branch. Amorphous morphology including bleb may impede the definite size selection of the WEB device (5). Inappropriate sized WEBs may also occlude critical branches originating from the aneurysm sac or residual filling may remain because of the undersized device. It has been reported that the PulseRider assisted coiling procedure, which has a much lower metal load compared to Y-stenting, is a safe and applicable device for treating basilar and carotid bifurcation aneurysms. In our patient group, ACom and MCA aneurysms constituted 80% of the group (11,12).

For treating complex bifurcation aneurysms, dual-SAC techniques including in X, T, and Y configurations were previously performed via large-sized microcatheters (3). As described by Chow et al. (4) in 2004, Y-stent assisted coiling (Y-SAC) has been widely used to treat complex bifurcation aneurysms (12). However, because of the technical difficulties, risk of hemorrhage, and necessity of dual antiplatelet treatment Y-SAC technique has a higher complication risk (dissection, vasospasm) than primary coiling (13, 14). Catheterization of narrow-angle and tortuous vessels in Y-stenting can be technically difficult and risky. Complication rates were reported to be higher in previous studies due to the catheterization that had to be performed with a large profile microcatheter. Spiotta et al. (15) reported a 31.6% complication rate for Y-SAC procedures using the older generation Neuroform that can be placed over 0.027 and 0.021 inches. Bartolini et al. (16) reported a complication rate of 18% in their study with double stenting and attributed the high complication rate to difficult parent artery catheterization with large catheters. Akgül et al. (6) reported a morbidity rate of 9.1% in their study with the Neuroform and Enterprise stent combination (open cell-closed cell). Procedural complications were reported as 8.9% and 12% in Y stent meta-analysis studies in the literature (7,17). The complication rate in crossing Y stents has been reported to be lower than in kissing stents, whereas the complication rates were lower with the Enterprise stent (6.5%) than Neuroform and LVIS stents (7).

The risk of complications is reduced, as the low-profile stent can be placed via the low-profile micro-catheter. The number of patients treated with open-cell-open-cell Y-SAC configuration with two Neuroform Atlas stents is few. In the open cell-open cell study by Kubilay et al. (5), Y-SAC was performed using 2 Neuroform Atlas stents, and the complication and permanent morbidity rates were reported as 6.7% and 3.3% respectively. Our complication rate was lower (4.2%) than both the Neuroform Atlas Y-SAC series of Aydın et al. (5) and the Enterprise Y-SAC series of Cognazzo et al. (7).

Y-stenting may increase the risk of device thrombogenicity, and thromboembolic events due to procedural difficulties and increased metal load. In the study reported by Goertz et al. (18) the risk of thromboembolic events due to Y stenting was reported as 10.7% with an ischemic stroke rate of 1.5%. In our series, 3 (3%) acute stent thrombosis developed intraoperatively. In these patients, a third Atlas stent had to be placed in the thrombosed artery. These patients were discharged without any sequel. In our series, one patient had a thromboembolic event on the postoperative tenth day. This patient was followed up with a permanent neurological sequela with mRS 4.

Aneurysm occlusion rates were reported in the range of 60% and 87.5% in immediate angiograms after Y-SAC treatment (5, 8, 19). In studies using open cell-open cell stent combination, Kubilay et al. (5), and Ciccio et al. (8) reported immediate complete occlusion rates of 83.3%, and 60%, respectively. In the closed cell-closed cell combination, Limbucci et al. (19) reported an immediate occlusion rate of 87.5%. In this study, we observed a complete occlusion rate (RROC I) of 86% and a near-complete occlusion rate (RROC II) of 14% in immediate angiograms. In the meta-analysis of Y-SAC treatment, Cognazzo et al. (7) reported the immediate complete occlusion rate as 82.2%. The long-term

occlusion rates were similar between the Enterprise, Neuroform, and LVIS stents. The rate of near-complete/complete occlusion was 95.4% with Enterprise, Neuroform, and LVIS stents (7). There were two recent reports for long-term Y-SAC and two Neuroform Atlas stents. Aydın et al. (5) reported a complete occlusion rate of 93.3%. In the latest study in the literature, Kim D (9) reported a complete occlusion rate of 73.3%, 6.7% neck remnant, and 20% incomplete occlusion. In our series, the complete occlusion rate (RROC I) was 92%, and the near-complete occlusion rate (RROC II) was 7% at the final DSA angiograms.

Recanalization rates are reported to be lower in SAC treatment because the stent facilitates aneurysm thrombosis (20). The recanalization rate was reported as 3% in the Cognazzo et al. (7) series. No recanalization was observed in our series. However, one patient was retreated because of the remaining RROC III grading on control angiograms. The patient underwent a second operation for coiling.

Limitations of the Study

It is single-center series and it is not a population-based study. The data were analyzed retrospectively. But the main limitation in our study is that the follow-up period of patients was limited to less than 3 years.

CONCLUSION

This study demonstrated that Y-SAC configuration deployment by low-profile Neuroform Atlas stents is feasible for treating challenging complex bifurcation aneurysms. This technique is safe and effective with high aneurysm occlusion rates, low complication, and low recurrence rates.

ETHICAL DECLARATIONS

Ethics committee approval: The study was initiated with the approval of the Adıyaman University Clinical Researches Ethics Committee (Date 6.01.2021, Decision No: 1358).

Informed consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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Examining the long-term effects of COVID-19 on the umbilical cord

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ABSTRACT

Introduction: It is known that COVID-19 in pregnancy causes some negative consequences. Although some studies have been conducted on the possible effects of COVID-19 seen in late pregnancy, its effects in the previous trimesters are not clearly known. This study aimed to examine the umbilical cords of pregnant women who did not have COVID-19 and those who had in the second and third trimesters, after delivery using histopathological and immunohistochemical methods.

Material and Method: The study included 27 pregnant women who had never had COVID-19 (n:9), who had had COVID-19 in the second trimester (n:9) and had had COVID-19 in the third trimester (n:9). After delivery, sections were taken from the umbilical cords of the pregnant women and examined with histopathological and immunohistochemical (VEGF and vimentin antibodies) methods. H-scores were determined for statistical analysis of immunohistochemical staining results. Group means were analyzed using the non-parametric Kruskal Wallis Test.

Results: In cases that had COVID-19 in the third trimester of pregnancy, histopathological findings were more significant than in the other groups. Hemorrhage, thinning of the tunica intima layer, and deterioration in its integrity were observed in the umbilical vascular structures of this group. VEGF and vimentin expression levels were higher in the third-trimester group than in the other groups.

Conclusion: The COVID-19 disease has both acute and long-term effects. The presence of histopathological and immunohistochemical findings in the umbilical cord during the third trimester of pregnancy supports this information. Moreover, the high levels of expression of VEGF and vimentin in the umbilical cords of pregnant women may contribute to the understanding of the pathogenesis of COVID-19 and the post-acute effects of these proteins.

Keywords: COVID-19, umbilical cord, VEGF, vimentin, long COVID

INTRODUCTION

The novel coronavirus disease (COVID-19) has emerged due to the Sars-CoV2 virus and has spread to many countries, causing an outbreak (1). The disease may progress asymptomatically as well as may lead to serious life-threatening conditions such as sepsis (2). Although the most frequently affected system due to this disease is the respiratory system, organs in many systems such as nervous, circulatory, digestive, and urogenital systems are also adversely affected. Damages that occur in the organs may occur in the acute period as well as in the later periods (3). The COVID-19 disease results in recovery in many cases whereas in some cases, although the PCR test result turns negative, some symptoms may persist. This condition is defined as "post-COVID-19 syndrome" or "long COVID" (4).

Pregnant women are among the risk groups affected by the COVID-19 disease. When this disease occurs during pregnancy, it can cause negative effects on both maternal and infant health (5). The COVID-19 infection during the active period of pregnancy leads to immunohistochemical and histopathological degenerative effects on the placenta (6). In addition, it is known that pregnant women experience mental complications such as anxiety, depression, and obsessive-compulsive disorder during the Covid-19 pandemic (7). In order to prevent these effects, necessary measures should be taken to reduce the rate of Sars-CoV2 infection during pregnancy and perinatal periods. Also, pregnant patients should be monitored closely (5, 8).

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The umbilical cord has an important role in fetal development (9). It is stated that perinatal and intrapartum complications are associated with umbilical cord abnormalities (10). Inflammation in the umbilical cord is defined as funisitis (11). Infection in the umbilical cord may cause neonatal mortality or morbidity and unhygienic practices performed during delivery lead to the development of the disease (12). COVID-19 infection can also cause perinatal transmission if the correct hygienic measures are not taken (13).

Vascular endothelial growth factor (VEGF) is a specific mitogen for vascular endothelial cells. VEGF, also known as vascular permeability factor, increases the proliferation of vascular endothelial cells, microvascular permeability, and angiogenesis (14). Angiogenesis is involved in many physiological and pathological processes. The VEGF family plays a significant role in the development of the vascular system through angiogenesis and is also involved in tissue repair (15). Umbilical cord cells widely express VEGF and these proteins play a role in the growth of the umbilical cord (16). It is known that VEGF levels increase in feline infectious peritonitis (FIP), an infection caused by coronaviruses (17).

Vimentin is an intermediate filament (IF) protein of mesenchymal cells and plays a significant role in organ homeostasis (18). One of the organs in which this protein is expressed and takes part is the umbilical cord (19). Vimentin is also a regulator of proteins involved in cell signaling and migration (18). It is thought that these proteins work as a cytoprotective structure that prevents the movement of some viruses into the cytoplasm (20). Despite this information, the functions of vimentin are not fully known and further studies are needed.

We could not find any information in the literature on how the umbilical cord is affected in pregnant women who have had COVID-19 infection in the second and third trimesters. This research aims to examine the umbilical cords of pregnant women who had not had COVID-19 and those who had had it in the second and third trimesters, after delivery with histopathological and immunohistochemical (VEGF and vimentin antibodies) methods.

MATERIAL AND METHOD

The study was carried out with the permission of Siirt University Non-Interventional Clinical Research Ethics Committee (Date: 13.04.2022, Decision No: 42191). The procedures applied in the study were carried out in line with the ethical rules and the principles of the Declaration of Helsinki. Participants included in the research were informed and a written informed consent form was taken.

A total of 27 pregnant women, nine who had had and recovered from COVID-19 in the second trimester of pregnancy, nine who had had and recovered from COVID-19 in the third trimester, and nine who had never had a COVID-19 infection, were included in the study. Pregnant women with a secondary disease or any chronic disease were not included in the study. Tissues (umbilical cord) were taken from pregnant women who applied to the Department of Obstetrics and Gynecology of Siirt Training and Research Hospital, right after delivery.

Histological Follow-up

Tissues taken from the groups were put into 10% buffered neutral formalin and kept for 16 hours for fixation. Then, the fixed tissues were washed with running water for about 12 hours and the formalin solution was eliminated. Then, the tissues were incubated in alcohols of 30%, 50%, 70%, 80%, 90%, and 96%, respectively, for 8 hours. The dehydration process of the tissue was completed after they were kept in absolute alcohol (99.9%) for 2X20 minutes. After the dehydration process, the tissues were kept in xylol for 2X15 minutes to make the tissues transparent and discard the alcohol from the tissues. The tissues then were incubated in melted paraffin containing 50% xylol in an incubator at 58°C for approximately 1.5 hours. Then the tissues were put into pure paraffin and left for approximately 4 hours for paraffin to penetrate the tissues. Then, the tissues embedded in liquid paraffin were kept at room temperature to dry and sections of 5 µm were taken from the dried tissues using a microtome (Leica RM 2265, Germany).

Hematoxylin-Eosin Staining Protocol

Sections kept in xylol in two series of 15 minutes were deparaffinized. Then, the sections were passed through the decreasing alcohol series for about 5 minutes, ending with the distilled water step. The sections were then incubated in Harris hematoxylin for 8 minutes. Sections were taken under the running water and kept here for 5 minutes, then immersed in 1% acid-alcohol solution to ensure differentiation and washed with distilled water for 5 minutes. After washing, the sections were kept in eosin-floxin solution for 2 minutes for counterstaining. Then, the sections were passed through increasing alcohol series, kept in xylol for two series of 15 minutes, and mounted with entellan.

VEGF and Vimentin Immune Staining Methods

Sections with a thickness of 5 µm were first kept in xylol for 2x15 minutes. Sections recovered from paraffin in xylol were then kept in decreasing alcohol series for 5 minutes each and taken into distilled water for a while. Then, the sections were put into EDTA solution and kept in the microwave oven for approximately 3

minutes. Sections taken from the microwave were left at room temperature for 15 minutes for cooling. At the end of incubation, the sections were placed in distilled water again and then dried. The parts with the tissues were drawn with hydrophobic pencils. Then, PBS-T was added and the sections were kept for 3x15 minutes. During these procedures, special care was taken to keep the immunohistochemistry box moist. After the PBS-T on the sections was discarded, hydrogen peroxide solution was dripped onto them and the sections were kept for an average of 20 minutes. Tissues were divided into two sections: some of the sections were incubated with 1/100 diluted with VEGF primary antibodies (cat no: MA5-13182, Thermofisher Scientific, US) and the remaining sections were incubated in 1/100 diluted Vimentin primary antibodies (cat no: MA5-11883, Thermofisher Scientific, US) overnight at +4°C. The sections were kept at room temperature for approximately 1 hour the next day and were washed with PBS-T for 3x5 minutes. The sections were then incubated with anti-rabbit secondary antibody for approximately 15 minutes. Streptavidin-peroxidase was dripped onto the sections washed with PBS-T for 3x5 minutes and the sections were kept for 15 minutes. They were washed with PBS-T for 3x5 minutes and DAB was dripped onto the sections. The reactions of the tissues were monitored with a light microscope and the reaction of those showing involvement was terminated with PBS-T. The sections were re-washed with PBS-T for 3x5 minutes, counterstained with Gill III hematoxylin for approximately 45 seconds, and washed in tap water for 5 minutes. Then, the sections passed through increasing alcohol series were kept in xylol for 2x15 minutes and mounted with entellan.

Immunohistochemical staining results were analyzed with H scoring (HS). The formula used is HS= Σ (1 + i) x pi. Here, 'pi' represents the intensity of the percentage of staining; 'i' corresponds to the staining intensity (0=no expression, 1=mild, 2=moderate, 3=intense, and 4=very intense) (6).

Statistical Analysis

Statistical analysis was made in the IBM SPSS program. Non-parametric Kruskal Wallis Test was performed to analyze the group means. Data were presented as mean and standard deviation (SD). Statistically, $p<0.05$ was considered significant.

RESULTS

Pregnant women who participated in the study did not develop any complications and no fetal abnormality was detected in their babies.

Histopathological Findings

Hematoxylin-eosin staining of umbilical cord tissues of the control and experimental groups (who had had COVID-19 in the second and third trimesters) are shown in **Figure 1**. **Figure 1A** represents the control group; **Figure 1B** represents the group who had had COVID-19 in the second trimester; **Figure 1C** represents the groups who had had COVID-19 in the third trimester. In the histopathological images of the umbilical cords of the control group, the muscles in the tunica media layer of the umbilical vessel were a regular structure. The histological structure of the Wharton gel was normal. There were occasional ruptures between the muscle fibers in the tunica media of the umbilical vessel in the umbilical cords of pregnant women who had had COVID-19 in the second trimester of pregnancy. The integrity of the tunica intima layer was lost. Hemorrhagic findings were detected in the umbilical vascular structure of the umbilical cords of pregnant women who had had COVID-19 in the third trimester of pregnancy. Furthermore, the tunica intima layer was thinner and the integrity partly deteriorated.

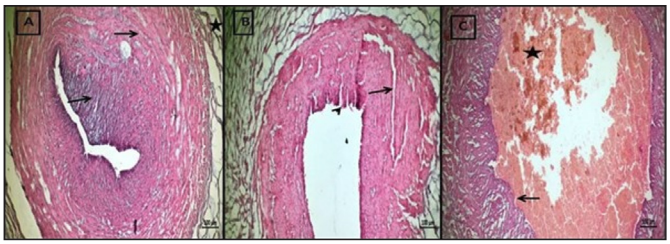

Figure 1. HE staining of sections of the umbilical cord (A) Normal histological appearance in the tunica intima, media (arrows), and adventitia (star) in the control group (B) Structural deterioration in the tunica media (arrow) and intima (arrowhead) layers in the group who had had COVID-19 in the second trimester (C) Hemorrhage (star) and thinning of the tunica intima (arrow) and deterioration in the integrity in the group who had had COVID-19 in the third trimester (X10)

Immunohistochemical Findings

VEGF immunohistochemical staining of the umbilical cords of the control and experimental groups are shown in **Figure 2**. In the umbilical cords of the control group, the expression of VEGF in the tunica intima and adventitia layers of the umbilical vessel was mild (**Figure 2A**). VEGF expression was positive in endothelial cells in the tunica intima of the umbilical vessel and at the border of the tunica media and tunica adventitia in the umbilical cord tissues of pregnant women who had had COVID-19 in the second trimester of pregnancy (**Figure 2B**). VEGF expression was higher in the second-trimester group than in the control group. In umbilical cord tissues of pregnant women who had had COVID-19 in the third trimester of pregnancy, VEGF expression was positive in endothelial cells in the tunica intima of the umbilical vessel, at the border of the tunica media and adventitia, and in the

tunica adventitia (**Figure 2C**). VEGF expression was higher in the group who had had COVID-19 in the third trimester than in both the control group and the group who had had COVID-19 in the second trimester.

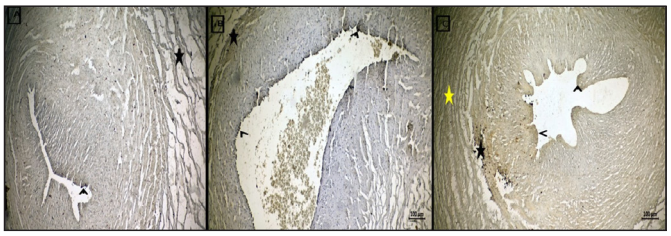

Figure 2. Immunohistochemical staining of VEGF in umbilical cord sections (A) Control group: Mild expression in the tunica intima (arrowhead) and adventitia (black star) of the umbilical vessel (B) Group who had had COVID-19 in the second trimester: Positive expression in the tunica intima (arrowheads) of the umbilical vessel, at the border of the tunica media and adventitia (star) (C) Group who had had COVID-19 in the third trimester: Positive expression in the tunica intima (arrowheads), at the border of the tunica media and adventitia (black star), and tunica adventitia (yellow star) of the umbilical cord (X10)

Vimentin immunohistochemical staining of the umbilical cords of the control and experimental groups are shown in **Figure 3**. In the umbilical cord of the control group, vimentin expression was positive in endothelial cells in the tunica intima of the umbilical vessel and mild in the tunica media layer (**Figure 3A**). Vimentin expression was positive in the tunica intima and media layers of the umbilical vessel and mild in the tunica adventitia layer in the umbilical cord tissues of pregnant women who had had COVID-19 in the second trimester of pregnancy (**Figure 3B**). Vimentin expression was higher in the second-trimester group than in the control group. In the umbilical cord tissues of pregnant women who had had COVID-19 in the third trimester of pregnancy, vimentin expression was positive in the tunica media and adventitia layers of the umbilical vessel and mild in the tunica intima layer (**Figure 3C**). Vimentin expression was higher in the third-trimester group than in both the control group and second-trimester group.

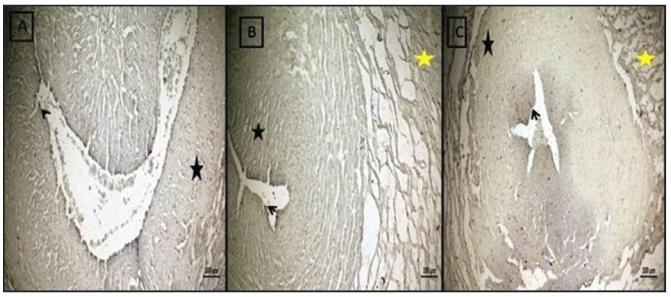

Figure 3. Immunohistochemical staining of vimentin in umbilical cord sections (A) Control group: Positive expression in the tunica intima (arrowhead), mild expression in the tunica media (star) of the umbilical vessel (B) Group who had had COVID-19 in the second trimester: Positive expression in the tunica intima (arrow) and media (black star) of the umbilical vessel and mild expression in the adventitia (yellow star) (C) Group who had had COVID-19 in the third trimester: Positive expression in the tunica media (black star) and adventitia (yellow star) of the umbilical vessel and mild expression in the intima (arrow) (X10)

Immunohistochemical Statistics

Statistical analysis of immune activities of VEGF and vimentin was shown in **Table 1**. The change in VEGF expression was statistically significant between groups. The significance of VEGF in the second group compared to the control group was less than the significance of the third group compared to the second group. The expression was the highest in the third-trimester group. Similarly, the change in vimentin expression was significant between control, second and third-trimester groups. Vimentin expression was significantly higher in the second-trimester group than in the control group. Vimentin reaction was higher in the third-trimester group than in the control and second-trimester group, this increase was statistically significant.

Table 1. Comparison of VEGF and Vimentin expression between groups

Parameters	Control group	2nd trimester	3rd trimester	p value
	Median (min-max)	Median (min-max)	Median (min-max)	
VEGF	0.00 (0.00-1.00)	1.00 (0.00-2.00)	2.00 (1.00-3.00)	*p=0.0019 ** p<0.001 ***p<0.001
Vimentin	0.00 (0.00-1.00)	2.00 (0.00-3.00)	3.00 (2.00-3.00)	*p<0.001 ** p<0.001 ***p=0.027

* control vs second, **control vs third, ***second vs third

DISCUSSION

The clinical findings of pregnant women with COVID-19 are generally similar to other COVID-19 patients. However, it is known that COVID-19 in pregnancy causes some negative consequences such as preterm birth, fetal distress, and neonatal asphyxia. Although COVID-19 does not usually cause fetal and neonatal adverse effects, COVID-19 infection, especially seen in the third trimester, may cause more risky and negative consequences (21). Furthermore, there is a need for further comprehensive studies, including other trimesters to understand the long-term effects of COVID-19 on maternal and infant health during pregnancy. In our study, we examined the long-term effects of COVID-19 in the second and third trimesters of pregnancy, using histopathological and immunohistochemical methods on the umbilical cord.

Immunohistochemical and histopathological disorders are seen in the placentas of pregnant women with active COVID-19. In a study, it was seen that IL-6 and Bax expression levels were higher in the postpartum placentas of pregnant women with active COVID-19 compared to the control group (6). In another study, fetal and maternal vascular malformation, villitis, thrombus formation, and chorangiosis were histopathologically reported in

the placentas of pregnant women with COVID-19 (22). This information suggests that COVID-19 should be evaluated not only in terms of clinical outcomes but also in terms of histopathological outcomes. Although there are studies on the possible effects of COVID-19 seen in the last period of pregnancy, its effects in the previous trimesters are not fully known (21). Studies examining the relationship of this disease with the umbilical cord are limited (23). Previous studies mostly reported that mesenchymal stem cells taken from the umbilical cord are successful in the treatment of this disease (24, 25).

A study in which the umbilical cords of pregnant women with COVID-19 were examined immunohistochemically reported antibody involvement against Sars-CoV-2 N protein. It was stated that the cells with involvement were macrophages and fibroblast-like cells in Wharton gel (23). The fact that there was no involvement in the umbilical cords in the control group whereas there was an involvement in the active COVID-19 group is important. This shows that the umbilical cord is affected by the Sars-CoV-2 virus, like many other organs. Accordingly, the presence of the Sars-CoV-2 virus in the umbilical cords and its long-term effects should be considered when evaluating the effects of COVID-19 on the mother-placenta-fetus system.

In our study, structural deteriorations were histopathologically observed in the layers of the umbilical vessels in the umbilical cords of pregnant women who had had COVID-19 in the second and third trimesters. There was more histopathological damage with hemorrhage in the third-trimester group. Given that the Sars-CoV-2 virus can be transmitted to the umbilical cord, the apparent histopathological findings, especially in the third trimester, can be explained by the histopathological effects of this infection. As a matter of fact, the pregnant women in the third-trimester group included in the study mostly consisted of cases who were near delivery. This suggests that the effects of the virus on the umbilical cord remain in the post-acute period. On the other hand, milder histopathological findings seen in the second-trimester group can be explained by the fact that the effects of COVID-19 infection seen in earlier periods on the umbilical cord decrease. At this point, it is important to ensure new pathogenic mechanisms to provide fetal protection against COVID-19 infections, especially near delivery.

VEGF is one of the major factors for fetal and placental development (26). A study comparing VEGF expressions between normal placenta and placenta increata cases found that VEGF expression levels were higher in placenta increata cases. Overexpression of VEGF and abnormal villous vessel formation seen in placental adhesion anomalies were found to be associated with the pathogenesis of placenta increata (27). In another study examining normal pregnancies and pregnancies with severe preeclampsia, VEGF expression levels in the umbilical cord were compared and VEGF expression was found to be higher in the preeclampsia group. It is known that VEGF affects NO synthesis by regulating eNOS production (28). Therefore, the increase in VEFG expression in the preeclampsia group may be a tolerant mechanism to dilate blood vessels and improve fetal blood flow against developing hypoxic conditions. In another study, it was stated that VEGF reduces the expression of proinflammatory cytokines such as TNF-α and IL-1. Moreover, VEGF was reported to reduce tissue damage by inhibiting inflammation (29).

In our study, VEGF expression in the vascular structures that form the basic structure of the umbilical cords and are embedded in the Wharton gel was higher in the group who had had COVID-19 in the third trimester. This suggests that VEGF may be involved in the pathogenesis of the COVID-19 infection, especially in the period near delivery. COVID-19 is known to cause arterial hypoxia due to pulmonary involvement (30). The increase in VEGF expression, especially seen in the third-trimester group, might be a defense mechanism that supports the blood circulation of the fetus against hypoxic conditions. Another finding in COVID-19 infection is the increase of proinflammatory cytokines such as TNF-α (31). Considering the effects of VEGF such as reducing the expression of proinflammatory cytokines such as TNF-α and inhibiting inflammation (29), it can be suggested that this protein has a regulatory and protective role in COVID-19 infection.

Vimentin has many functions such as cell physiology, cellular interactions, and tissue homeostasis. Another important function of vimentin is that it takes part in cases of infection (18). It was reported that vimentin has a cytoprotective role that prevents the entry of viral components into the cytoplasm. Indeed, this effect of vimentin was demonstrated in a study conducted on African swine fever virus (ASFV) infection (20). The increase in vimentin expression observed in the third-trimester group in our study may be due to the cytoprotective role of vimentin against viral infections. The decreasing vimentin expression from the second-trimester group to the control group can be explained by the fact that the effect of the disease and the viral load gradually decrease.

In a study in which the placentas of preeclamptic and normotensive pregnant women were compared in terms of vimentin expression, it was found that the vimentin expression was higher in the preeclamptic group and that vimentin has a role in the pathogenesis of preeclampsia (32). In another study, it was stated that the expression levels of

vimentin and VEGF in these patients were correlated with each other. Moreover, both proteins were held responsible for trophoblast invasion, angiogenesis, and vascular permeability (26). In our study, the expression levels of vimentin and VEGF in the umbilical cord were found to be higher in the third-trimester group. This suggests that both proteins can be co-evaluated in the pathogenesis of COVID-19. On the other hand, new studies are required to explain the molecular mechanisms by which vimentin and VEGF play a role in the relationship between COVID-19 and the umbilical cord.

Limitations

This study has some limitations. Primarily, each group included in the study consisted of nine cases. The decreasing trend of the number of COVID-19 cases in our region made it difficult to find pregnant patients who had COVID-19, especially in the third trimester. Comprehensive studies can be conducted with a larger number of pregnant patients who have had COVID-19 by increasing the period for the study. Secondly, cases who had had the disease in the second and third trimesters were included in the study to investigate the long-term effects of COVID-19. Further studies to examine postpartum umbilical cords can be planned with cases who have had COVID-19 in the first trimester and even before pregnancy. Lastly, the use of different primary antibodies may provide important results when evaluating the expression of other proteins important for umbilical cord development besides VEGF and vimentin primary antibodies.

CONCLUSION

Today, both the acute and long-term effects of the COVID-19 disease are still a matter of discussion. One of the most researched subjects among these effects is maternal and infant health. Histopathological and immunohistochemical findings in the umbilical cord, especially in the third trimester of pregnancy, were more prominent, suggesting that more attention should be paid to COVID-19 infection near delivery. Furthermore, the high levels of VEGF and vimentin expression in the umbilical cords of pregnant women in this period may be useful in understanding the post-acute effects of these proteins in the pathogenesis of COVID-19.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Siirt University Non-Interventional Clinical Research Ethics Committee (Date: 13.04.2022, Decision No: 42191).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: All authors state that there is no conflict of interest.

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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The role of MPV/albumin ratio in determining disease severity in acute cholangitis in the emergency medicine

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ABSTRACT

Background: We aimed to examine the relationship of mean platelet volume (MPV) / albumin ratio (MAR) with disease and disease severity in patients with acute cholangitis.

Material and Method: Cases older than 18 years of age, who applied to the emergency department and were diagnosed with acute cholangitis after detailed evaluation were included in the study.Tokyo 2018 criteria are used to determine the severity of acute cholangitis.

Result: There was a positive correlation between MAR levels and Tokyo severity,and increased MAR levels were determined as an independent predictor for each risk group.The cut-off value of the MAR level in predicting moderate risk compared to the mild risk group was found to be >20.9% with 73.3% sensitivity and 70.6% specificity (AUC±SE=0.785±0.03; +PV= 51.3%, -PV= 86.2%; p< 0.001). The cut-off value of the MAR level in predicting severe risk compared to the moderate risk group was found to be >23.2% with 77.2% sensitivity and 59.1% specificity (AUC±SE=0.744±0.03; +PV= 64.5%, -PV= 72.9%; p<0.001). Mean MAR levels were found to be higher in patients admitted to the ICU compared to those who were not admitted (25.2±6.0 vs 21.3±4.6; p<0.001) and increased MAR levels were a potential risk factor for mortality (HR= 1.09; p<0.001).

Conclusion: We found that the MAR level is a very good marker in determining the severity of acute cholangitis.

Keywords: Abdominal pain,acute cholangitis, jaundice, mean platelet volume, Tokyo 2018

INTRODUCTION

Acute cholangitis is one of the emergencies with rapid onset and high mortality (1). It is associated with obstruction and infection in the biliary system (2). The clinical situation in acute cholangitis is very variable.While the patient may present with mild symptoms and minimal findings; It can also be brought to the emergency room with widespread systemic involvement and septic shock.For this reason, the patient should be evaluated in terms of the severity of acute cholangitis at the time of admission to the emergency department.

Determining the severity in patients diagnosed with acute cholangitis allows timely and effective treatment to be made (3,4). For example, in patients presenting with severe acute cholangitis, rapid and effective biliary drainage plays a major role in improving the clinical situation and reducing morbidity and mortality (5).

Tokyo 2018 criteria are used to determine the severity of acute cholangitis. According to these criteria, it is classified into three groups as mild, moderate and severe acute cholangitis (6). When determining the severity of acute cholangitis in the Tokyo 2018 criteria, extensive systemic evaluation, extensive laboratory evaluation and radiological evaluation are required. Because of this wide evaluation, acute cholangitis severity classification may not be possible in the early period in acute cholangitis patients who apply to the emergency department. For this reason, there is a need for inexpensive and reliable markers that can be measured quickly within the first few hours of admission to the emergency department, which determine the severity of acute cholangitis.

In our literature review, we could not find any study on mean platelet volume (MPV) / albumin ratio (MAR). MPV rate is an increasing parameter in both the

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gastrointestinal system and the infection rates in other foci (7-12). Albumin is a negative acute phase reactant that decreases in case of infection.Therefore, we think that the MAR will be an index with high sensitivity and specificity in acute cholangitis.

Therefore, in this study, we aimed to examine the relationship of MAR with disease and disease severity in patients with acute cholangitis.

MATERIAL AND METHOD

Study Population

This study was planned as a retrospective study in Ankara City Hospital Internal Medicine Clinic. The study was carried out with the permission of Ankara City Hospital No:2 Clinical Researches Ethics Committee (Date: 06.07.2022, Decision No: E2-22-2155). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Cases older than 18 years of age, who applied to the emergency department with complaints such as abdominal pain, jaundice, fever, nausea, vomiting, back pain, and were diagnosed with acute cholangitis after detailed evaluation were included in the study. Patients were included in our hospital from March 2019 to January 2022 according to the order of admission, regardless of gender and age.

Cases with infection in another known focus, active rheumatic disease, acute liver failure, chronic liver failure, diagnosed malignancy, malnutrition, using immunosuppressive therapy for any reason, and severe alcohol dependence were excluded from the study. The clinical demographic findings and laboratory parameters of the patients were obtained from the electronic files of the patients.

The severity of acute cholangitis in the emergency department was determined according to the Tokyo 2018 criteria (6).

The MPV / albumin ratio was calculated by dividing the MPV level by the direct albumin level.

Statistical Analysis

Statistical analysis was performed using SPSS 20 for Windows (IBM Corp., Armonk, NY, USA). The normal distribution of data was evaluated by the Shapiro-Wilk test. Numeric variables with and without normal distribution were plotted as mean±standard deviation and median (25^{th} and 75^{th} interquartile range (IQR)), respectively. Categorical variables were indicated as numeric and percentile values. Chi-square, Yates correction and Fischer's exact tests were used for comparison of categorical data. Student-T test or Mann-Whitney U test were used for comparison of numeric variables between the two groups according to the distribution of normality. ANOVA test (post-hoc: Bonferroni test) or Kruskall Wallis H test (post-hoc: Dunn's test) were used for comparison of numeric variables between the Tokyo severity groups according to the distribution of normality. Logistic regression analysis was used to identify independent predictors of Tokyo severity and ICU admission. Cox regression analysis was used to identify independent predictors of in-hospital mortality. Diagnostic performance evaluation of MAR was done by receiver operating characteristics curve analysis and the cut-off values were determined according to the Youden index method. P values $p<0.05$ (*) were considered significant in statistical analysis.

RESULT

The study population consisted of 454 patients, 340 of which were choledocholithiasis (74.9%), 44 benign biliary stenosis (9.7%), 55 malignancy (12.1%) and 15 other causes of cholangitis (3.3%).The characteristics findings of the patients are shown in **Table 1** in detail. According to Tokyo severity, 54.6% of patients had mild acute cholangitis, 23.1% moderate, and 22.1% severe acute cholangitis. It was determined that the incidence of malignant etiology, median urea levels, median CRP levels, median procalcitonin levels, mean MPV levels and mean MAR levels were increased as Tokyo severity increased, while median platelet levels, median alanine aminotransferase (ALT) levels an mean albumin levels were decreased (**Table 1**).

In the multivariable regression model, in which the variables associated with Tokyo 2018 severity were included; independent predictors of the moderate risk group compared to the mild risk group were found to be increasing age, increasing total bilirubin, increasing procalcitonin and increasing MAR levels. Independent predictors of severe risk group compared to moderate risk group were increased urea, and increased MAR levels. According to this; compared to the mild risk group, a %1 increase in the MAR level increased the moderate risk 1.38 folds (OR=1.38; $p<0.001$), compared to the moderate risk group, it increased the severe risk 1.14 folds (OR=1.14; $p<0.001$) (**Table 2**). The cut-off value of the MAR level in predicting moderate risk compared to the mild risk group was found to be >20.9% with 73.3% sensitivity and 70.6% specificity (AUC±SE=0.785±0.03; +PV= 51.3%, -PV= 86.2%; $p< 0.001$) (**Figure A**). The cut-off value of the MAR level in predicting severe risk compared to the moderate risk group was found to be >23.2% with 77.2% sensitivity and 59.1% specificity (AUC±SE=0.744±0.03; +PV= 64.5%, -PV= 72.9%; $p<0.001$) (**Figure 1B**).

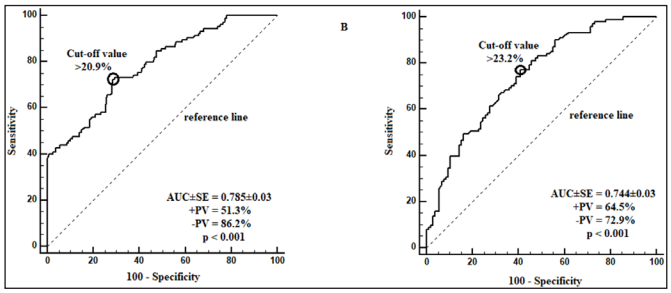

Figure 1. Diagnostic performance assessment of MAR levels in predicting moderate **(A)** and severe **(B)** of TOKYO severity

Findings related to ICU admission and mortality are shown in **Table 3** and **Table 4** in detail. MeanMAR levels were found to be higher in patients admitted to the ICU compared to those who were not admitted (25.2±6.0 vs 21.3±4.6; p<0.001) and increased MAR levels were a potential risk factor for mortality (HR= 1.09; p<0.001).

Table 1. Demographic and clinical findings of patients with acute cholangitis

Variables	All population n=454	TOKYO Severity			p
		Mild n=248	Moderate n=105	Severe n=101	
Demographicfindings					
Age, years	66.3±16.5	59.0±16.1	75.4±12.7	74.9±11.8	<0.001*
Gender, n(%)					0.500
Female	215(47.4)	114(46.0)	55(52.4)	46(45.5)	
Male	239(52.6)	134(54.0)	50(47.6)	55(54.5)	
Etiology, n(%)					0.002*
Choledocholithiasis	340(74.9)	195(78.6)	76(72.4)	69(68.3)	
Benign biliary stenosis	44(9.7)	29(11.7)	7(6.7)	8(7.9)	
Malignancy	55(12.1)	18(7.3)	15(14.3)	22(21.8)	
Other	15(3.3)	6(2.4)	7(6.7)	2(2.0)	
Duration of service, days	8(5-12)	8(5-10)	8(6-12)	8(5-15)	0.449
ICU hospitalization, n(%)	104(22.9)	21(8.5)	24(22.9)	59(58.4)	<0.001*
Duration of ICU, days	6(4-10)	5(2-7)	6(3.5-10)	6(4-11)	0.104
Laboratuar findings					
WBC, $\times 10^3$ /mL	10.2(7.6-13)	9.2(7.2-11.2)	12.9(10.2-16.2)	11.1(8-15)	<0.001*
Platelet, $\times 10^3$ /mL	240(191-312)	269.5(213.5-329)	231(191-294)	161(106-235)	<0.001*
Hemoglobin, g/dL	13.2±1.9	13.6±1.8	12.9±1.7	12.4±2.2	<0.001*
URE, mg/dL	37(27-54)	29(23-38)	42(32-54)	66(47-89)	<0.001*
ALT, U/L	188.5(98-340)	237(123.5-392)	162(105-284)	132(69-230)	<0.001*
AST, U/L	171.5(87-307)	185(90-329.5)	155(95-278)	135(77-259)	0.145
ALP, U/L	253.5(184-420)	240.5(168.5-391)	310(199-457)	257(194-354)	0.021*
GGT, U/L	445(245-708)	532(267.5-769)	457(272-741)	330(181-511)	<0.001*
Total bilirubin, mg/dL	4.6(2.9-7)	3.7(2.2-5.8)	5.9(4.1-8.4)	5.6(3.9-9.6)	<0.001*
Direct bilirubin, mg/dL	3.2(1.9-5.1)	2.5(1.3-4)	4.2(2.6-5.9)	4.1(2.8-6.9)	<0.001*
Albumin, g/dL	39.4±5.3	42.1±3.9	37.7±4.8	34.5±4.8	<0.001*
CRP, mg/dL	60(24-120)	34(17-87)	90(48-132)	110(57-179)	<0.001*
Procalcitonin, mcg/L	0.7(0.2-5.5)	0.3(0.1-1.6)	1.6(0.3-7.4)	5.4(1-32.1)	<0.001*
INR	1.2±0.5	1.1±0.2	1.2±0.1	1.6±0.5	<0.001*
MPV, fL	8.5±1.1	8.2±0.8	8.7±1.2	9.2±1.3	<0.001*
MAR	22.2±5.3	19.5±2.3	23.5±4.8	27.4±6.3	<0.001*
Composite outcome, n(%)	56(12.3)	8(3.2)	13(12.4)	35(34.7)	<0.001*
Mortality, n(%)	25(5.5)	4(1.6)	4(3.8)	17(16.8)	<0.001*
Duration of hospitalization, day	9(6-13)	8(6-11)	10(6-13)	13(8-18)	<0.001*

Data are mean±standard deviation or median (IQR), or number (%). *p<0.05 indicates statistical significance. Bold characters show the difference between groups. Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; CRP, C reactive protein; GGT, gamma glutamyl transferase; ICU, intensive care unit; INR, international normalized ratio; MAR, MPV to Albumin ratio

Table 2. Independent predictors forTOKYO severity

Variables	OR	95% CI		p
		Lower	Upper	
TOKYO Severity				
Moderate (ref: mild)				
Age	1.08	1.05	1.11	<0.001*
Total bilirubin, mg/dL	1.20	1.10	1.30	<0.001*
Procalcitonin, mcg/L	1.04	1.01	1.07	0.013*
MAR	1.38	1.22	1.57	<0.001*
Nagelkerke R2= 0.528, p< 0.001				
Severe (ref: moderate)				
UREA, mg/dL	1.03	1.02	1.05	<0.001*
MAR	1.14	1.07	1.22	<0.001*
Nagelkerke R2= 0.395, p< 0.001				

*p<0.05 indicates statistical significance. Abbreviations: CI, confidence interval; MAR, MPV to Albumin ratio; OR, odds ratio.

Table 5. Independent predictors for endpoints

Variables	OR	95% CI		p
		Lower	Upper	
ICU admission				
Age	1.03	1.01	1.05	0.016*
Etiology, n(%)				
Bening	ref			
Malignancy	2.78	1.39	5.54	0.004*
Tokyo Severity				
Mild	ref			
Moderate	1.96	0.97	3.98	0.062
Severe	9.48	4.96	18.14	<0.001*
MAR	1.08	1.03	1.06	0.002*
Nagelkerke R2= 0.380, p< 0.001				
Mortality	HR			
Etiology, n(%)				
Bening	ref			
Malignancy	2.62	1.03	6.64	0.043*
ICU admission	5.14	1.05	10.41	0.018*
MAR	1.05	1.01	1.10	0.021*
-2Log Likelihood=188.2, p<0.001				

*p<0.05 indicates statistical significance. Abbreviations: CI, confidence interval; MAR, MPV to Albumin ratio; HR, hazard ratio; ICU, intensive care unit; OR, odds ratio.

Table 3. Factors associated with hospitalization of the intensive care unit in patients with acute cholangitis

Variables	ICU admission		p
	No n=350	Yes n=104	
Demographic findings			
Age, years	63.8±16.7	74.8±12.6	<0.001*
Gender, n(%)			0.999
Female	166(47.4)	49(47.1)	
Male	184(52.6)	55(52.9)	
Etiology, n(%)			<0.001*
Benign	320(91.4)	79(76.0)	
Malignancy	30(8.6)	25(24.0)	
TOKYO severity, n(%)			<0.001*
Mild	227(64.9)	21(20.2)	
Moderate	81(23.1)	24(23.1)	
Severe	42(12.0)	59(56.7)	
Duration of service, days	8(6-11)	7(1-13)	0.003*
Duration of ICU, days	-	6(4-10)	-
Laboratuar findings			
WBC, x10^3 /mL	10(7.5-12.6)	11.45(8.4-14.85)	0.007*
Platelet, x10^3 /mL	247.5(197-315)	215(145-296)	0.002*
Hemoglobin, g/dL	13.3±1.9	12.9±1.9	0.107
URE, mg/dL	34(25-48)	52(36-72)	<0.001*
ALT, U/L	201(104-348)	168.5(84-296)	0.145
AST, U/L	165(82-302)	189(98.5-308.5)	0.202
ALP, U/L	244(176-402)	311.5(198.5-444)	0.033*
GGT, U/L	464.5(251-731)	391(233.5-619)	0.172
Total bilirubin, mg/dL	4.3(2.7-6.6)	5.7(3.95-9.2)	<0.001*
Direct bilirubin, mg/dL	2.9(1.6-4.7)	4.2(2.75-6.8)	<0.001*
Albumin, g/dL	40.3±5.0	36.4±5.3	<0.001*
CRP, mg/dL	55(22-105)	100(35.5-160)	<0.001*
Procalcitonin, mcg/L	0.5(0.2-4.2)	1.95(0.4-14.95)	<0.001*
INR	1.2±0.47	1.37±0.4	0.001*
MPV, fL	8.4±1.1	8.9±1.2	<0.001*
MAR	21.3±4.6	25.2±6.0	<0.001*
Composite outcome, n(%)	3(0.9)	53(51.0)	<0.001*
Mortality, n(%)	3(0.9)	22(21.2)	<0.001*
Duration of hospitalization, day	8(6-11)	13(8-19)	<0.001*

Data are mean±standard deviation or median (IQR), or number (%). *p<0.05 indicates statistical significance. Bold characters show the difference between groups. Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; CRP, C reactive protein; GGT, gamma glutamyl transferase; ICU, intensive care unit; INR, international normalized ratio; MAR, MPV to Albumin ratio

Table 4. Factors associated with in-hospital mortality in patients with acute cholangitis

Variables	Survival		Univariable Regression			
	Alive n=429	Exitus n=25	HR	95% CI lower	95% CI upper	p
Demographic findings						
Age, years	65.8±16.5	74.3±14.4	1.02	0.99	1.05	0.228
Gender, n(%)						
Female	206(48.0)	9(36.0)	ref			
Male	223(52.0)	16(64.0)	1.10	0.46	2.61	0.831
Etiology, n(%)						
Bening	387(90.2)	12(48.0)	ref			
Malignancy	42(9.8)	13(52.0)	5.38	2.30	12.60	<0.001*
TOKYO severity, n(%)						
Mild	244(56.9)	4(16.0)	ref			
Moderate	101(23.5)	4(16.0)	1.54	0.38	6.27	0.547
Severe	84(19.6)	17(68.0)	4.36	1.39	13.69	0.012*
Duration of service, days	8(5-12)	9(1-15)	1.04	1.01	1.08	<0.001*
ICU hospitalization, n(%)	82(19.1)	22(88.0)	12.08	3.50	41.76	<0.001*
Duration of ICU, days	6(4-9)	4.5(2-13)	0.95	0.90	1.01	0.115
Composite outcome, n(%)	31(7.2)	25(100.0)	52.60	4.40	635.50	0.010*
Duration of hospitalization, day	9(6-13)	11(5-20)	-	-	-	-
Laboratuar findings						
WBC, x10^3 /mL	10.1(7.6-12.9)	11.8(7.9-15.3)	1.02	0.96	1.08	0.583
Platelet, x10^3 /mL	240(193-308)	219(152-355)	1.00	1.00	1.01	0.166
Hemoglobin, g/dL	13.3±1.8	11.8±2.4	0.77	0.64	0.94	0.010*
URE, mg/dL	36(26-53)	44(36-87)	1.02	1.01	1.03	0.001*
ALT, U/L	207(109-348)	71(38-108)	0.99	0.98	1.00	<0.001*
AST, U/L	175(91-311)	115(58-222)	1.00	0.99	1.00	0.147
ALP, U/L	249(179-393)	415(282-518)	1.00	1.00	1.00	0.788
GGT, U/L	457(256-710)	308(169-583)	1.00	1.00	1.00	0.314
Total bilirubin, mg/dL	4.5(2.8-6.9)	7.9(4.5-11.1)	1.02	0.97	1.08	0.366
Direct bilirubin, mg/dL	3.1(1.8-4.9)	5.8(3.2-8.4)	1.04	0.97	1.11	0.296
Albumin, g/dL	39.8±4.9	31.8±6.5	0.84	0.79	0.90	<0.001*
CRP, mg/dL	59(24-119)	96(29-131)	1.00	1.00	1.01	0.521
Procalcitonin, mcg/L	0.6(0.2-5.5)	1.3(0.6-5.3)	1.00	0.98	1.01	0.719
INR	1.2±0.4	1.5±0.4	1.05	0.61	1.82	0.864
MPV, fL	8.5±1.1	8.9±1.3	1.06	0.77	1.46	0.721
MAR	21.8±4.6	29.6±9.0	1.09	1.05	1.13	<0.001*

Data are mean±standard deviation or median (IQR), or number (%). *p<0.05 indicates statistical significance. Bold characters show the difference between groups. Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; CRP, C reactive protein; GGT, gamma glutamyl transferase; ICU, intensive care unit; INR, international normalized ratio; MAR, MPV to Albumin ratio

DISCUSSION

In this study, we examined the relationship between acute cholangitis and MAR and the role of MARin determining the severity of acute cholangitis. In our opinion, this study is the first study in this field.

We did not find any study on the MAR in any field. Although MPV levels have been studied in many disease groups, we could not find any study on acute cholangitis. In the study conducted by Şeker et al. (13) in a limited number of cholecystitis cases, MPV levels were found to be lower in acute cholecystitis cases compared to chronic cholecystitis cases. Bozkurt et al. (14), in a study conducted in acute appendicitis cases, found that MPV level was not a good marker for the diagnosis of acute appendicitis. In a study in inflammatory bowel disease, MPV was found to be a good marker for the activity of inflammatory bowel disease (8). There are also studies showing the relationship between mpv and mortality in intensive care patients (15).

When we examine the studies made with the indexes created with albumin, we often come across studies on the CRP/albumin ratio. In a study conducted by Behera et al. (16) in patients with acute pancreatitis, it was determined that the CRP/albumin ratio was correlated with prognostic scores such as Ranson and Atlanta, which predict the prognosis of acute pancreatitis. In a study of 876 people including ulcerative colitis and crohn's patients, CRP/albumin ratio was found to be associated with inflammatory bowel disease disease activity (17). Apart from these studies, it has been determined that the CRP/albumin ratio is closely related to prognosis in many gastrointestinal system malignancies (18-22).

In our study, when we classified acute cholangitis patients according to Tokyo 2018, the MAR level was found to be highest in patients with severe acute cholangitis, lower in patients with moderate cholangitis compared to patients with severe cholangitis, and higher in patients with mild cholangitis. In our regression analysis, it was determined that the MAR level predicted the severity of acute cholangitis classification according to Tokyo 2018 separately in each group. When we reviewed a meta-analysis published in the literature, the role of procalcitonin in determining the severity of acute cholangitis was examined. Six studies were included in this study. We found that the sample size was lower in all studies than in our study.In all studies, procalcitonin level was found to be higher in severe acute cholangitis patients compared to mild and moderate acute cholangitis patients (23). In our study, similar to the above studies, procalcitonin level was found to be highest in severe acute cholangitis patients, then in moderate acute cholangitis patients and lowest in mild cholangitis patients. However, in our regression analysis, procalcitonin level was found to be associated with severe acute cholangitis, but not with mild or moderate acute cholangitis. MAR was found to be better than procalcitonin in this sense. In addition, MAR level was found to be a risk factor associated with mortality in our study, while proclacytonin was not found to be associated with mortality.

In our study, we classified acute cholangitis according to the Tokyo 2018 criteria. Although MAR level and Tokyo 2018 criteria could predict admission to intensive care unit, MAR level was found to be associated with mortality, Tokyo 2018 was not associated with mortality. When we look at this secondary result of our study, we can say that the MAR level is a marker that makes the classification of acute cholangitis severity well, and that it is also a good marker that predicts both mortality and admission to the intensive care unit.In addition, in our study, together with procalcitonin, platelet, ALT, CRP, MPV, albumin and urea levels also differed in mild, moderate and severe groups of acute cholangitis, similar to MAR levels. However, no parameter was found to be associated with mild, moderate and severe groups of acute cholangitis, similar to the MAR level in the regression analysis.

The main limitation of our study is its retrospective nature. However, vital signs, physical examination findings, laboratory tests and radiological images of our patients are recorded regularly from the emergency department to the final hospitalization. This minimizes this limitation. Another limitation of ours is that it is not known when the acute cholangitis started in the cases and how long after the symptom onset the cases were admitted to the hospital.However, considering the nature of the disease, this limitation is valid for all studies conducted in this field.

CONCLUSION

In conclusion, in our study, we found that the MAR was an index related to the severity of acute cholangitis. We found that the MAR increased with the increase in the severity of acute cholangitis and the cut-off values predicting the severity. In addition, determining the MAR as an index predicting admission to intensive care and mortality is among the secondary gains of our study. More prospective studies are needed for the MAR level to be an important predictor of the severity of acute cholangitis..

ETHICAL DECLERATIONS

Ethics Committee Approval: The study was carried out with the permission of Ankara City Hospital No:2 Clinical Researches Ethics Committee (Date: 06.07.2022, Decision No: E2-22-2155).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All the authors declare that they have all participated in the design, statistical evaluation and writing and that they have approved the final version.

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Evaluation of strain echocardiography and atrial electromechanical delay in patients with idiopathic carpal tunnel syndrome

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ABSTRACT

Aim: Carpal tunnel syndrome (CTS) could be an early marker for amyloidosis before developing of overt symptoms of cardiac amyloidosis (CA). CA characterized with left ventricular (LV) diastolic dysfunction and impairment of LV deformation-based parameters. There is limited data about echocardiographic parameters such as strain value of LV, diastolic parameters and atrial EMD in patients with idiopathic CTS. In this study, we investigated LV strain values, diastolic parameters of LV and atrial EMD in patients with idiopathic CTS. Then, we compared these parameters in CTS patients to control group.

Material and Method: Thirty-four patients with idiopathic CTS and twenty-four aged and sex matched volunteers were enrolled to study. Patients with known amyloidosis, heart failure, diabetes mellitus and secondary etiologic states for CTS such as trauma or rheumatologic disease were excluded from the study. ECG and echocardiographic examination of each patient were performed and recorded by cardiology specialist. Conventional and strain imaging echocardiography were performed. Atrial electromechanical delays (EMD) were measured.

Results: Baseline characteristics features were not different in groups. Mitral inflow velocities (mitral E and A wave), mitral E wave deceleration time, tissue Doppler velocities (lateral annular E' and A wave), E/A and E/E' ratios were similar in two groups. Septal basal strain values increased in CTS group (-21.3±4.83% vs -25.7±2.96%, p<0.001). Septal apical to base ratio (SAB) and relative apical sparing (RELAPS) were increased in CTS group compared to control group (0.94±0.43 vs 0.66±0.12, 0.90±0.31 vs 0.73±0.08, p=0.004, p=0.013, respectively). PA lateral, PA septal, inter-atrial EMD and intra-atrial EMD were significantly higher in CTS group compared to control group (78.2±12.3 ms vs 70.6±9.9 ms, 64.1±8.42 ms vs 58.3±10.1 ms, 25.8±9.09 ms vs 20.7±5.31 ms, 11.68±5.11 ms vs 8.46±3.02 ms, p=0.015, p=0.023, p=0.009 and p=.008, respectively).

Conclusion: In CTS group, mean basal strain decreased compared to control group. SAB and RELAPS which associate with CA, decreased in CTS group. Atrial EMD prolonged in CTS group. These changes may associate with increased risk of CA and AF in patients with CTS.

Keywords: Carpal tunnel syndrome, strain echocardiography, atrial electromechanical delay

INTRODUCTION

Carpal tunnel syndrome (CTS) is a peripheral nerve entrapment syndrome which is sometimes associated with amyloidosis. Amyloids accumulate over the flexor tenosynovium and transverse carpal ligament of the hand and make symptoms of CTS (1). Recent studies showed that CTS could be an early marker for amyloidosis before developing of overt symptoms of cardiac amyloidosis (CA) (1,2).

Heart failure with preserved ejection fraction (HFPEF) negatively affect quality of life and life expectation (3). Frequency of HFPEF increase in the population from years to years(4). CA is often missed cause of HFPEF (5). CA characterized with left ventricular (LV) diastolic dysfunction and impairment of LV deformation-based parameters (6, 7). Diastolic dysfunction associate with higher LV filling pressure and consequence of left atrial (LA) enlargement which makes electrical instability (8). Atrial electromechanical delay (EMD) is non-invasive method that is used marker for developing atrial fibrillation (AF) (9). There is limited data about echocardiographic parameters such as strain value of LV, diastolic parameters and atrial EMD in patients with idiopathic CTS.

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In this study, we investigated LV strain values, diastolic parameters of LV and atrial EMD in patients with idiopathic CTS. Then, we compared these parameters in CTS patients to control group.

MATERIAL AND METHOD

The study was carried out with the permission of Kayseri City Education and Research Hospital, Noninvasive Clinical Ethics Committee (Date: 04.03.2021, Decision No: 318). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Thirty-four patients who examined in electroneuromyography (EMG) and diagnosed CTS enrolled to CTS group. Twenty-four aged and sex matched volunteers were enrolled to study as a control group. Detailed neurological and EMG examinations were performed by neurology specialist. Patients with idiopathic CTS which defines as Katz diagnostic definition were included to study (10). Patients with known amyloidosis, heart failure, diabetes mellitus and secondary etiologic states for CTS such as trauma or rheumatologic disease were excluded from the study. ECG and echocardiographic examination of each patient were performed and recorded by cardiology specialist. Informed consent was signed by the participants.

Electroneuromyography

EMG studies were achieved at room temperature, using the EMG device (Dantec Keypoint system, by Natus Neurology) and the skin temperature was 32°C.

Both upper extremity median and ulnar nerves were used for conduction properties. Neurology specialist agreed with general standards of EMG. Motor conduction properties were calculated using superficial disc electrodes and orthodromic method, sensory conduction studies were performed using ring electrodes and antidromic method. Conduction velocity and amplitudes were recorded as well as motor and sensory latencies of the median and ulnar nerves.

Echocardiography

Echocardiographic examinations of all participants were performed by a cardiology specialist. Left lateral position and apical 2 and 4 cavity images were obtained from the parasternal short and long axes. M-mode was used to measure the left ventricular (LV) end-systolic and end-diastolic diameters from the parasternal long axis (at the mitral chordal level perpendicular to the long axis of the ventricle). Eccentricity index calculated as the ratio of interventricular septum thickness at end diastole over the posterior wall thickness at end diastole. Relative wall thickness's (RWT) formula is calculated as; 2x(posterior wall thickness at end diastole) /LV end-diastolic diameter. Longitudinal strain analyses of the LV were performed offline (QLab 7.0, Philips Medical Systems, USA) using zoom mode images of the LV in four and two chamber views. Point and select method was used to manually trace the endocardial edge of the LV. The region of interest was fitted to the thickness of the ventricular myocardium. In each view, the LV was automatically divided into six segments giving longitudinal strain curves from a sum of 12 segments. Septal apical to base ratio (SAB) and relative apical sparing (RELAPS) were calculated and defined as below. SAB is ratio of septal apical strain to septal basal strain. RELAPS is ratio of average apical strain values of LV to average basal and mid strain values of LV.

Atrial EMD was defined as the time interval from the onset of atrial electrical activity (P wave on surface ECG) to the beginning of mechanical atrial contraction (late diastolic A wave). All values were averaged over 3 consecutive beats. Atrial EMD was measured from the lateral mitral annulus and called 'PA lateral', from the septal mitral annulus, called 'PA septal', and from the right ventricle tricuspid annulus, called 'PA tricuspid' (9).

Statistical Analyses

The Statistical Package for Social Sciences software program (SPSS, version 25,0 for Windows) was used for statistical analysis. Continuous variables were given as means±SD; categorical variables were defined as percentages. The Shapiro-Wilk or Kolmogorov-Smirnov tests were used to test the normality of the distribution of continuous variables. Continuous variables were compared between groups using the Student's t test or Mann-Whitney U test as appropriate. The $\chi 2$ test was used for univariate analysis of the categorical variables. A probability value of $p < 0.05$ was considered significant, and 2-tailed p values were used for all statistics.

RESULTS

Baseline characteristics features were shown in **Table 1**. Age and female sex ratios were not different in two groups, statistically (52.8±11.6 years vs 53.6±11.0 years, %44 vs %41, respectively). Echocardiographic parameters were shown in **Table 2**. LA diameter and area were higher in CTS group, but the changes did not reach to statistical significancy (3.46±0.35 cm vs 3.32±0.29 cm, 13.45±2.91 cm^2 vs 12.37±2.00 cm^2 , p=0.109, p=0.120, respectively). LV diameters were similar in two groups. Mitral inflow velocities (mitral E and A wave), mitral E wave deceleration time, tissue Doppler velocities (lateral annular E' and A wave), E/A and E/E' ratios were similar in two groups. Strain values were shown in **Table 3**. Septal basal strain values increased in CTS group (-21.3±4.83 % vs -25.7±2.96 %,

p<0.001) (**Figure 1**). SAB and RELAPS were increased in CTS group compared to control group (0.94±0.43 vs 0.66±0.12, 0.90±0.31 vs 0.73±0.08, p=0.004, p=0.013, respectively) (**Figure 2**).

Table 1. Baseline demographic and clinical features of groups

	CTS group n=34	Control group n=24	P
Age, years	52.8±11.6	53.6±11.0	0.793
Female sex	15 (44%)	10 (41%)	0.424
Hypertension	16 (47%)	16 (66%)	0.183
Smoking	13 (38%)	8 (33%)	0.786
BMI, kg/m²	25.9±3.2	26.3±3.3	0.649
Systolic blood pressure, mmHg	124.7±14.7	124.6±15.0	0.801
Diastolic blood pressure,mmHg	77.7±8.4	79.2±9.9	0.549

BMI= Body mass index

Table 2. Echocardiographic parameters of groups

	CTS group n=34	Control group n=24	P
LA Diameter, cm	3.46±0.35	3.32±0.29	0.109
LA Area, cm²	13.45±2.91	12.37 2.00	0.120
LVSd, cm	3.26±0.30	3.33±0.30	0.382
LVDd, cm	4.89±0.38	5.05±0.45	0.132
IVSd, cm	1.10±0.13	1.07±0.15	0.514
PWd, cm	1.06±0.11	1.02±0.10	0.222
LVEF, %	62.60±3.72	62.8±3.10	0.808
Eccentricity index	1.03±0.053	1.05±0.077	0.493
RWT	0.435±0.048	0.405±0.036	0.014
Mitral E wave velocity, cm/s	0.78±0.16	0.80±0.12	0.645
Mitral A wave velocity, cm/s	0.67±0.08	0.63 0.9	0.149
DT, ms	176.1±23.5	170±24.4	0.398
Lateral E', cm/s	10.2±2.65	10.9 2.49	0.299
Lateral A', cm/s	8.15 1.94	7.92±1.71	0.643
E/A	1.18±0.27	1.29±0.30	0.154
E/E'	0.79±0.017	0.75±0.14	0.372
Global longitudinal strain,%	-20.2±2.4	-21.0±1.37	0.118
Mean basal strain, %	-21.3±4.83	-25.7±2.96	<0.001
Mean mid strain,%	-20.77±3.95	-20.45±2.71	0.737
Mean apical strain,%	-18.46±5.22	-16.95±1.98	0.183
SAB	0.94±0.43	0.66±0.12	0.004
RELAPS	0.90±0.31	0.73±0.08	0.013

LA=Left atrium; LVDD=Left ventricle end-diastolic dimension; LVSD=Left ventricle end-systolic dimension; IVS=interventricular septum thickness; PW=posterior wall thickness; LVEF=Left ventricle ejection fraction; DT=deceleration time; IVRT=isovolumic relaxation time; RWT= Relative wall thickness; SAB= septal apical to base longitudinal strain; RELAPS= relative apical sparing (ratio of apical longitudinal /sum of base and mid longitudinal strain)

Table 3. Atrial electromechanical delay values of groups

	CTS group n=34	Control group n=24	P
PA lateral, ms	78.2±12.3	70.6±9.9	0.015
PA septal, ms	64.1±8.42	58.3±10.1	0.023
PA tricuspid, ms	52.4±6.44	49.9±10.2	0.255
Inter-atrial EMD, ms	25.8±9.09	20.7±5.31	0.009
Intra-atrial EMD, ms	11.68±5.11	8.46±3.02	0.008
Left-atrial EMD, ms	14.15±5.47	12.25±4.79	0.177

EMD= Electromechanical delay

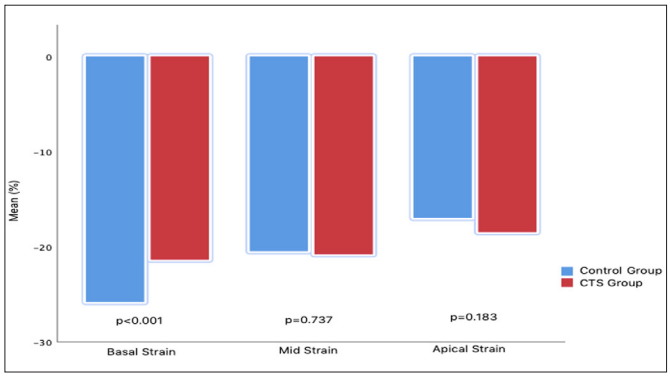

Figure 1. Longitudinal strain values of left ventricular segments of groups

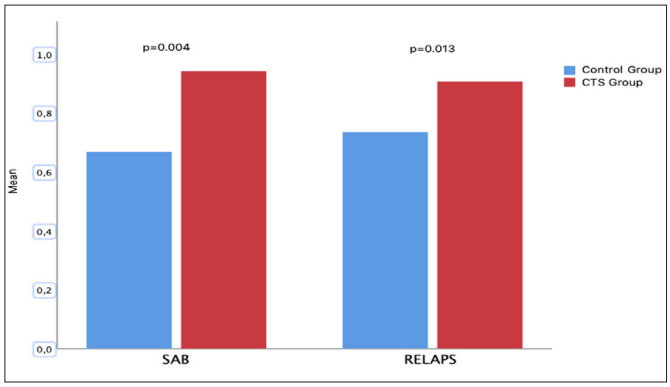

Figure 2. Septal apical to base ratio (SAB) and relative apical sparing (RELAPS) values of groups

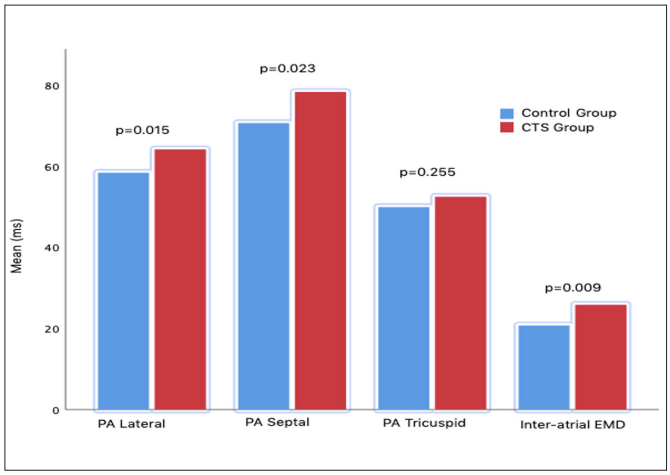

Figure 3. Atrial electromechanical delay values of groups

Table 3 showed atrial EMD values of the groups. PA lateral, PA septal, inter-atrial EMD and intra-atrial EMD were significantly higher in CTS group compared to control group (78.2±12.3 ms vs 70.6±9.9 ms, 64.1±8.42 ms vs 58.3±10.1ms, 25.8±9.09 ms vs 20.7±5.31 ms, 11.68±5.11 ms vs 8.46±3.02 ms, p=0.015, p=0.023, p=0.009 and p=.008, respectively). PA tricuspid and left-atrial EMD were also higher in CTS group but not statistically significant (52.4±6.44 ms vs 49.9±10.2 ms, 14.15±5.47 ms vs 12.25±4.79 ms, p=0.255 and p=0.177, respectively). Figure 3 showed the atrial EMD parameters of the groups.

DISCUSSION

In our knowledge, this is the first study which evaluate echo parameters in patients with idiopathic CTS. Three important findings were revealed in this study. First, there is no differences in conventional echocardiography parameters in two groups. Second, LV basal segments strain were decreased in CTS group. Another finding is that atrial EMD delayed in CTS group.

Systemic wild-type ATTR (ATTRwt) amyloidosis whose prevalence increase with age, is a disease occurred by the extracellular deposition of amyloid fibrils composed of wild-type transthyretin (TTR)(11). Systemic ATTRwt amyloidosis is rarely diagnosed in general population. Nakagawa et al. (12) found that CTS is the most common first symptom of systemic (ATTRwt) amyloidosis. CA can be diagnosed years after CTS symptoms (13). So, patients with CTS may prone to CA.

Previous studies showed basal strain values significantly decrease patients with CA. Phelan et al. (7) found global longitudinal strain significantly decreased in patients with CA. Especially basal segments were more affected from the decreasing. They found the mean value of %-8.9 for global longitudinal strain and %-3.3 for basal segments longitudinal strain. In our study, although global longitudinal strain did not decrease, basal strain values decreased in patients with CTS compared to control. We found %-21.3 value for mean basal segment longitudinal strain for CTS group. However, mean basal segments strain decreased in CTS group compared to control group, these changes did not reach to CA's values. These results may be related to initial changes.

Another important finding is that atrial EMD time prolonged in CTS group. Atrial EMD is an excellent non-invasive method which is used for predicting atrial arrhythmias such as AF (14). Previously, atrial EMD prolonged in various situations such as diastolic dysfunction, hypertension, psoriasis and Behcet disease (9, 15–17). LV hypertrophy is hypothesized mechanism for prolongation atrial EMD in hypertension, diastolic dysfunction, and other disease(15). LV hypertrophy associated with low oxygen demand to myocardium (18). This ischemia is thought link to prolongation of atrial EMD. In our study, we found that LV wall thickness increased in CTS group but not statistically significant. These changes may contribute to prolong of atrial EMD in CTS group.

CA associated with diastolic and systolic dysfunction of LV therefore it is a cause of heart failure. Recently, Sood et al. (19) reported systemic amyloidosis can be diagnosed after carpal tunnel release procedure and heart failure increased 4,68-fold the risk of systemic amyloidosis diagnosis after carpal tunnel release. Heart failure is a risk factor for atrial enlargement and AF(20). Also, heart failure associated with prolonged atrial EMD(20). In our study, the prolongation of atrial EMD in CTS group may link to tendency to heart failure in patients with CTS.

Many inflammatory statements connect to prolongation atrial EMD (9, 17). Inflammatory cells have been observed in the atria of patients with AF. Inflammatory indicators in plasma (e.g., plasma C-reactive protein, interleukin-6 and tumor necrosis factor-α) seem to associate with the achievement of electrical cardioversion and recurrence of AF following cardioversion (21). Karimi et al. (22) found that IL-1, IL-6, IL-10 and tumor necrosis factor-α increase in patients with idiopathic CTS. Increased inflammatory markers in idiopathic CTS may associate with prolonged atrial EMD and higher AF incidence. Our findings support this hypothesis

Future studies which will evaluate inflammatory markers such as IL-1, IL-6, IL-10, tumor necrosis factor- α, atrial EMD and strain echocardiography in patients with idiopathic CTS, may clearly reveal the association between inflammation and echocardiographic changes. Another study that will investigate echocardiographic parameters and carpal tunnel biopsy exhibit amyloidosis, amyloidosis severity and type, will be valuable for understanding association between idiopathic CTS and CA.

In this study, there are several limitations. First, amyloidosis can detect invasively with carpal tunnel release biopsy. If we did carpal tunnel release biopsy, echocardiographic changes could have attributed to CA. Another limitation is relatively small sample size. Data from larger sample size can give more reliable results.

CONCLUSION

In CTS group, mean basal strain decreased compared to control group. SAB and RELAPS which associate with CA, decreased in CTS group. Atrial EMD prolonged in CTS group. These changes may associate with increased risk of CA and AF in patients with CTS

ETHICAL DECLARATIONS

Ethics committee approval: The study was carried out with the permission of Kayseri City Education and Research Hospital, Noninvasive Clinical Ethics Committee (Date: 04.03.2021, Decision No: 318).

Informed consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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Association of COVID-19 vaccine with lymph node reactivity: An ultrasound-based study

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ABSTRACT

Aim: Millions of people worldwide have been infected and died due to the pandemic caused by COVID-19. Vaccination is the most effective way to deal with the pandemic. Though vaccines are safe, they are not completely risk-free, and some side effects can occur after vaccination such as lymphadenopathy. This study, it was aimed to measure the lymph node reactivity that may develop after mRNA vaccination.

Material and Method: A total of 50 healthy people were included in the study. Left axillary and supraclavicular ultrasound examinations were performed before and one week after the administration of the mRNA vaccine. Each patient was assessed for supraclavicular and level 1 axillary lymph region in terms of the presence, size (long and short axis), and cortex thickness of the lymph nodes.

Results: Of the patients participating in the study, 23 (46 %) were male, 27 (54 %) were female, and the median age was 33. In comparison, the difference in long, short axis and cortex diameter measurements of the supraclavicular lymph node before and after vaccination was found to be statistically significant (p=0.034, 0.021, 0.004, respectively). Similarly, the difference in the long, short axis, and cortex thickness of the left axillary lymph node before and after vaccination was statistically significant (p<0.001, <0.001, <0.001, respectively).

Conclusion: Anti-Covid-19 vaccines may cause lymphadenopathy as a result of reactivation in lymph nodes in the left axillary and supraclavicular regions. When lymphadenopathy is detected in these regions, the vaccine should be questioned in the clinical history and ultrasound follow-up should be performed on the patient.

Keywords: Lymphadenopathy, COVID-19 vaccines, ultrasound

INTRODUCTION

According to the statistics of the World Health Organization (WHO), millions of people around the world were infected and died due to the pandemic caused by COVID-19. One of the effective methods of dealing with the epidemic is vaccination (1). With the mass adoption of vaccination, both prevention of COVID-19 infection and reduction in morbidity and mortality can be achieved (2). However vaccines are highly safe methods, they are not completely risk-free, and some side effects can occur after vaccination (3). The development of lymphadenopathy in patients receiving the COVID-19 vaccine has recently been described in the literature (4). In addition, unilateral axillary and supraclavicular lymphadenopathy cases have been reported in recent articles (5). Sometimes, supraclavicular and axillary lymph nodes (LN) may mimic pathological lymph nodes after the COVID-19 vaccine. Ultrasound (US) examination of lymph nodes may represent a first-line imaging modality due to its rapid application, low cost, and reproducibility (6). The sonologists should consider that anti-COVID-19 vaccines may be involved in the etiology of supraclavicular and axillary lymphadenopathy with suspicious US features. This study, it was aimed to measure the lymph node reactivity that may develop after vaccination.

MATERIAL AND METHOD

This study was approved by the Lokman Hekim University Non-interventional Clinical Researches Ethics Committee (Date 15.02.2022, Decision No: 2022017). All procedures applied in the study were carried out in accordance with the Declaration of Helsinki and ethical principles.

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Before and one week after the administration of mRNA vaccine to the health personnel working in the hospital, the presence of lymph nodes in the left axillary and supraclavicular regions, if any, the short and long axis (**Figure 1 a-b**) and the thickest part of the cortex were measured (**Figure 1 c-d**). Patients with any known oncological, hematological, or autoimmune diseases were not included in the study.

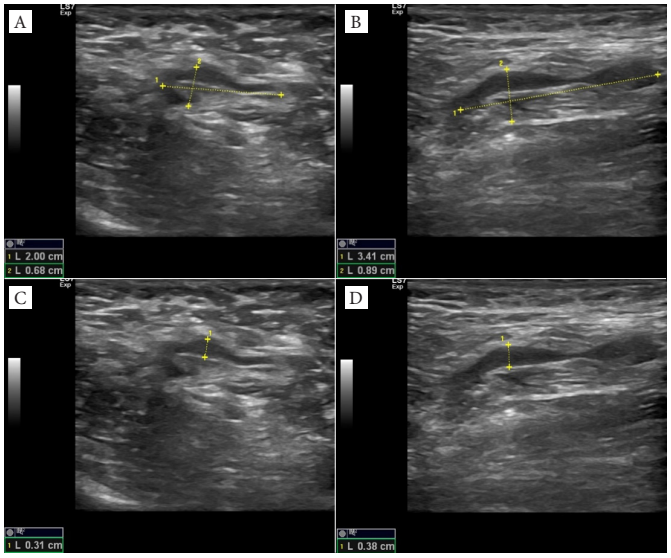

Figure 1. A-36-year-old female patient has reactive lymph nodes (a-b) and cortex thicknesses (c-d) in the left axillary region before and after vaccination.

US protocol

All ultrasonographic procedures were performed by the same radiologist (19 years of experience) using a GE Logiq S7 Expert with a linear 9L-D MHz array probe. The presence of lymph nodes in the level 1 left axillary and supraclavicular regions, the size of the largest lymph node (long and short axis), location, and cortex thickness were recorded in detail for each patient.

Statistical Analysis

Distribution was determined by the Shapiro-Wilk test, skewness and kurtosis values, and histogram graphics. The median (min-max) values of the numerical variables that do not fit the normal distribution are given. Categorical variables were expressed as numbers (percentage distributions). Wilcoxon Signed Ranks Test was used to compare lymph node US measurements before and after vaccination. A p-value of <0.05 was considered statistically significant in all analyzes and these analyzes were performed using the SPSS 25.0 program.

RESULTS

Of the patients participating in the study, 23(46 %) were male, 27 (54 %) were female, and the median age was 33 years. The median time to US controls after vaccination was 7 days.

The comparison of left axillary lymph node measurements measured by the US before and after vaccination is given in **Table 1**. In comparison, the difference in supraclavicular LN long, short axis, and cortex diameter measurements before and after vaccination was found to be statistically significant (p=0.034, 0.021, and 0.004, respectively) (**Table 2**). Similarly, the difference in the long, short axis, and cortex thickness of the axillary LN before and after vaccination was statistically significant (p<0.001, <0.001, <0.001, respectively). There was no statistically significant difference between the long/ short axis ratios of both evaluated lymph nodes before and after vaccination.

Table 1. Comparison of left axillary LN measurements before and after vaccination (n=48)

	Before vaccination median (min-max)	After vaccination median (min-max)	Z value	P-value*
Left Axillary LN long axis	18.0 (12.0-28.0)[1]	22.0 (15.0-37.0)	-5.663	<0.001
Left Axillary LN short axis	6.0 (4.0-11.0)[1]	8.0 (5.0-11.0)	-4.921	<0.001
Left Axillary L/S ratio	2.7 (1.7-4.6)[1]	2.6 (1.8-5.2)	-0.435	0.664
Left Axillary LN cortex thickness	2.0 (1.0-4.0)[1]	3.0 (1.3-6.4)	-5.908	<0.001

* Wilcoxon Signed Ranks Test

Table 2. Comparison of people with positive supraclavicular LN before vaccination with post-vaccine measurements (n=16)

	Before vaccination median (min-max)	After vaccination median (min-max)	Z value	P-value*
Supraclavicular LN long diameter	4.0 (0.0-12.0)	8.5 (0.0-12.0)	-2.123	0.034
Supraclavicular LN short diameter	1.5 (0.0-6.0)	4.0 (0.0-6.0)	-2.307	0.021
Supraclavicular L/S ratio	0.7 (0.0-3.0)	2.0 (0.0-2.6)	-1.790	0.073
Supraclavicular LN cortex thickness	0.7 (0.0-2.7)	2.4 (0.0-5.0)	-2.870	0.004

* Wilcoxon Signed Ranks Test

DISCUSSION

In our study, it was found that mRNA vaccines may cause reactivity in axillary and supraclavicular lymph nodes ipsilateral to the injection site. However, distinguishing lymphadenopathy from abnormal lymph nodes after COVID-19 vaccines has posed a diagnostic challenge (7). We observed those who are generally suspicious of malignancy, such as cortical thickening in some of the patients studied.

The most important criteria to distinguish between normal and abnormal lymph nodes are; the shape of the lymph nodes, their hilum features, and their cortical thickness. Cortical thickness >3 mm, round shape, and change in the echogenic hilum often suggest a pathological process (8,9). Axillary lymphadenopathy has been observed following vaccination with influenza, human papillomavirus vaccines, and recently with COVID-19 mRNA vaccines (10). Garreffa et al. (11) reported in a study they conducted that the frequency of lymphadenopathy detected by imaging varies between 14.5% and 53%.

Hanneman et al. (12) showed that a patient with no history of malignancy who underwent cardiac fluorine 18 F- FDG) PET/MRI investigation the day after injection of the COVID-19 vaccine caused unilateral left axillary lymphadenopathy with moderately increased FDG uptake.

In patients with malignancy, lymphadenopathy may be found incidentally on routine screening or imaging tests such as mammography, CT, or MRI. In such cases, the US examination becomes important (13,14). In some patients, the lymph nodes inthe US have a rounded shape, there is no echogenic hilum. These US features may be of concern, especially in cancer patients (15).

In their study, El-Sayed et al. (16) emphasized that if the lymph node reactivity after the COVID-19 vaccination is not taken into account in malignancy patients who underwent PET CT, it would lead to errors in upgrading. Johnson et al. (17) emphasized that considering the vaccination history in patients who underwent 18F-FDG PET/CT for cancer staging or control would be important in the differential diagnosis. Hagen et al. (18) performed needle biopsy in 5 patients who were admitted with post-vaccine lymph node enlargement with a diagnosis of malignancy and proved that lymph node enlargement was caused by the vaccine.

Özütemiz et al. (19) presented five cases of axillary lymphadenopathy mimicking metastasis after the COVID-19 vaccination. Histopathological evaluation was performed in two cases. However, other cases were recently associated with vaccination. Xu et al. (20) presented that axillary lymphadenopathy cluster in a follow-up FDG PET/CT scan of a patient with mantle cell lymphoma who had a complete metabolic response to treatment was due to COVID-19 vaccine. It is important to know the potential for ipsilateral lymphadenopathy associated with the COVID-19 vaccine to avoid unnecessary biopsy and/or treatment changes. A multidisciplinary expert panel recommended deferring imaging for at least 6 weeks after completion of vaccination to avoid misdiagnosis (21).

Mehta et al. (22) evaluated four cases vaccinated with Pfizer and Moderna vaccine. Unilateral axillary lymphadenopathy was detected in the cases. They concluded that in such cases, in addition to breast cancer, the COVID-19 vaccine should be added to the differential diagnosis. In one case, lymphadenopathy developed 9 days after vaccination. However, other cases were found incidentally. It is not known when the lymph node reaction started after receiving the COVID-19 vaccine. In this study, we found that lymph node reactivity appeared within the first 7 days.

This study has some limitations. First of all, the number of patients included is small. Second, the study has been limited by the absence of a histopathological correlation. Moreover, studies with a longer duration may give us an idea about the outcomes of those lymphadenopathies.

CONCLUSION

Anti-COVID-19 vaccines may cause lymphadenopathy in the axillary and supraclavicular regions. Knowing those patients' history of recent vaccination prevents radiologists from performing unnecessary and costly histopathological evaluations of lymph nodes. Short-interval US control facilitates the follow-up of patients and would give us data about the outcomes of these patients.

ETHICAL DECLARATIONS

Ethics Committee Approval: This study was approved by the Lokman Hekim University Non-interventional Clinical Researches Ethics Committee (Date 15.02.2022, Decision No: 2022017).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version

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Original Article

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Analysis of the factors that affect survival among patients who developed subcutaneous emphysema monitored on COVID-19 diagnosis: single-centred research

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ABSTRACT

Aim: The COVID-19 patients with pulmonary involvement frequently develop pneumothorax, pneumomediastinum and subcutaneous emphysema due to barotrauma. Reviewing the literature, pneumothorax, pneumomediastinum and subcutaneous emphysema it can be observed among ICU patients due to the pulmonary involvement of the COVID-19 disease, and therefore, can cause mortality and morbidity. This study aims to analyse the factors that affect mortality in COVID-19 patients in ICUs who develop subcutaneous emphysema.

Material and Method: A total of 854 COVID-19 patients who were consulted from all branches in the Chest Surgery Clinic of Ankara City Hospital between September 1, 2020 - March 1, 2021 were retrospectively analyzed. Demographic characteristics, comorbid diseases and COVID-related tests (LDH, D-dimer, procalcitonin, ferritin, CRP, IL-6, lymphocyte percentage and neutrophil and lymphocyte ratio) imaging results and survival of 66 patients with subcutaneous emphysema were analyzed.

Results: Of the patients, 41 (62%) were male and 25 (38%) were female. The mean age was 63 years. 55 (83%) of these patients were followed up with invasive ventilation support due to general health impairment, increased oxygen demand and heart problems. Age, intubation and NLR were found to be statistically significant in terms of survival and death, on survival. It was discovered that age and intubation variables could be risk factors. The mortality rates were 1.01 times higher for the elderly compared to the younger patients and 13.8 times higher for the intubated compared to the non-intubated patients.

Conclusion: Age of patient and intubation can be regarded as risk factors for mortality in COVID-19 patients with subcutaneous emphysema, monitored in ICUs. Furthermore, comorbid diseases increase mortality rates.

Keywords: COVID-19, mortality, subcutaneous emphysema

INTRODUCTION

COVID-19, a viral disease, first appeared in Wuhan, China in December 2019. The disease spread rapidly and caused serious health expenses around the world (1,2). The World Health Organization declared a viral pandemic in March 2020, which is also the date the first case was announced by the Ministry of Health of Turkey (3-5). Thorax CT is crucial for the early diagnosis for COVID-19 (6). The most common CT findings include ground-glass appearance with consolidations (6,7). According to the published data, around 20% of the patients were monitored in intensive care units (ICUs) and they showed vascular and pulmonary complications due to COVID-19 (1-4).

The COVID-19 patients with pulmonary involvement frequently develop pneumothorax, pneumomediastinum and subcutaneous emphysema (8). The severe COVID-19 patients monitorized by invasive and non-invasive mechanical ventilation and positive pressure devices (9). Especially in patients with parenchymal involvement, there may appear life-threatening complications such as barotrauma-related pneumothorax, haemothorax, pneumomediastinum and subcutaneous emphysema (8-10). Subcutaneous emphysema is defined as abnormal level of air and gas in subcutaneous tissues. It may develop due to high pressure ventilation (barotrauma) in case of obstructive pulmonary disease, interstitial pulmonary disease and infective diseases with pulmonary parenchymal involvement (11,12).

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This study aimed to analyze the factors affecting mortality in COVID-19 patients who developed subcutaneous emphysema in intensive care units.

MATERIAL AND METHOD

After obtaining the ethics committee approval (E-21-2021), 6187 patients were consulted to the Ankara City Hospital Thoracic Surgery Clinic from the clinics and polyclinics of our hospital between September 1, 2020 - March 1, 2021; 854 patients diagnosed with COVID-19 were retrospectively reviewed. Consultations that unrelated with COVID-19 excluded. Also those who were COVID-19 negative and developed subcutaneous emphysema for any reason were not included in the study. It was found that 66 patient developed subcutaneous emphysema. The demographic characteristics, comorbid diseases and laboratory analysis (LDH, D-dimer, procalcitonin, ferritin, CRP, IL-6, lymphocyte percentage, and neutrophil and lymphocyte ratio (NLR)) and imaging results were analysed. All computer tomography (CT) images of the thorax of the that admitted to the ICU service and included in this study showed widespread pulmonary parenchymal involvement on the day of their admission. Since the additional disease status of four of the patients was unknown, they were excluded from the survival comparison. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki

Statistical Analysis

The data were analysed using IBM SPSS 25.0 (IBM Corp, Armonk, NY) and MedCalc 15.8 statistics software. Definitive statistical methods (frequency, percentage, average, standard deviation, median, min-max) were employed in the assessment of the research data as well as Chi-Square ($\chi 2$) test in the comparison of qualitative data. The eligibility of the data to normal distribution was evaluated with Kulmogorov-Smirnow test, skewness-kurtosis and graphical methods (histogram, Q-Q Plot, Stem and Leaf, Boxplot). On the other hand, the research applied Independent Samples t test for the intergroup comparisons of the qualitative data that showed normal distribution. Similarly, Mann-Whitney U test was used for the intergroup comparisons of the data that did not show normal distribution. The Kaplan-Meier method was used for the evaluation of the survival of the patients. A logistic regression analysis was performed to identify the effects of the variables (age, intubation and NLR) on survival. Statistical significance level was accepted as $p< 0.05$.

RESULTS

Of 66 patients who developed subcutaneous emphysema the number of male and female patients were 41 (62%) and 25 (38%), respectively. Their ages varied between 20 and 92 while the average was 63. Of these patients, 55 (83%) were intubated due to general impairment of health, increased oxygen need and cardiac issues while the remaining 11 (16.6%) were provided with non-invasive ventilation support (**Table 1**).

Table 1. Characteristics of the patients that developed subcutaneous emphysema during COVID-19

		n=66 Mean.±SD	% Median (Min-Max)
Intubation [a]	Yes	55	83.3
Survival [a]	Dead/Alive	56/10	84.8/15.2
Pneumothorax [a]	Yes/No	28/38	42.5/57.5
Side [a]	Left/Right	16/12	57.1/42.9
Tube Thoracostomy [a]	Yes/No	19/47	28.8/71.2
Lab data			
Ldh (U/L) [b]		600.3±250.1	543.0 (201.0-1253.0)
D-Dimer (mg/L) [b]		6.7±7.6	3.7 (0.2-35.2)
Procalcitonin (µg/L) [b]		1.7±6.0	0.2 (0.0-46.8)
Ferritin (µg/L) [b]		1344.7±2343.5	802.5 (39.0-17641.0)
Lymphocyte % [b]		5.5±4.2	4.5 (0.8-18.0)
Neutrophil/ Lymphocyte Ratio [b]		28.3±21.8	20.4 (4.1-116.5)
Crp (mg/L) [b]		101.9±93.0	71.0 (0.9-427.0)
Il-6 (mg/mL) [b]		458.7±1572.1	50.6 (2.0-10488.0)

[a]: n/%, [b]: Average±SD/Median (Min-Max),

56 patients (84.8%) died during monitoring. There were pneumothorax in 28 (42.5%) patients. The comparison between the patients who died (n=56) and the patients who survived (n=10) concluded that there was a statistically significant difference in terms of age, intubation, lymphocyte and neutrophil/lymphocyte ratio values (**Table 2**). The additional disease of 4 of the 56 patients who died could not be reached. The patients who died were elderly and had higher intubation rates, lower lymphocyte values and higher neutrophil/lymphocyte ratio ($p<0.001$, $p=0.001$, $p=0.046$, $p=0.045$, respectively) (**Table 2**). The effect of additional diseases on survival was not statistically significant ($p=0.478$) (**Table 3**).

Table 2. The effect of variables on survival

		Dead (n=56)	Alive (n=10)	P
Sex	Female/ Male	23/33 (41.1%/58.9%)	2/8 (20%/80%)	0.297 [a]
Age (Month)		798.3±168.6	529.7±206.3	0.000 [b]
Intubation	Yes	51 (91.1%)	4 (40%)	0.001 [a]
Pneumothorax	Yes/No	25/31 (44.6%/55.4%)	3/7 (30%/70%)	0.498 [a]
Side	Left/ Right	15/10 (26.8%/17.9%)	1/2 (10%/20%)	0.560 [a]
Tube Thoracostomy	Yes/No	16/40 (28.6%/71.4%)	3/7 (30%/70%)	1.000 [a]
Lymphocyte %		3.7 (2.2-7.2)	7.8 (4.1-9.5)	0.046 [c]
Neutrophil/Lymphocyte Ratio		25.3 (12.2-42.6)	11.1 (8.5-22.3)	0.045 [c]

[a]: Chi-Square Test (n/%), [b]: Independent Samples t Test (Mean±SD), [c]: Mann-Whitney U Test (Median /IQR))

Table 3. Survival comparison of 62 patients with additional disease information available

Additional Disease	Survival		P*
	Dead (n=52)	Alive (n=10)	
No	18 (34.6%)	5 (50.0%)	0.478
Yes	34 (65.4%)	5 (50.0%)	
HT, DM	8 (23.5%)	--	
HT	4 (11.8%)	1 (20.0%)	
HT, CVD, DM	3 (8.8%)	1 (20.0%)	
HT, DM, Asthma	2 (5.9%)	--	
HT, CeVD	2 (5.9%)	--	
Alzheimer , Parkinson	1 (2.9%)	--	
DM	1 (2.9%)	--	
HT, Asthma	1 (2.9%)	--	
HT, DM, AF, Hypothyroidism	1 (2.9%)	--	
HT, DM, CRF	1 (2.9%)	--	
HT, DM, Multiple myelom	1 (2.9%)	--	
HT, CVD, DM, COPD	1 (2.9%)	--	
HT, CVD, CRF	1 (2.9%)	--	
HT, COPD	1 (2.9%)	--	
CVD	1 (2.9%)	--	
CRF	1 (2.9%)	--	
Multiple myeloma	1 (2.9%)	--	
Rheumatoid arthritis	1 (2.9%)	--	
Cerebral palsy	1 (2.9%)	--	
CVD	1 (2.9%)	--	
HT, Epilepsy	--	1 (20.0%)	
HT, CVD	--	1 (20.0%)	
Wegener disease	--	1 (20.0%)	

*: Chi-Square Test (n/%)
HT hypertension, DM Diabetes mellitus, CVD Coronary vascular disease, AF Atrial fibrilation, CeVD cerebrovascular disease, CRF Chronic renal failure

A logistic regression analysis was performed to identify the effects of the variables (age, intubation and NLR), which were found to be statistically significant in terms of survival and death, on survival. It was discovered that age and intubation variables could be risk factors. It was also found out that the mortality rates were 1.01 times higher for the elderly compared to the younger patients and 13.8 times higher for the intubated compared to the non-intubated patients. No significant difference was found for neutrophil/lymphocyte ratio (**Table 2** and **Table 4**).

Table 4. Possible risk factors for survival

Risk Factor	OR (95% CI)	P*
Age (Month)	1.01 (1.00 - 1.01)	0.008
Intubation	13.78 (1.92 - 99.05)	0.009
N/L Ratio	1.05 (0.98 - 1.13)	0.156

* Binary Logistic Regression
Nagelkerke R^2=0.521, Hosmer and Lemeshow Test=0.530

In this study, an analysis of the time was also conducted from the development of subcutaneous emphysema until the death of patients who died. When divided into two groups as the intubated and non-intubated patients, the patients who died did not show a statistically significant difference in terms of the time between the development of subcutaneous emphysema and death ($p<0.01$). In other words, intubation has no effect on the time from the development of subcutaneous emphysema to death in the patients who died.

DISCUSSION

Declared to be a viral pandemic around the world in March 2020, the COVID-19 disease takes a mortal course in patients who are elderly or has weaker immune system, comorbid diseases and/or pulmonary diseases (1-3).

Pulmonary parenchymal damage among COVID-19 patients brings about complications related to barotrauma (9-11). In a study carried out by Jones et al. (13), barotrauma was found to be a frequent complication in severe COVID-19 patients, and it was predicated that ventilation support to these patients should be adjusted in optimal level. It can be claimed that the patients included in the present study developed pneumothorax, pneumomediastinum and subcutaneous emphysema secondary to barotrauma (9,11).

In the intubated COVID-19 patients with pulmonary parenchymal involvement, there is decreased ventilation and an increased need for oxygen due to the fibrosis effect of parenchymal involvement. Therefore, increased volume and pressure of mechanical ventilation lead to barotrauma effect which, in turn, causes pneumothorax, pneumomediastinum and subcutaneous emphysema. Similarly, non-invasive pressure ventilation (CPAP, hi-flow oxygen support) creates barotrauma effect and brings about complications in patients who are monitored without intubation (12). This study revealed that pneumothorax emerged in the mechanically-ventilated patients during ventilation due to increased pressure and volume. It was also established that 3 (27%) patients in non-invasive mechanical ventilation received oxygen

support only with a nasal oxygen cannula and, therefore, the subcutaneous emphysema developed by the patients was not due to barotrauma but arose spontaneously, and that all of these patients survived.

A study performed by Lemmers et al. (14) indicates that patients with pulmonary parenchymal involvement become dependent on mechanical ventilation within a short period of time, while subcutaneous emphysema develops 7 times more compared to other patients due to parenchymal tissue damage and the barotrauma effect of mechanical ventilation. In this study, the average of the ICU admission and intubation periods of the intubated patients was 3.7 days (1 to 8 days). The intubated patients showed 4.1 times more subcutaneous emphysema which was lower compared to Lemmers et al. This is because the hospital in which this study was held had a considerable experience after accommodating the highest number of COVID-19 patients in Ankara, Turkey and the international monitoring and treatment algorithms for ICU patients took shape as the time progressed.

However, subcutaneous emphysema does not develop only due to barotrauma. The study by Mana et al. (15) identified subcutaneous emphysema and spontaneous pneumomediastinum in 11 non-intubated patients. However, this requires further and wider research as it is believed to be secondary to parenchymal damage. It can be stated that the non-intubated patients in this study developed subcutaneous emphysema as a result of barotrauma which, in turn, came into question due to CPAP applied in order to get over with the respiratory distress of parenchymal damage.

Cut et al. (16) revealed that the male patients who develop subcutaneous emphysema due to COVID-19 experience more severe prognosis compared to female patients. In the present study, compared to the female patients, the mortality rates were found to be higher for the male patients who developed subcutaneous emphysema but the difference was not statistically significant. More reliable results may be obtained as regards to the relation between sex and mortality rates with further studies and meta-analyses to be performed on a wider patient population.

There are also studies which show that the emergence of subcutaneous emphysema adversely affect the prognosis of patients. In this regard, Al-Azzawi et al. (17) published a paper on 3 cases and expressed that subcutaneous emphysema is limited in patients who are not infected with COVID-19 but lead to rather complicated and mortal consequences in COVID-19 patients. The research by Ozsoy et al. (18) divided patients into two groups as patients with and without spontaneous pneumomediastinum and concluded that the need for tube thoracostomy and mechanical ventilation was higher, while there was increased hospital stay and mortality rates in the former group. The meta-analysis by Nasa et al. (19) suggested that COVID-19 patients may develop subcutaneous emphysema, pneumothorax or pneumomediastinum spontaneously without positive pressure ventilation, and that mortality rates were higher for the patients with complications (barotrauma) which develop due to positive pressure ventilation. As specified above, the research by Ozsoy et al. (18) grouped COVID-19 patients as the patients with and without spontaneous pneumomediastinum and concluded that prognosis was poorer in the patients with pneumomediastinum compared to the other group. The present study identified that intubation was particularly risky for mortality in patients with subcutaneous emphysema; however, no significant difference was found in terms of mortality rates for the non-intubated patients although the prognosis was poor (extended hospital stay and additional pathologies). The evaluation between the development of emphysema and the period to death inferred that the period to death was shorter in the intubated patients compared to the non-intubated but the differences was not statistically significant.

We found out that subcutaneous emphysema developed to be more lethal with poorer prognosis in both the intubated and non-intubated patients with chronic diseases, while on the other hand, no statistically significant difference was obtained in terms of survival for the patients without comorbid diseases. It can be indicated that this is because already existing and severe pulmonary parenchymal damage progresses together with cytokine storm and hyperinflammation, and therefore, the condition of patients worsens due to pulmonary fibrosis and shortness of breath which are difficult to reverse. In consequence, patients pass away independent of their chronic diseases.

A retrospective observational study that included 119 COVID-19 patients emphasized that higher NLR is a poor prognostic factor and a reliable parameter that can be easily measurable . Similarly, in the present study we found that higher NLR values were associated with worse outcomes (20).

The limitations of the study include the lack of clear numerical values relating to the mechanical ventilation pressure applied to the patients, failure to include the intubation tube diameters in the study, the exclusion of the data whether non-invasive pressure ventilation support had been given before mechanically-ventilating the intubated patients, failure to identify subcutaneous emphysema in the examination of the patients who developed minimal pneumomediastinum and subcutaneous emphysema, and the limited number of research population.

CONCLUSION

Although not rare, COVID-19 patients who are monitored in ICUs do not frequently develop subcutaneous emphysema. Advanced age and intubation can be regarded as risk factors for mortality in patients with subcutaneous emphysema. Prognosis takes a very poor course for these patients. Furthermore, comorbid diseases increase mortality rates. However, more comprehensive studies are required in this regard with wider case series.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Ankara City Hospital Noninvasive/ Clinical Researches Ethics Committee (Decision No: E-21-2021)

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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The relationship between acute physiology and chronic health evaluation-II, sequential organ failure assessment, Charlson comorbidity index and nutritional scores and length of intensive care unit stay of patients hospitalized in the intensive care unit due to chronic obstructive pulmonary disease

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ABSTRACT

Aim: It is known that disease severity and nutritional status are determinants of prognosis in patients hospitalized in the intensive care unit (ICU). Different scoring systems are used to evaluate the nutritional status and disease severity of intensive care patients. It will be very useful in clinical practice to determine the intensive care scores that are in harmony with the nutritional parameters and affect the length of stay in the ICU in patients hospitalized with the diagnosis of chronic obstructive pulmonary disease (COPD). It was aimed to determine the relationship between acute physiology and chronic health evaluation-II (Apache-II), sequential organ failure assessment (SOFA), and Charlson comorbidity index (CCI) with nutritional scores in intensive care patients with a diagnosis of COPD. Also, it was aimed to determine the scoring systems that affect the length of stay in the ICU.

Material and Method: Nutritional risk score-2002 (NRS-2002), prognostic nutritional index (PNI), modified nutritional risk in critically ill (mNutric) score, albumin, Apache-II, SOFA and CCI values and intensive care unit length of stay of the patients hospitalized in the intensive care unit due to COPD were recorded. The scoring systems that affect the length of stay in the ICU and the relationship between nutritional scores and Apache-II, SOFA and CCI was analyzed using statistical methods.

Results: A significant correlation was found between only CCI and all nutritional scores. Only the CCI value was found to be significantly higher in those found to be at high risk compared to all nutritional scoring systems. CCI cut-off value determined according to nutritional scoring was determined as 4.5 according to PNI and albumin, and 5.5 according to mNutric score and NRS-2002. It was determined that CCI affects the length of stay in the intensive care unit.

Conclusion: CCI is a scoring system that is compatible with nutritional parameters and affects the length of stay in the intensive care unit. Therefore, we think that CCI can be used to predict prognosis and nutritional risk in patients with COPD in the intensive care unit and to predict the length of stay in the intensive care unit. In terms of malnutrition risk, a cut-off value of ≥6 can be used for CCI.

Keywords: Charlson comorbidity index, chronic obstructive pulmonary disease, COPD, intensive care, nutritional scores

INTRODUCTION

Intensive care units (ICU) are high-tech special treatment units developed for close follow-up, rapid intervention, and treatment of acute disease (1). Prolongation of intensive care stays not only affects morbidity and mortality but also brings with it an increase in cost (2). It is known that disease severity and nutritional status are determinants of prognosis in patients hospitalized in the ICU. Different scoring systems are used to evaluate the nutritional status and disease severity of intensive care patients. While the nutritional risk score-2002 (NRS-2002), modified nutritional risk in critically ill (mNutric) score, and albumin are used to evaluate the nutritional status of patients, recently the prognostic nutritional index (PNI) has been evaluated as a prognostic risk

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score based on albumin and lymphocyte (3-6). Acute physiology and chronic health evaluation-II (Apache-II), sequential organ failure assessment score (SOFA) evaluates patients in terms of acute physiology, disease severity, and organ failure, while Charlson comorbidity index (CCI) evaluates patients in terms of comorbidity (7-10,11). However, there is no definite consensus on which scoring system should be used to determine the risk tendency in intensive care patients.

Patients with a diagnosis of chronic obstructive pulmonary disease (COPD) often have to stay in intensive care, especially during acute exacerbations. Malnutrition is a common condition in these patients (12). Malnutrition, on the other hand, affects the length of stay in the hospital and ICU and is a determinant in prognosis and mortality (12-14). Therefore, it will be very useful in clinical practice to determine the intensive care scores that are in harmony with the nutritional parameters and affect the length of stay in the intensive care unit in patients hospitalized with the diagnosis of COPD.

In this study, it was aimed to determine the relationship between NRS-2002, m Nutric score, PNI, and albumin, which shows the nutritional status of patients hospitalized in the intensive care unit with chronic obstructive pulmonary diagnosis, and Apache-II, sequential organ failure assessment, and Charlson comorbidity index (CCI). In addition, it is aimed to determine the scoring systems that affect the length of stay in the intensive care unit..

MATERIAL AND METHOD

The study was initiated with the approval of the Ankara Keçiören Training and Research Hospital Clinical Researches Ethics Committee (Date: 11.01.2022, number: 2012-KAEK-15/2451). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

The data of patients admitted to the intensive care unit with COPD between January 2018 and November 2018 were scanned retrospectively from patient files. Demographic data such as age, gender, body mass index, length of stay in intensive care, whether the patient received invasive mechanical ventilator support during admission to the intensive care unit, lymphocyte count, albumin value, Apache-II, SOFA, CCI, NRS- 2002, PNI, and mNutric Score values were recorded from patient files. Nutric score calculation is based on patient's age, Apache-II score, SOFA score, number of co-morbidities, Interleukin-6(IL-6), and the length of hospital stay before admission to the intensive care unit (15). In our study, the modified Nutric score (mNutric score) calculated without taking into account IL-6 was used. PNI was calculated from the formula 10 × serum albumin (g/dL) + 0.005 × lymphocyte count/mm3 (16). The nutritional risk status of the patients was determined as follows: PNI≥ 45; (low risk), PNI< 45;(High Risk), Albumin≥35 g/L (Low Risk), Albumin<35 g/L (High Risk), NRS-2002 ≤4; (Low Risk), NRS-2002>4; (High Risk), Nutric score≤4; (Low Risk), Nutric score>4;(High Risk) (15-18).

Those who were admitted to the intensive care unit for a reason other than COPD, those with a diagnosis of malignancy, those under the age of 18, those who were hospitalized in the intensive care unit for less than 24 hours, and those who lacked the necessary tests for the study were excluded from the study.

Statistical Analyses

Data analyses were performed by using SPSS for Windows, version 22.0 (SPSS Inc., Chicago, IL, United States). Whether the distribution of continuous variables was normal or not was determined by the Kolmogorov Smirnov test. Levene test was used for the evaluation of homogeneity of variances. Unless specified otherwise, continuous data were described as mean±SD for normal distributions, and median (interquartile range) for skewed distributions. Categorical data were described as the number of cases (%). Statistical analysis differences in not normally distributed variables between two independent groups were compared by the Mann Whitney U test. Categorical variables were compared using Pearson's chi-square test or fisher's exact test. Univariate and multivariate linear regression analyzes were performed to determine the factors affecting the length of stay in the intensive care unit. İt was evaluated degrees of the relationship between variables with Spearman correlation analysis. Receiver operating characteristic (ROC) curve analysis was used to determine the cutoff value of the charlson comorbidity index associated with the risk of PNI, albümin, NRS-2002, mNutric score. It was accepted p-value <0.05 as a significant level on all statistical analyses.

RESULTS

A total of 216 patients admitted to the intensive care unit for COPD were identified. 11 patients were excluded because their data were missing. Data from a total of 205 patients hospitalized in the intensive care unit due to COPD were analyzed. The demographic data of the patients, intensive care scores and the proportion of patients receiving invasive mechanical ventilator support are given in **Table 1**.

The risk distribution of the patients according to nutritional scores and Apache-II, SOFA, Charlson comorbidity scores of the patients are shown in **Table 2**.

Table 1: The demographic data of the patients, length of intensive care stay and the proportion of patients receiving mechanical ventilator support

		All patients (n:205)	
		±SD	Median (Q_1-Q_3)
Age, year		70.80±11.56	
Gender, n(%)	Male	125 (61.0%)	
	Female	80 (39.0%)	
BMI			24.8 (7.6)
Intensive care stay, days			3 (4)
MV support, n(%)	No	134 (65.4%)	
	Yes	71 (34.6%)	

Continuous variables were expressed as either the mean±standard deviation (SD) and median (interquartile range). Categorical variables were expressed as either frequency (percentage). BMI: body mass index, MV: mechanical ventilation,

Table 2. The risk distribution of the patients according to nutritional scores and Apache-II, SOFA, charlson comorbidity scores of the patients

	All Patients	
PNI		
High-risk	180 (87.8%)	
Low-risk	25 (12.2%)	
ALBUMIN		
High-risk	133 (64.9%)	
Low-risk	72 (35.1%)	
NRS -2002		
High-risk	112 (54.6%)	
Low-risk	93 (45.4%)	
mNUTRIC SCORE		
High-risk	134 (65.4%)	
Low-risk	71 (34.6%)	
	±SD	Med (IQR)
APACHE-II	21.08±6.23	20(8)
CCI	5.70±1.95	6(3)
SOFA	6.17±1.84	6(2)

Continuous variables were expressed as either the mean±standard deviation (SD) and median (interquartile range). Categorical variables were expressed as either frequency (percentage). APACHE-II: Acute Physiology and Chronic Health Evaluation-II, CCI: Charlson comorbidity index, SOFA: Sequential Organ Failure Assessment Score, PNI: prognostic nutritional index, NRS-2002: nutritional risk score-2002, mNUTRIC: modified nutritional risk in critically ill

There is a statistically significant negative correlation between PNI and the Charlson comorbidity index (r:-0.332 p:<0.001). There is a statistically significant negative correlation between PNI and SOFA (r:-0.174 p:0.013) (Table 3). There is a statistically significant negative correlation between albumin and Apache-II (r:-0.186 p:0.008) and Charlson comorbidity index (r:-0.338 p:<0.001) (Table 3). There is a statistically significant negative correlation between albumin and SOFA (r:-0.273 p:<0.001) (**Table 3**).

There is a statistically significant positive correlation between NRS-2002 and Apache-II (r:0.189 p:0.007) and the Charlson comorbidity index (r:-0.174 p:0.013) (Table 3). There is a statistically significant positive correlation between mNutric Score and Apache-II (r:0.761 p:<0.001). There is a statistically significant positive correlation between the mNutric Score and the Charlson comorbidity index (r: 0.534 p: <0.001). There is a statistically significant positive correlation between mNutric Score and SOFA (r:0.701 p:<0.001) (**Table 3**).

Table 3. Correlation analysis between Apache-II, CCI, SOFA and nutritional scores.

		PNI	Albumin	NRS -2002	mNutric Score
APACHE-II	r	-0.109	-0.186	0.189	**0.761**
	p	0.120	0.008	0.007	<0.001
CCI	r	-0.332	-0.338	0.465	**0.534**
	p	<0.001	<0.001	<0.001	<0.001
SOFA	r	-0.174	-0.273	0.098	**0.701**
	p	0.013	**<0.001**	0.161	<0.001

r:correlation coefficient. Statistically significant p-values were in bold. APACHE-II: Acute Physiology and Chronic Health Evaluation-II, CCI: Charlson comorbidity index, SOFA: Sequential Organ Failure Assessment Score

The Charlson comorbidity index values of patients with high risk for PNI were found to be significantly higher than those with low risk for PNI (Table 4). The Charlson comorbidity index and SOFA values of patients with high risk for albumin were found to be significantly higher than those with low risk for albumin (Table 4). Apache-II and Charlson comorbidity index values of patients with high risk according to NRS-2002 level were found to be significantly higher than those with low risk (Table 4). Apache-II, Charlson comorbidity index, and SOFA values were found to be significantly higher in cases with high risk according to the mNutric score level compared to cases with low risk (**Table 4**).

Table 4. Apache-II, SOFA and CCI by nutritional risk grouping

	±SD	Med (IQR)	±SD	Med (IQR)	p
PNI	**Low-risk**		**High-risk**		
APACHE II	22.60±5.72	21 (6)	20.87±6.29	20 (8)	0.140
CCI	4.84±2.34	4 (1)	5.82±1.86	6 (3)	0.002
SOFA	6.20±1.71	6 (2)	6.16±1.87	6 (2)	0.763
ALBUMIN	**Low-risk**		**High-risk**		
APACHE II	20.06±4.83	19 (5)	21.64±6.83	20 (8)	0.206
CCI	4.97±1.59	5 (2)	6.10±2.01	6 (2)	<0.001
SOFA	5.68±1.17	5 (1)	6.43±2.08	6 (2)	0.014
NRS -2002	**Low-risk**		**High-risk**		
APACHE II	19.86±6.13	19 (7)	22.10±6.16	21 (7)	0.005
CCI	4.83±1.66	5 (1)	6.43±1.87	6 (2)	<0.001
SOFA	5.98±1.77	5 (1)	6.32±1.90	6 (2)	0.065
mNUTRIC Score	**Low-risk**		**High-risk**		
APACHE II	16.18±3.30	16 (5)	23.68±5.86	22 (7)	<0.001
CCI	4.58±1.43	4 (1)	6.30±1.92	6 (2)	<0.001
SOFA	5.17±0.79	5 (0)	6.69±2.02	6 (2)	<0.001

Continuous variables were expressed as either the mean±standard deviation (SD) and median (interquartile range). Continuous variables were compared with mann whitney u test. Statistically significant p-values were in bold. APACHE-II: Acute Physiology and Chronic Health Evaluation-II, CCI: Charlson comorbidity index, SOFA: Sequential Organ Failure Assessment Score, PNI: prognostic nutritional index, NRS-2002: nutritional risk score-2002, mNUTRIC: modified nutritional risk in critically ill

In the ROC analysis performed to determine a cut-off value for CCI according to nutritional parameters, the area under the treatment characteristic curve (AUC) for PNI, albumin, NRS-2002, mNutric score was calculated

as 0.692, 0.668, 0.768, and 0.767, respectively. CCI cut-off value was determined as 4.5 according to PNI and albumin, and 5.5 according to NRS-2002 and mNutric score (**Table 5**).

Table 5. Cut-off values for CCI determined by ROC analysis

	SE	p	AUC (95% CI)	Cut Off	Senstivity	Specifity
PNI	0.059	0.002	0.692 (0.575-0.809)	4.5	73.9%	60%
ALBUMIN	0.039	<0.001	0.668 (0.590-0.745)	4.5	58.6%	65.3%
NRS-2002	0.034	<0.001	0.768 (0.700-0.835)	5.5	73.2%	77.4%
mNUTRIC SCORE	0.035	<0.001	0.767 (0.699-0.835)	5.5	65.7%	78.9%

SE:Standard Error, AUC: Area under the ROC Curve, CI: Confidence interval, CCI: Charlson comorbidity index, PNI: prognostic nutritional index, NRS-2002: nutritional risk score-2002, mNUTRIC: modified nutritional risk in critically ill

Univariate and multivariate linear regression analysis was applied to determine the factors affecting the length of stay in the intensive care unit. According to the results of the 5th step, which is the last step, the need for MV support and the increase in the Charlson comorbidity index were determined as the factors affecting the length of stay in the intensive care unit (**Table 6**).

Table 6. Factors affecting the length of stay in intensive care unit according to univariate and multivariate linear regression analysis

	Univariate Lineer Regression			
	t	p	β	95,0% for β
Age	0.333	0.740	0.023	(-0.065-0.091)
Gender (reference: male)	-0.965	0.336	-0.068	(-2.735-0.938)
BMI	1.069	0.286	0.075	(-0.061-0.206)
MV Support	6.770	<0.001	0.429	(4.148-7.558)
PNI	-0.563	0.574	-0.039	(-0.141-0.078)
ALBUMIN	-2.066	0.040	-0.144	(-0.329--0.008)
NRS-2002	-1.135	0.258	-0.079	(-1.867-0.503)
mNUTRIC SCORE	3.33	0.001	0.226	(0.370-1.457)
APACHE-II	2.704	0.007	0.186	(0.053-0.336)
CCI	2.524	0.012	0.174	(0.127-1.038)
SOFA	3.202	0.002	0.219	(0.297-1.249)
	Multivariate Lineer Regression (Backward Step 5)			
MV Support	6.553	<0.001	0.415	(3.956-7.361)
CCI	2.024	0.044	0.128	(0.011-0.845)

t: test statistics, β:coefficient, CI: Confidence interval. Statistically significant p-values are in bold. BMI: body mass index, MV: mechanical ventilation, APACHE-II: Acute Physiology and Chronic Health Evaluation-II, CCI: Charlson comorbidity index, SOFA: Sequential Organ Failure Assessment Score, PNI: prognostic nutritional index, NRS-2002: nutritional risk score-2002, mNUTRIC: modified nutritional risk in critically ill

DISCUSSION

In our study, a negative correlation was found between CCI and PNI and albumin, and a positive correlation between mNutric score and NRS-2002. There is no correlation between Apache-II and PNI, SOFA, and NRS-2002. Among the scorings, only the CCI value was significantly higher in those found to be at high risk compared to all nutritional scoring systems. In ROC analysis, the cut-off value determined according to nutritional scoring for CCI was determined as 4.5 according to PNI and albumin, and 5.5 according to mNutric score and NRS-2002. According to the regression analysis, it was determined that CCI affects the length of stay in the intensive care unit.

Critical diseases seen in patients hospitalized in intensive care are seen as an important public health problem due to high mortality and high health expenditures (19). For this reason, it is aimed to reduce the length of stay in the intensive care unit by increasing the quality of medical care.

It is stated that scoring systems used in intensive care are effective in clinical decisions, evaluation of treatment effectiveness, and optimizing the use of resources (20). However, very few of the scoring systems developed for this purpose are used effectively in clinical practice (20). The reason for this may be the use of many different scoring systems and the fact that each of these scoring systems gives the risk status of patients with different numerical values. In our study, in the nutritional risk classification made according to PNI, NRS-2002, mNutric Score, and albumin, it was seen that each of these scoring systems determined patients in high and low-risk groups at different rates. In our study, 54.6% of the patients were at high risk according to NRS-2002, while 65.4% of the patients were at high risk according to the mNutric score. In PNI and albumin, these rates are 87.8% and 64.9%, respectively.

It is known that the nutritional status of the patients hospitalized in the intensive care unit is very important in the prognosis, nutritional support changes the course and outcome of the critical illness, yet malnutrition is a neglected condition in hospitalized patients (19,21). Scorings that evaluate organ failure, chronic disease, and morbidity conditions such as Apache-II, SOFA, and CCI, which are used to predict prognosis in intensive care units, are scoring systems that do not evaluate the nutritional status of patients. However, detecting a relationship or correlation between these scoring systems and nutritional scoring will contribute significantly to clinical practice. When we looked at the correlation between disease severity scores and nutritional scores in this study, we saw that only CCI had a significant correlation with all nutritional scores in our study. In addition, only the CCI value was found to be significantly higher in those with high risk compared to all nutritional scoring systems in our study. Therefore, we can say that CCI is in good agreement with nutritional scoring. The cut-off value determined according to nutritional scoring was determined as 4.5 according to PNI and albumin,

and 5.5 according to mNutric score and NRS-2002. We think that the cut-off value of 5.5 can be used in clinical practice since it also includes the cut-off value determined for PNI and albumin. CCI, which is the gold standard in comorbidity risk assessment, is a scoring system where a score ranging from 1 to 6 is given to each of the 19 comorbid disease categories and calculated by the sum of these scores (8,22,23). Therefore, the CCI value cannot be a decimal value. For this reason, we can say that if CCI ≥ 6 in COPD patients hospitalized in the intensive care unit, the patients are also at high risk in terms of malnutrition. In studies in the literature, values of 2 and above for CCI have been reported as high CCI (8,24,25). However, these studies are not studies that determine a cut-off value for CCI. Again, these studies do not evaluate the relationship of CCI with nutritional scoring. In addition, these studies do not address COPD patients in intensive care. The fact that it is the first study that deals with the relationship of CCI with nutritional scores and determines a cut-off value for CCI from a nutritional point of view distinguishes our article from other studies in the literature.

Many studies evaluate the effectiveness of scoring systems in predicting the mortality of patients (26-28). Although it is important to prevent mortality, shortening the length of stay in the intensive care unit is important in terms of demonstrating the quality of medical care and reducing costs.

Many studies have shown that CCI is useful in predicting the prognosis of patients and that high CCI is associated with mortality and disease severity (8,29,30). However, we could not find any study evaluating the relationship between CCI and length of stay in the intensive care unit. In one study, it was stated that higher CCI was associated with a longer hospital stay in older adults hospitalized for acute stroke (24). However, this study does not evaluate COPD patients hospitalized in intensive care. According to the univariate and multivariate regression analysis we performed to determine the factors affecting the length of stay in the intensive care unit in COPD patients, it was determined that CCI affects the length of stay in the intensive care unit.

The fact that our research is single-centered and retrospective is a limitation of this article.

CONCLUSION

CCI is a scoring system that is compatible with nutritional parameters and affects the length of stay in the intensive care unit. Therefore, we think that CCI can be used to predict prognosis and nutritional risk in patients with COPD in the intensive care unit and to predict the length of stay in the intensive care unit. In terms of malnutrition risk, a cut-off value of ≥6 can be used for CCI.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was initiated with the approval of the Ankara Keçiören Training and Research Hospital Clinical Researches Ethics Committee (Date: 11.01.2022, number: 2012-KAEK-15/2451).

Informed Consent: All patients were informed about the application and their informed consent was obtained.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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The effect of duct width and pancreatic gland structure on pancreatic fistula rates in patients who underwent pancreaticoduodenectomy for pancreatic cancer

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ABSTRACT

Introduction: One of the most important causes of morbidity in pancreaticoduodenectomy (PD) surgery is pancreatic anastomosis leakage. There is a possibility of pancreatic fistula even in the most experienced hands. After PD, pancreatic fistula occurs between 10% and 20% in various series. This study aims to evaluate the effects of pancreatic duct size and pancreatic tissue on the development of pancreatic fistula after PD is performed in our center.

Material and Method: Pancreatic duct size was categorized as small <3 mm and large >3 mm. Pancreatic gland tissue was categorized as a soft, medium, and hard. These variables were calculated preoperatively with the help of computed tomography (CT), ultrasonography(USG), and Endoscopic ultrasound (EUS), and postoperative pathology results. It was accepted that the 24-hour flow rate of the drain behind the pancreatic anastomosis was more than 50 ml during 3 days after PD and/or the amylase concentration of the drain content measured at 3 different times was 3 times higher than the serum amylase concentration.

Results: A total of 90 patients were included in the study, anastomotic leakage was not observed in 63 (70%) of 90 patients, and leakage was observed in 27 (30%) patients. The mean age was 71.22±10.78 years (p=0.615). There was no statistically significant difference between the ductus diameters between the two groups (p=0.240). There was no statistical difference between the groups formed according to pancreatic duct width. (p=0.059). It was observed that 60.3% of the patients in the non-leakage group had a hard appearance, and this rate was statistically significantly reduced to 29.6% in the patients with leakage (p=0.008).

Conclusion: In summary, our study showed that pancreatic fistula after PD is associated with soft pancreatic parenchyma. The surgeon should consider this risk factor when performing a PD and be more careful to reduce the rate of pancreatic fistula.

Keywords: Pancreaticoduedenectomy, fistula, duct width, pancreatic gland structure

INTRODUCTION

Although pancreaticoduodenectomy (PD) was first performed by Kausch, it was popularized by Whipple in 1935 (1,2). It was considered an operation that should not be performed for a period because it causes high morbidity and mortality, but it could not be abandoned because it is the only potentially curative treatment option for pancreatic head and periampullary tumors. The most important step of PD surgery is pancreaticojejunostomy anastomosis.

Many different methods have been tried to be developed to reduce this morbidity and mortality. These include ligation of the pancreatic stump and occlusion of the duct with prolamin or fibrin adhesives, pancreaticogastrostomy anastomosis, prophylactic pharmacological agents such as octreotide, and external drainage of the pancreatic duct, internal drainage of the pancreatic duct, and many different pancreaticojejunostomy anastomosis techniques (3-6). Despite this, the pancreatic fistula rate is inevitable even in the most experienced hands.

After PD, the most prevalent cause of prolonged hospitalization and morbidity is pancreatic fistula formation, which is seen in postoperative patients between 10% and 20% in various series (7-9).

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Several research reportson the fistula risk score after PD Both small pancreatic duct size and soft glandular tissue were identified as factors impacting fistula formation following PD (10-15). This study aims to evaluate the effects of pancreatic duct size and pancreatic tissue on the development of pancreatic fistula after PD is performed in our center.

MATERIAL AND METHOD

The study was carried out with the permission of Hitit University Erol Olçok Training and Research Hospital Non-Invasive Research Ethics Committee (Date: 10/01/2022, Decision No: 2021-88). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Study Population

Between January 2014 and March 2022, patients over the age of 18 who underwent PD due to pancreatic cancer, underwent duct mucosa anastomosis, were not considered inoperable and did not undergo vascular anastomosis were included in the study. Patients under the age of 18 who underwent PD for non-cancer reasons, who developed intraoperative complications, who could not undergo ductomucosal anastomosis, and who had different anastomosis techniques were excluded from the study.

PD anastomosis was performed by all surgeons with the same surgical technique.

Two independent variables were tested in our study. The first of these was the pancreatic duct size. Pancreatic duct size was categorized as small <3 mm and large >3 mm. The second variable was pancreatic gland tissue. It was categorized as a soft, medium, and hard. These variables were calculated preoperatively with the help of computed tomography (CT), ultrasonography (USG), and Endoscopic ultrasound (EUS), and postoperative pathology results. Other demographic and clinical variables included age, gender, preoperative Bilirubin, aspartate aminotransferase (AST), alanine transaminase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), carbohydrate antigen (CA) 19-9, and carcinoembryonic antigen (CEA) levels, and postoperative amylase level (units/liter).

Postoperative morbidity was determined as surgery-related or systemic complications. As pancreatic fistula definition, as defined in ISGPF (international study group for pancreatic fistula definition), the 24-hour flow rate of the drain behind the pancreatic anastomosis for 3 days after PD is more than 50 ml and/or the amylase concentration of the drain content measured at 3 different times, serum 3 times higher than amylase concentration was accepted as Pancreatic fistula grading was classified according to ISGPF (16).

Normal values of serum albumin levels between 3.5-5.2 g/dl and serum bilirubin between 0.2-1.1 mg/dl were accepted. The length of stay in the hospital was recorded as the number of days between the day of surgery and discharge.

Surgical Technique

Between the posterior capsule of the pancreatic stump parenchyma and the seromuscular layer of the jejunum, posterior sutures were created using 4/0 nonabsorbable sutures in the duct-to-mucosal pancreaticojejunostomy anastomosis approach. For pancreatic duct anastomosis, the antimesenteric wall of the jejunum was opened to match the duct diameter. The pancreatic duct and all layers of the jejunum were then sewn together one by one using 5/0 nonabsorbable sutures, with the nodes on the outside. Subsequently, the anterior capsule of the residual parenchyma of the pancreas and the seromuscular layer of the jejunum were closed using the same technique to form the anterior wall of the anastomosis. Duct-to-mucosal PJ anastomosis was performed without stenting in the pancreatic duct.

Statistical Analysis

Data analysis was evaluated with SPSS 22.0 for the Windows data analysis program. Numbers and percentages for categorical variables, the mean and standard deviation for numerical variables, and the median in parenthesis were provided as descriptive statistics. The Shapiro Wilks test was used to assess the data's normal distribution. The data distribution looked into relationships between variables using the Pearson or Spearman correlation coefficient. Comparison of numerical measurements for two independent groups according to research groups, using two-sample t-tests for MPV and Albumin by the data distribution, age, duration of operation, duration of postoperative hospitalization, monocytes (MO), platelet distribution width (PDW), lymphocyte (LY), platelet (PLT), C-reactive protein (CRP), CA 19- 9, albumin, amylase values, pancreatic duct width and time to mortality were evaluated with Mann Whitney U test. Chi-square and Fisher exact tests were performed to compare the categorical variables such as gender, ASA, duct groups, pancreatic morphology, mortality rates at the same hospitalization and during the whole follow-up, and the rate comparisons between the study groups separated according to the presence of an anastomotic leak. The Kaplan-Meier Survival Analysis was used to compute the patients' predicted survival, and the Log-Rank test was used to determine statistical significance between the two groups. $P<0.05$ was considered statistically significant.

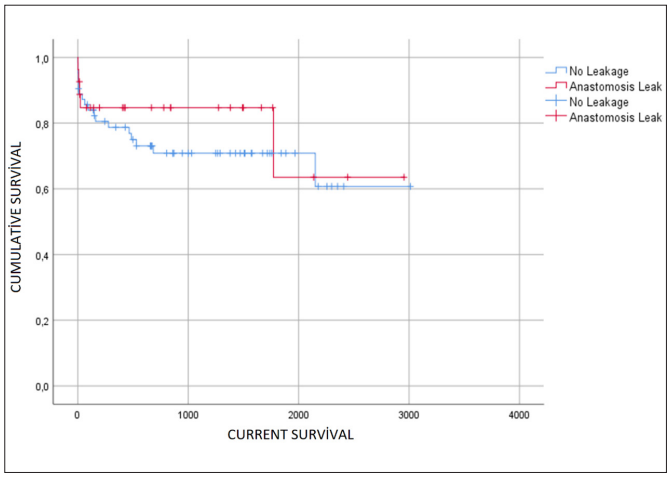

Figure 1. Kaplan-Meier curve showing no difference was observed between the groups with and without anastomotic leakage

Kolmogorov-Smirnov test was performed and it was shown that the parameters fit the normal distribution. Descriptive statistics (frequency, percentage distribution, etc.) were used as statistical analysis. In the comparison of the two groups; the Chi-square test was used for qualitative values and the Student-t-test was used for quantitative data. P<0.05 was considered statistically significant.

RESULTS

The study included 90 patients; anastomotic leakage was not seen in 63 (70%) of the 90 patients, while leakage was observed in 27 (30%) of the 90 patients. An additional intervention was required in only 1 (3.7%) of the patients with leakage.

When the mean ages of the two groups were compared, no statistically significant difference was observed, and the mean age of the whole group was 71.22±10.78 (73.5) years (p=0.615). The rate of female patients in the whole group was found to be 40%, this rate was 39.7% in the group without anastomotic leakage and 40.7% in the group with leakage, However, the difference was not statistically significant (p=0.925).

There was no significant difference between the operation times of both groups, the mean duration of all operations was found to be 308.63±71.8 minutes (p=0.784). However, when the post-operative hospitalizations of these patients were compared, it was observed that patients with anastomotic leakage were hospitalized for approximately 6 days longer, with a statistically significant difference of 14.73±9.28 vs 20.63±14.46, as expected(p=0.029). There was no significant difference between the two groups when the distribution of ASA scores was compared. In the whole group, 20 patients were ASA2 (22.2%), 53 patients were ASA3 (58.9%), 15 patients were ASA4 (16.7%), and 2 patients were ASA5. (2.2%) was evaluated.

When the patients' monocyte, MPV, PDW, LY, and PLT were examined, there was no discernible difference between the two groups. The mean monocyte of the whole group was 0.62±0.25 , the mean of MPV was 10.29±1.16. The mean PDW was found to be 13.9±2.6, lymphocyte mean 1.74±0.76 and platelet mean 263.88±103.21 (p=0.940; p=0.262; p=0.191; p=0.567; p=0.860, respectively).

When the albumin values of the patients were compared, the mean of the group without anastomotic leakage was 3.37±0.61, and the mean of the group with leakage was 3.37±0.51, no statistical significance was observed (p=0.975). When the CRP values were examined, the mean of Group 1 was 21.18±27.93, the mean of Group 2 was 37.95±65.52, and no significant difference was found (p=0.715).

The mean pre-operative CA19-9 level of the group without anastomotic leakage was 882.77±2662.23 and the mean of the patients with leakage was 229.23±455.1, although the average of the patients with leakage was lower. this difference was not statistically significant (p=0.122). There was no significant difference in amylase values between the groups. The mean amylase value of all patients was observed as 62.78±68.3 (p=0.476).

When the relationship between the ductus diameters between the two groups was evaluated numerically, the duct width of the patients without leakage was 4.13±2.26 mm (3.4) and that of the patients with leakage was 3.91±3.03 mm. Although a difference of 1 mm was observed between the median values, no statistically significant difference was found in the Mann-Whitney U test (p=0.240). When the relationship between the groups formed according to the pancreatic duct width being less than 3 mm and 3 mm and above and the anastomotic leakage relationship is examined, the rate of duct width of 3 mm and above in the group without leakage was 41.27%, while this rate was significantly higher with 62.96% in the group with leakage, but it was statistically significant. no difference could be found, which was thought to be related to the low number of patients (p=0.059).

When the appearance of the patients on CT was evaluated, it was seen that 60.3% of the patients in the non-leakage group had a rigid appearance, and this rate was statistically significantly reduced to 29.6% in the patients with leakage (p=0.008).

The mean duration of observation of all patients was found to be 922.84±809.155 days. When the observation times of the two groups were evaluated, no significant difference was observed between the two groups (p=0.570). When the patients were examined in terms of mortality, there were 12 (13.3%) patients with mortality at the same hospitalization in the whole group, no statistical difference was observed between the two groups (p=0.787). Disease-

related mortality was observed during the postoperative period in 25.6% of the patients who were followed up, and no statistically significant difference was observed between the two groups (p=0.316).

The mean time to mortality in patients with mortality was 302.22±564.42, and no difference was observed between the groups with and without anastomotic leakage (p=0.628). As a result of the Kaplan Meier analysis performed to determine the estimated average survival time, the estimated average survival time of the patients in the whole group was determined as 2151.437 days (±155.468). The estimated survival time of the group without anastomotic leakage was 2103,849 days (±180.75) and the estimated survival of the group with anastomotic leakage was 2253,698 days (±286.06). (p=0.473).

DISCUSSION

It is a general opinion that the quality of pancreatic tissue and the width of the pancreatic duct may be related to the rates of postoperative pancreatic fistula (POPF). Pancreatic anastomosis, which is included in a complex surgery such as PD, is an issue that needs to be emphasized, both in terms of its location and the tissue it contains.

Pancreatic tissue evaluated preoperatively, perioperatively, and postoperatively is an important risk factor for anastomotic leakage. In a study by Yeo et al.(17), they showed that the softness of the pancreatic tissue statistically increased the risk of pancreatic anastomotic leakage. Lin et al. (18) found that soft pancreatic parenchyma is the most frequently accepted risk factor for pancreatic fistula in a study of

Table 1. Comparison of variables between groups

Variable	All Patients (n=90)	No Leakage (n=63)	Anastomosis Leak (n=27)	Statistical Significance
Age	71.22±10.78	71.43±11.38	70.74±9.4	0.615
Gender				0.925
Male	54 (60.0%)	38 (60.3%)	16 (59.3%)	
Female	36 (40.0%)	25 (39.7%)	11 (40.7%)	
Post-operative hospitalization time	16.5±11.34	14.73±9.28	20.63±14.46	0.029
Operation duration	308.63±71.8	305.35±68.67	316.3±79.48	0.784
ASA				0.285
ASA 2	20 (22.2%)	11 (17.5%)	9 (33.3%)	
ASA 3	53 (58.9%)	38 (60.3%)	15 (55.6%)	
ASA 4	15 (16.7%)	12 (19%)	3 (11.1%)	
ASA 5	2 (2.2%)	2 (3.2%)	0 (0%)	
MO	0.62±0.25	0.61±0.23	0.64±0.3	0.940
MPV	10.29±1.16	10.38±1.24	10.08±0.96	0.262
PDW	13.9±2.6	14.1±2.57	13.43±2.66	0.191
LY	1.74±0.76	1.73±0.65	1.77±0.99	0.567
PLT	263.88±103.21	266.98±109.12	256.63±89.39	0.860
ALB	3.37±0.58	3.37±0.61	3.37±0.51	0.975
CRP	26.21±43.1	21.18±27.93	37.95±65.52	0.715
CA 19-9	686.71±2255.78	882.77±2662.23	229.23±455.1	0.122
Amylase	62.78±68.3	62.23±70.76	64.07±63.47	0.476
Ductus diameter (mm)	4.07±2.5	4.13±2.26	3.91±3.03	0.240
Ductus diameter groups				0.059
<3 mm	47 (52.22%)	37 (58.73%)	10 (37.04%)	
≥3 mm	43 (47.78%)	26 (41.27%)	17 (62.96%)	
Pancreas morphology				0.008
Soft	44 (48.9%)	25 (39.7%)	19 (70.4%)	
Hardened	46 (51.1%)	38 (60.3%)	8 (29.6%)	
Anastomosis leak incidence	27 (30.0%)			
Leak Type (n=27)				
Leak w/o Intervention	26 (96.3%)			
Leak w/ Intervention	1 (3.7%)			
Same admission mortality	12 (13.3%)	8 (12.7%)	4 (14.8%)	0.787
Overall survey duration	922.84±809.155	944.21±793.209	873±858.579	0.570
Overall mortality	23 (25.6%)	18 (28.6%)	5 (18.5%)	0.316
Overall days to mortality	302.22±564.42	285±514.86	364.2±787.03	0.628
Estimated survival duration (SE)	2151.437 (155.468)	2103.849 (180.75)	2253.698 (286.06)	0.473

1891 patients. This demonstrated that patients with a soft pancreas were much more likely to develop a POPF following PD than those with a hard pancreas. The data on the increased risk of anastomosis in patients with soft pancreatic tissue was found in our study in line with the literature.

We discuss many possible explanations for the link between soft pancreatic tissue and the probability of fistula formation. First, a soft pancreas is more easily injured, either directly or as a result of ischemia caused by sutures placed between the pancreatic parenchyma and the seromuscular layer of the jejunum; second, and perhaps most importantly, the soft pancreas has a better exocrine function, secreting more pancreatic juice rich in proteolytic enzymes (18-20).

Mazzafero et al. (21), Van Berge et al. (22) and Yang et al.(23) stated that the size of the pancreatic duct is effective in pancreatic leakage, and the risk of pancreatic leakage increases in ducts with a size of ≤3 mm, as a result of their large-scale studies. In a study, it was shown that each 1 mm shrinkage of the pancreatic duct increases the risk of anastomotic leakage by 68% (24). In this study, when the relationship between the ductus diameters between the two groups was evaluated numerically, the canal width was 4.13±2.26 mm (3.4) in patients without leakage and 3.91±3.03 mm (2.5) in patients with leakage. Although a difference of 1 mm was observed between the median values, no statistically significant difference was found in the Mann-Whitney U test (p=0.240). We believe that the limited number of our patients is to blame for this condition, which differs from the literature.

There are certain limitations to our research. Our research was a retrospective one with a small number of participants. In addition, Therefore, there were patients that we could not include in the study.

CONCLUSION

In summary, our study showed that pancreatic fistula after PD is associated with soft pancreatic parenchyma. The surgeon should consider this risk factor when performing a PD and be more careful to reduce the rate of pancreatic fistula.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Hitit University Erol Olçok Training and Research Hospital Non-Invasive Research Ethics Committee (Date: 10/01/2022, Decision No: 2021-88).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

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Author Contributions: The author declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Evaluating mental health literacy in a university hospital: A cross-sectional study

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ABSTRACT

Objective: Mental state and social condition are integrally linked to a person's physical health. The present study investigates the mental health literacy levels of patients aged 30–50 years who presented to the outpatient clinics of training and research hospital, as well as the relationship between mental health literacy and sociodemographic characteristics.

Material and Method: This cross-sectional study included 522 patients aged 30–50 years who presented to the Adult Outpatient Clinics of Karabuk University Training and Research Hospital between October and December 2021. The participants were administered a two-part, 33-item face-to-face questionnaire, in which the first part included 11 items assessing sociodemographic characteristics, and the second part included a 22-item scale comprising three (knowledge, belief, and resource-oriented) subscales for the measurement of the level of Mental Health Literacy (MHL).

Results: The mean scores of knowledge, belief and resource-oriented subscales were 8.92±0.98, 1.16±0.92 and 3.37±0.71, respectively, and the mean total MHL scale score was 13.46±1.39. The participants' education level, employment status, financial status, presence of chronic and psychiatric diseases, and psychiatric medication were significantly associated with the MHL scale scores (p=0.013, p=0.023, p=0.024, p=0.000, p=0.000 and p= 0.000, respectively).

Conclusion: As the level of MHL increases, so does the person's awareness of the symptoms of mental health disorders and the correct use of appropriate treatment resources. It is believed that training programs aimed at improving mental health literacy will improve health-related social outcomes, thereby reducing the burden of disease.

Keywords: Mental disorders, health literacy, mental health, humans

INTRODUCTION

Taking a holistic approach, health can be defined as the state of physical, mental and social well-being (1). Mental health, given a general definition, is the state of harmony and balance between oneself and others (2,3). The World Health Organization (WHO) defines mental health as the "state of well-being in which the individual realizes his or her abilities can cope with the normal stresses of life, can work productively and fruitfully, and is able to make a contribution to his or her community" (4). The concept of Mental Health Literacy (MHL) has been introduced to the domain of health literacy, supporting the protection and promotion of the health of both the individual and society (5). MHL was first conceptualized by A. F. Jorm (6) in 1997 as knowledge and beliefs knowledge and beliefs about mental disorders which aid their recognition, management, or prevention. Improving MHL supports the early recognition of mental disorders, and consequentially, the timely provision of appropriate treatment and care, and decreased stigma while enhancing professional help-seeking behaviors. When MHL is low, mental problems progress, and the use of non-adaptive coping methods such as alcohol and inappropriate medication increases to the detriment of the mental health of the person and society (7).

We consider it necessary to study this issue in our country to establish and monitor the level and to direction of knowledge, beliefs and the attitudes of society toward mental health. Accordingly, the present study assesses the mental health literacy levels and the association with sociodemographic characteristics in a specific age group by administrating the MHL scale to patients aged 30–50 who presented to the Outpatient Clinics of Karabuk University Training and Research Hospital.

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MATERIAL AND METHOD

The study was approved by the Karabük University Non-Interventional Clinical Researches Ethics Committee (Date: 18.11.2021, Decision No: 2021/715) and all procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

This cross-sectional study was conducted with people aged 30–50 who presented to the Outpatient Clinics of Karabuk University Training and Research Hospital between October and December 2021. The selection of this specific age group was made based on the fact that this is the age group that presents to the hospital most frequently, that can express itself best, that is open to communication and that has reading comprehension.

The study population amounted to 575 people, all of whom were included in the study without sampling, although 53 were subsequently excluded due to missing or erroneous data, meaning that the study was completed with 522 people. A face-to-face survey was employed for the study using a two-part, 33-item questionnaire. The first part included 11 items seeking to garner data on such sociodemographic characteristics as age, gender and education level, while the second part included 22 items scored from the three subscales of the MHL scale. The original MHL Scale was developed by Jung et al. (7) and comprised 26 items. The Turkish validity and reliability study of the MHL Scale was conducted by Göktaş et al. (8). There are 10 items (items 1–10) in the Knowledge-Oriented MHL subscale, eight items (items 11–18) in the Beliefs-Oriented MHL subscale and four items (items 19–22) in the Resource-Oriented MHL subscale. The total score range of the scale is 0–22. In the first two subscales, 18 items are rated on a 6-point Likert scale in which the answer options are: "strongly agree, agree, neutral, disagree, strongly disagree, not sure". The four items in the resource-oriented MHL subscale are answered "yes" and "no". The responses "strongly agree", "agree" and "yes" are assigned "1 point", and other responses are assigned "0 points". Items 11–18 in the beliefs-oriented subscale are coded and scored in reverse (8). Participation in the study was voluntary and written consent was obtained from all participants before the study.

The validity and reliability study of the scale reported a Cronbach's alpha of 0.71, while the present study recorded a Cronbach's alpha of 0.74. The data were normally distributed, presented using descriptive statistics, and analyzed using an Independent Samples t-test, a one-way analysis of variance (ANOVA) and Tukey's test for further analysis. The statistical analyses were made using IBM SPSS Statistics (Version 25.0. Armonk, NY: IBM Corp). A p-value of <0.05 was considered statistically significant.

RESULTS

Of the study participants, 54.8% (n=286) were female and 45.2% (n=236) were male with mean age, respectively, of 38.59±1.99 years and 31.21±3.02 years, with an overall mean age of 39.07±7.51 years. While 60.2% of the participants were university graduates, 61.7% were married and 68.0% were employed. Financial status was moderate in 52.9%, there was no chronic disease in 72.0% and there was no psychiatric disorder in 80.8% of the participants (**Table 1**).

Table 1. Distribution of participants by sociodemographic characteristics

Variables	n	%
Gender		
Male	236	45.2
Female	286	54.8
Age		
30–35	184	35.2
36–41	148	28.4
42–50	190	36.4
Education level		
Primary school	78	14.9
High school	130	24.9
Higher education	314	60.2
Marital status		
Single	166	31.8
Married	322	61.7
Divorced/widowed	34	6.5
Employment		
Employed	356	68.2
Job-seeker	54	10.3
Student	62	11.9
Housewife	50	9.6
Financial status		
Income less than expenses	154	29.5
Income equal to expenses	276	52.9
Income more than expenses	92	17.6
Chronic disease		
Yes	146	28.0
No	376	72.0
Interest in mental health-related subjects		
Yes	202	38.7
No	320	61.3
Psychiatric disorders		
Yes	100	19.2
No	422	80.8
Psychiatric medication		
Yes	75	14.4
No	447	85.6
Knowing anyone with mental disorders		
No - no one	260	49.8
Yes - family members, relatives, friends, neighbors with psychiatric disorders	262	50.2

No significant relationship was identified between the MHL scale score and the gender of the participants (p=0.446); and no statistically significant relationship was found between age and the total MHL Scale score

(p=0.231). Education level and MHL scale scores were statistically significantly associated (p=0.013). This relationship was due to the significant association only between the resource-oriented MHL scale score and education groups (p<0.001). When the MHL scale score was calculated according to education subgroups, the score was highest among primary school graduates and lowest among high school graduates. There was no statistically significant relationship between marital status and MHL scale score (p=0.920). A statistically significant relationship was found between employment status and MHL scale score (p=0.023). This relationship was statistically significant only between the beliefs-oriented MHL scale score and employment groups (p=0.009), and this difference was highest among job seekers and lowest among employees. A statistically significant difference was noted between financial status and the MHL scale score (p=0.024). There was a statistically significant difference only in the beliefs-oriented MHL scale scores of the financial status groups (p=0.011), and this difference was highest in the group with an income equal to expenses, and lowest in the group with income less than expenses. A statistically significant relationship was found between the presence of chronic disease and MHL scale score (p<0.001). The belief- and resource-oriented scale scores were associated significantly with the presence of chronic disease (p=0.026, p<0.001). There was no statistically significant association between an interest in mental health-related subjects and the MHL scale score (p=0.097). A statistically significant relationship was found between the presence of psychiatric disorders and the MHL scale score (p<0.001). The knowledge- and resource-oriented subscale scores were significantly associated with the respondent's interest in mental health-related subjects (p=0.043, p<0.001). A statistically significant relationship was found between psychiatric medication and the MHL scale score (p<0.001) that was due to the significant association that exists between the knowledge- and resource-oriented subscale scores and an interest in mental health-related subjects (p=0.003, p= 0.028). There was no statistically significant relationship between knowing someone with a mental disorder and the MHL scale score (p=0.385). In the study, the mean total MHL scale score was 13.46±1.39 and the mean subscale scores were 8.92±0.98, 1.16±0.92 and 3.37±0.71 (for knowledge, beliefs, and resource-oriented subscales) respectively (**Table 2**).

The post-hoc Tukey's test was used in the study. The distributions of the MHL scale score according to the descriptive characteristics of the participants were analyzed by the Independent Samples t-test for two groups, and the One-Way Analysis of Variance (ANOVA) for three or more groups.

DISCUSSION

In the presedent study, the mean MHL scale total score of the participants was 13.46±1.3, the mean knowledge subscale score was 8.92±0.98, the mean beliefs subscale score was 1.16±0.92, and the mean resource subscale score was 3.37±0.71. The study by Öztaş and Aydoğan (9), which included 239 healthcare professionals not working in mental health units in training and research hospital, found the mean knowledge, beliefs, and resource subscale scores to be 8.45±1.69, 5.32±1.70 and 3.19±1.25, respectively and the total scale score to be 16.96±3.30. The study by Seki Öz (10) with 388 participants living in a city center found the mean knowledge, beliefs and resource-oriented subscale scores to be 7.20±2.39, 4.76±1.76 and 2.80±1.25, respectively, and the total scale score to be 14.76±3.67. The different mean scores found in the present study may be attributable to the size of the selected sample and the differences in sociodemographic characteristics.

A statistical relationship was also identified between education level and mental health literacy scale score in the present study. In contrast, Öztaş and Aydoğan (9) identified a relationship only between the resource subscale score and MHL according to education level. Kaneko and Motohashi (11) examined the variables affecting mental health literacy in their study of 8,163 participants, and identified a strong relationship between education level and MHL. Lee et al.'s (12) study of 732 participants in Minnesota, on the other hand, determined that people with higher education levels had higher levels of MHL. We thus concluded that the findings of the present study was consistent with those of earlier studies.

In the present study, a statistical relationship was established between employment status and the mental health literacy scale score. Similarly, Seki Öz (10) found that employment status had an effect on the MHL scale scores. This can be explained by the fact that those who are employed tend to have a wider social circle than the unemployed, and that the problems experienced in working environments have an effect on mental status, leading to greater interest and awareness.

Our study revealed a statistical relationship between the presence of chronic disease and the mental health literacy scale score. The study by Al-Yateem et al. (13) of 317 healthcare professionals in the United Arab Emirates found the mental health literacy level of children and young people with chronic diseases to be low. This difference may be because while the participants were getting information about physical diseases, it was not possible for them to get mental health information and find time to research this subject.

Table 2. The Relationship between sociodemographic characteristics, mental health literacy subscale and total scale scores

	Knowledge-Oriented MHL		Beliefs-Oriented MHL		Resource-Oriented MHL		Scale Score	
	X±SD	Test p	X±SD	Test p	X±SD	Test p	X±SD	Test p
Gender		t=0.410 0.682		t: 1.63 0.114		t=3.043 0.002		t=0.762 0.446
Women	8.91±0.92		1.21±0.87		3.30±0.69		13.42±1.33	
Men	8.94±1.07		1.08±0.99		3.49±0.71		13.52±1.49	
Age		F=2.72 0.067		F=4.96 0.007		F=0.555 0.575		F=1.46 0.231
30–35	9.04±0.05		1.17±0.94		3.33±0.70		13.55±0.35	
36–41	8.80±0.99		1.33±0.97[a]		3.36±0.67		13.51±0.45	
42-70	8.88±0.87		1.01±0.82[b]		3.41±0.75		13.31±0.37	
Total	8.92±0.98		1.16±0.92		3.37±0.71		13.46±1.39	
Education Level		F=2.26 0.105		F=1.93 0.146		F=2.26 0.000		F=4.36 0.013
Primary school	8.82±1.02		1.27±0.84		3.58±0.65[a]		13.68±1.28	
High school	9.06±.087		1.04±0.71		3.54±0.61[b]		13.66±1.33[a]	
Higher education	8.88±1.02		1.19±1.02		3.23±0.74[a],[b]		13.30±1.43[b]	
Total	8.92±0.98		1.16±0.92		3.37±0.71		13.46±1.39	
Marital Status		F=2.47 0.085		F=0.960 0.384		F=5.59 0.004		F=0.083 0.920
Single	8.80±0.97		1.22±0.91		3.47±0.62[b]		13.49±1.39	
Married	8.98±0.99		1.12±0.93		3.33±0.74[a]		13.44±1.39	
Divorced/Widowed	9.20±0.78		1.400.84		2.80±0.78[a]		13.40±1.42	
Total	8.92±0.98		1.16±0.92		3.37±0.71		13.46±1.39	
Employment		F=0.759 0.517		F=3.98 0.009		F=0.846 0 .469		F=3.19 0.023
Employed	8.90±0.98		1.07±0.94[a]		3.35±0.76		13.32±1.39[a]	
Job-seeker	8.80±1.14		1.41±0.93[b]		3.40±0.49		13.61±1.39	
Student	9.03±0.95		1.23±0.81		3.50±0.66		13.76±1.43[b]	
Housewife	9.01±0.86		1.370.83		3.34±0.64		13.74±1.24	
Total	8.92±0.98		1.16±0.92		3.37±0.71		13.46±1.39	
Financial status		F=1.27 0.280		F=4.50 0.011		F=0.624 0.536		F=3.77 0.024
Income less than expenses	8.90±1.05		1.02±0.86[a]		3.42±0.66		13.32±1.34[a]	
Income equal to expenses	8.89±0.97		1.17±0.94		3.34±0.67		13.42±1.44	
Income more than expenses	9.09±0.88		1.41±0.90[b]		3.36±0.91		13.86±1.23[b]	
Total	8.92±0.98		1.16±0.92		3.37±0.71		13.46±1.39	
Chronic diseases		t=0.414 0.679		t=2.22 0.026		t=3.63 0.000		t=3.63 0.000
Yes	8.95±0.99		1.31±0.91		3.56±0.61		13.83±1.37	
No	8.91±0.98		1.11±0.92		3.30±0.73		13.33±1.37	
Interest in mental health-related subjects		t=-0.071 0.943		t=-0.112 0.911		t=3.02 0.002		t=1.66 0.097
Yes	8.92±0.92		1.16±0.92		3.26±0.68		13.34±1.32	
No	8.92±1.02		1.17±0.92		3.45±0.72		13.55±1.39	
Total	8.92±0.98		1.16±0.92		3.37±0.71		13.46±1.39	
Psychiatric disorders		t=-2.03 0.0043		t=-1.36 0.174		t=-4.71 0.000		t=5.03 0.000
Yes	8.74±1.05		1.05±0.99		3.07±0.64		12.86±1.26	
No	8.96±0.96		1.19±0.90		3.44±0.70		13.60±1.38	
Psychiatric medication		t=-2.98 0.003		t=-1.14 0.251		t=-2.23 0.028		t=-4.19 0.000
Yes	8.61±0.99		1.05±1.01		3.21±0.66		12.88±1.29	
No	8.97±0.97		1.18±0.90		3.40±0.71		13.56±1.38	
Knowing a person with mental disorders		F=1.18 0.160		F=2.02 0.133		F=1.49 0.226		F=0.956 0.385
No - no one	8.85±0.95		1.14±0.82		3.41±0.71		13.41±1.46	
Yes, family member or relative	9.02±1.06		1.92±1.10		3.290.72		13.61±1.28	
Yes, friend or neighbor	9.00±0.95		1.060.93		3.34±0.69		13.42±1.30	
Total	8.92±0.98		1.16±0.92		3.37±0.71		13.46±1.39	

t: Independent Samples t-test, F: Multivariate post-hoc parameter.

The present study identified no relationship between gender and mental health literacy scale score, while the study by Kaneko and Motohashi (11) found a low level of mental health literacy in males compared to females. Miles et al. (14) found a higher level of MHL among female students in their study. While most studies in the literature report a relationship between gender and MHL, no such relationship was identified in our study. The differences in the reported relationships between gender and MHAL may be due to the differences in the gender distribution and the cultural characteristics of the sample groups.

In the present study, an evaluation of the participants based on age revealed a statistical difference in the MHL scale scores of the 36–41 and 42–70 age groups. Farrer et al.'s (15) study of Australian adults aged 18 and over found the participants in the 18–24 age group to have the highest MHL levels. The differences in relationships between age and MHL in literature may be due to differences in age distribution and ethnic origin, and the cultural characteristics of the sample groups.

An examination of the relationship between marital status and MHL scale scores in the present study revealed a statistical difference in the MHL scale scores between marital status groups. In a statistical assessment, Öztaş and Aydoğan (9) found a difference in the mean scale scores of those with different marital statuses, with the mean scores of those who were married being higher. It is believed that the different distributions of marital status in the study groups affect MHL awareness.

An examination of the relationship between an interest in mental health-related issues and MHL scale score in the present study revealed a statistical difference. A study by Mehrotra et al. (16) comparing the MHL levels of family members caring for people with mental disorders between 1993 and 2016 years. It was found that the level of MHL of caregiver family members significantly increased across the 23-year study period. A study by Wang and Lai (17) of 3,047 cases in Canada investigating the MHL level for depression showed that the level of MHL was higher in those who communicated with people with mental problems than those who did not. It can thus be understood that knowing a person with mental health problems compel people to study the issue, thus increasing the level of MHL.

The limitation of the study is its single-center design.

CONCLUSION

Mental health literacy (MHL) remains a developing field in health literacy. The level of MHL plays a decisive role in the mental health of the individual and society. As the level of MHL increases, so does their awareness of the symptoms of mental health disorders and the correct use of appropriate treatment resources. Increased levels of MHL improve people's knowledge and attitudes toward mental health, as well as behaviors toward people with mental health disorders, thus reducing stigma. It is believed that training programs aimed at improving mental health literacy will improve health-related social outcomes, thereby reducing the burden of disease.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was approved by the Karabük University Non-Interventional Clinical Researches Ethics Committee (Date: 18.11.2021, Decision No: 2021/715).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version

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Clinical comparison of omicron and delta variants in older COVID-19 patients and the effect of vaccination status

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ABSTRACT

Aim: It was aimed to investigate the clinical course of the Omicron vand Delta variant among the SARS-CoV-2 vaccinated and unvaccinated COVID-19 patients over 65 years old and to compare their effects on patients.

Material and Method: The study was conducted on 567 COVID-19 patients over 65 years old. All patients' gender, age, medical history, COVID-19 PCR test results, blood test results, thorax CT images, vaccination status, hospitalization status, and treatment results were recorded. When evaluating the chest CT images, a semiquantitative scoring system was used. The patients were divided into the Omicron and Delta variant subgroups, and vaccinated and unvaccinated groups. Comparisons were made between the Delta variant and Omicron variant groups, the vaccinated and unvaccinated patient groups,and SARS-CoV-2 mRNA vaccinated and inactivated SARS-CoV-2 vaccinated patient groups.

Results: A total of 519 patients were included in the study.337 patients were in the Omicron variant group, 182 were in the Delta variant group.The hospitalization rate, ICU admission rate, mortality rate, rate of symptomatic patients,and the median thorax CT severity score was significantly higher in the Delta variant group than the Omicron variant group.The hospitalization rate, ICU admission rate, mortality rate, median thorax CT score and the rate of asymptomatic patients was significantly higher in the unvaccinated patient group than in the vaccinated group.There was no significant difference in the mortality rates and in the ICU admission rates between the inactivated SARS-CoV-2 vaccinated group and the SARS-CoV-2 mRNA vaccinated group.

Conclusion: The SARS-CoV-2 Omicron variant compared to the Delta variant and the SARS-CoV-2 vaccinated patients compared to the unvaccinated patients had a milder clinical course and less mortality in COVID-19 patients over 65 years old.

Keywords: Chest CT, COVID-19, delta variant, older patients, omicron variant, SARS-CoV-2 vaccine

INTRODUCTION

Cases of corovirus disease 2019 (COVID-19) were first reported in December 2019 in Wuhan, China, and then it has been spread around the world. According to WHO statistics, COVID-19 has led to more than 580 million cases and more than 5.6 million deaths worldwide (1). Continuing as a global public health problem today, COVID-19 also causes significant social and economic global burdens.

COVID-19 can manifest itself in various clinical forms, ranging from asymptomatic to severe pneumonia (2). People of all ages are at risk of infection and severe illness. However, geriatric patients and patients with underlying medical comorbidities are at risk of developing severe illnesses (2,3).

Since COVID-19 was first reported, different variants of SARS-Cov-2 have been reported as a result of various mutations in the S protein (3). These variants have become the pre-dominant variants in the world from time to time. Features such as different clinical courses, vaccine responses, and contagiousness have been reported for each variant (4).

The term variant of concern (VOC) for the COVID-19 agent SARS-COV-2 refers to viral variants with mutations in the spike protein receptor binding site (RBD) that significantly increase binding affinity (5). According to WHO's latest epidemiological update as of 11 December 2021, five VOCs have been identified since the start of the pandemic (6). SARS-COV-2 Alpha variant is the first known VOC, reported in the UK in late December 2020.

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The Beta variant was first reported in South Africa in December 2020. The Gamma variant was first reported in Brazil in early January 2021. The Delta variant was first reported in India in December 2020. The Omicron variant was first reported in South Africa in November 2021 (5).

Currently, SARS-CoV-2 has spread worldwide as the Omicron variant. This variant carries 32 mutations on the spike (S) protein, which is the main antigenic target of antibodies produced by infections or vaccination (7). The Delta variant, which has spread globally and caused a severe clinical course, had 5 mutations in the S protein (8). Large changes in the RBD region of the Omicron variant may contribute to its high binding ability with ACE2, resulting in a higher spreading rate and a significant effect on pathogenesis compared to the Delta variant. There is limited information on the current status of the Omicron variant, such as its contagiousness, clinical course, treatment, management and efficacy of vaccines.

Since COVID-19 was first identified, several subunit proteins and inactivated virus vaccines have been offered for use in humans. Due to the mutations that lead to new variants, there is a debate about resistance to these vaccines or a decrease in the effectiveness of vaccines (8,9). Although we have various vaccines, the fight against this pandemic is getting harder worldwide due to mutations.

The Omicron variant has now become the pre-dominant variant in COVID-19 disease worldwide (12,13). It is thought to be more contagious than the previous pre-dominant variants, the Delta variant, but causes a milder clinical course. However, we have very little clinical data on this subject. We also have limited information on the effectiveness of vaccines in patients infected by these variants. Therefore, we need clinical studies on this subject. This study was aimed to examine the clinical course of the Omicron variant and the Delta variant in vaccinated and unvaccinated geriatric patients and compare their effects on this aged patient group.

MATERIAL AND METHOD

The study was carried out with the permission of Sancaktepe Şehit Prof. Dr. İlhan Varank Training and Research Hospital Scientific Researches Ethics Committee (Date:12.01.2022, Decision No: 2022/259). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. For this study, written consent was obtained from the patients. However, written consent was not obtained because the patients in September 2021 were analyzed retrospectively.

Patients

The study was conducted on 567 patients over 65 years old who applied to Prof. Dr. Feriha Oz Emergency Hospital. Patients over 65 years old with positive COVID-19 PCR test, who had not been diagnosed with COVID-19 before, and who had thorax CT at admission to the hospital were included in the study. The diagnose of COVID-19 in patients had been confirmed by a positive result for SARS-Cov-2 RNA in nasopharyngeal swabs by using real-time fluorescence reverse transcription-polymerase chain reaction (RT-PCR) before the patients were applied to the inpatient clinic. Patients under the age of 65, previously diagnosed with COVID-19, and with negative COVID-19 PCR test results were excluded from the study. Patients who had received a single dose of SARS-CoV-2 vaccine and had received different types of vaccines were excluded from the study. The vaccination information of 11 patients could not be reached, 17 patients were diagnosed with COVID-19 for the second time, 13 patients were vaccinated with a single dose of SARS-CoV-2 vaccine, 7 patients were vaccinated with one dose of SARS-CoV-2 mRNA and one dose of inactive SARS-CoV-2 vaccine. Therefore, these 48 patients were excluded from the study. Two types of SARS-CoV-2 vaccines are administered in our country, namely the SARS-CoV-2 mRNA vaccine and inactivated SARS-CoV-2 vaccine. The vaccination dates of the patients and the type of vaccine they were vaccinated with were examined. According to the date of diagnosis of COVID-19, patients who had received two doses of vaccine with an interval of 4-6 weeks in the last 6 months were considered vaccinated. 337 COVID-19 patients admitted between 13-20 January 2022 during the Omicron variant pre-dominant period and 182 COVID-19 patients between 16-23 September 2021 during the Delta variant pre-dominant period were included in the study. All patients' gender, age, medical history, COVID-19 PCR test results, blood test results, chest CT images, vaccination status, hospitalization status, and treatment results were examined.

Chest CT Severity Score: When evaluating the COVID-19 related pneumonia on chest CT, a semiquantitative scoring system based on the extent of lobar involvement was used called Chest CT Severity Score. In this scoring system: Each of the five lung lobes was visually scored on a scale of 0–5, with 0 indicating no involvement, 1 indicating less than 5% involvement, 2 indicating 5–25% involvement, 3 indicating 26–49% involvement, 4 indicating 50–75% involvement, and 5 indicating more than 75% involvement (9,10).

The patients were divided into the Omicron and Delta variant groups, and vaccinated and unvaccinated groups. Comparisons were made between: i) the Delta

variant and Omicron variant groups, ii) the vaccinated and unvaccinated patient groups, and iii) SARS-CoV-2 mRNA vaccinated and inactivated SARS-CoV-2 vaccinated patient groups.

Statistical Analysis

The IBM SPSS (Statistical Package for Social Sciences; version 25.0 for windows, Chicago, USA) was used for statistical analyses. While evaluating the study data, the compatibility of the parameters with the normal distribution was evaluated with Kolmogorov-Smirnov and Shapiro Wilks tests, and the homogeneity of the group variables was evaluated with the Levene test. Percentage and mean±standard deviation (±SD) or median (interquartile range [IQR]) methods were used to indicate the baseline characteristics of the data according to the evaluation of the normality distribution. Differences in the values of the variables between the groups were evaluated by the Independent samples t-test or Mann–Whitney U test. Chi-square test was used to analyze qualitative data. p<0.05 was considered significant for all statistical analyses.

RESULTS

A total of 519 patients (312 females and 207 males) were included in the study. 337 patients were in the Omicron variant group, and 182 were in the Delta variant group. The median age was 72 (67-77) years in the Omicron group, and 73 (68-79.25) years in the Delta group. The hospitalization rate was statistically significantly higher in the Delta variant group than in the Omicron variant group (49.5% vs. 17.8%, respectively; p<0.001). ICU admission rate was significantly higher in the delta variant group (40% vs. 21.7% , respectively, p=0.019). There was no significant difference in length of stay in hospital and ICU between the Delta and Omicron variant groups. The median thorax CT score was significantly higher in the Delta variant group than in the Omicron variant group (p=0.001). The rate of asymptomatic patients was significantly higher in the Omicron variant group than in the Delta variant group (34,7% vs.13,7%, respectively, p<0,001). The mortality rate was significantly higher in the Delta variant group than in the Omicron variant group (15.9% vs. 3.9%, respectively, p<0.001) (**Table 1**).

According to vaccination status, 85.7% of all patients were vaccinated. The hospitalization rate was significantly higher in the unvaccinated patient group than in the vaccinated patient group (21.8% vs. 71.6%, respectively, p<0.001). The ICU admission rate was significantly higher in the unvaccinated patient group than in the vaccinated patient group (52.8% vs. 21.6%, respectively, p<0.001). The median thorax CT score was significantly higher in the unvaccinated patient group than in the vaccinated patient group (p<0,001). The rate of asymptomatic patients was significantly higher in the vaccinated patient group than in the unvaccinated patient group (30,6% vs. 8,1%, respectively, p<0,001). The mortality rate was significantly higher in the unvaccinated patient group than in the vaccinated patient group (37.8% vs. 3.1%, respectively, p<0.001). (**Table 2**)

Table 1. Demographic characteristics of all participants, Delta and Omicron variant subgroups and comparative analyses of these subgroups.

	All Patients (n=519)	Delta Variant Group (n=182, 35.1%)	Omicron Variant Group (n=337, 64.9%)	p value
Median (IQR) or n (%)				
Gender				0.651[a]
Females	312 (60.1%)	107 (58.8%)	205 (60.8%)	
Males	207 (39.9%)	75 (41.2%)	132 (39. 2%)	
Age (year)	72 (67-78)*	73 (68-79.25)*	72 (67-77)*	0.213[b]
Hospitalization				<0.001[a]
No	369 (71.1%)	92 (50.5%)	277 (82.2%)	
Yes	150 (28.9%)	90 (49.5%)	60 (17.8%)	
Hospitalized in				0.019[a]
Ward	101 (67.3%)	54 (60%)	47 (78.3%)	
ICU	49 (32.7%)	36 (40%)	13 (21.7%)	
Length of Hospital Stay (day)	8 (6-14.50)*	9 (6-16)*	8 (5-12.75)*	0.142[b]
Length of ICU Stay (day)	7.50 (5-11)*	8 (5-11)*	7 (5.50-10.50)*	0.722[b]
Thorax CT score	6 (2-10)*	0 (0-7)*	0 (0-3)*	0.001[b]
Symptomatic				<0.001[a]
Yes	377 (72.6%)	157 (86.3%)	220 (65.3%)	
No	142 (27.4%)	25 (13.7%)	117 (34.7%)	
Result				<0.001[a]
Healed	477 (91.9%)	153 (84.1%)	324 (96.1%)	
Exitus	42 (8.1%)	29 (15.9%)	13 (3.9%)	

*Median (IQR), aChi-Square Testi, bMann-Whitney U Test, ICU:Intensive care unit,CT:Computerized tomography

Table 2. Demographic characteristics of all participants, the vaccinated and unvaccinated subgroups and comparative analyses of these subgroups.

	All Patients (n=519)	Vaccinated Group (n=445, 85.7%)	Unvaccinated Group (n=74, 14.3%)	p value
Median (IQR) or n (%)				
Gender				0.895[a]
Females	312 (60.1%)	267 (60%)	45 (60.8%)	
Males	207 (39.9%)	178 (40%)	29 (39.2%)	
Age (year)	72 (67-78)*	71 (67-77)*	76 (69.75-85)*	<0.001[b]
Hospitalization				<0.001[a]
No	369 (71.1%)	348 (78.2%)	21 (28.4%)	
Yes	150 (28.9%)	97 (21.8%)	53 (71.6%)	
Hospitalized in			.	<0.001[a]
Ward	101 (67.3%)	76 (78.4%)	25 (47.2%)	
ICU	49 (32.7%)	21 (21.6%)	28 (52.8%)	
Length of Hospital Stay (day)	8 (6-14.50)*	9 (6-14)*	8 (6-16)*	0.994[b]
Length of ICU Stay (day)	7.50 (5-11)*	9 (7-11)*	7 (5-10)*	0.147[b]
Thorax CT score	6 (2-10)*	0 (0-3)*	2.50 (0-10)*	<0.001[b]
Symptomatic				<0.001[a]
Yes	377 (72.6%)	309 (69.4%)	68 (91.9%)	
No	142 (27.4%)	136 (30.6%)	6 (8.1%)	
Result				<0.001[a]
Healed	477 (91.9%)	431 (96.9%)	46 (62.2%)	
Exitus	42 (8.1%)	14 (3.1%)	28 (37.8%)	

*Median (IQR), aChi-Square Testi, bMann-Whitney U Test, ICU:Intensive care unit, CT:Computerized tomography

Of the vaccinated patients, 51.5% were vaccinated with the inactivated SARS-CoV-2 vaccine and 48.5% with the SARS-CoV-2 mRNA vaccine. The hospitalization rate was significantly higher in the inactivated SARS-CoV-2 vaccinated group than in the SARS-CoV-2 mRNA vaccinated group (p<0.001). There was no significant difference in the ICU admission rates between the inactivated SARS-CoV-2 vaccinated group and the SARS-CoV-2 mRNA vaccinated group. The median thorax CT score was significantly higher in the inactivated SARS-CoV-2 vaccinated group than in the SARS-CoV-2 mRNA vaccinated group (p=0,001). The rate of asymptomatic patients was significantly higher in the SARS-CoV-2 vaccinated group than in the SARS-CoV-2 mRNA vaccinated group (p=0,001). There was no significant difference in the mortality rates between the inactivated SARS-CoV-2 vaccinated group and the SARS-CoV-2 mRNA vaccinated group (**Table 3**).

Table 3. Demographic characteristics of all participants and the inactivated SARS-CoV-2 vaccinated and SARS-CoV-2 mRNA vaccinated subgroups and comparative analyses of these subgroups.

	Vaccinated Group (n=445)	Inactivated SARS-CoV-2 vaccinated group (n=229, 51.5%)	SARS-CoV-2 mRNA vaccinated group (n=216, 48.5%)	p value
Median (IQR) or n (%)				
Gender				0.615[a]
Females	267 (60%)	140 (61.1%)	127 (58.8%)	
Males	178 (40%)	89 (38.9%)	89 (41.2%)	
Age (year)	71 (67-77)*	72 (67-78)*	71 (67-77)*	0.412[b]
Hospitalization				<0.001[a]
No	348 (78.2%)	159 (69.4%)	189 (87.5%)	
Yes	97 (21.8%)	70 (30.6%)	27 (12.5%)	
Hospitalized in				0.083[a]
Ward	81 (83.5%)	58 (82.9%)	18 (66.7%)	
ICU	16 (16.5%)	12 (17.1%)	9 (33.3%)	
Length of Hospital Stay (day)	9 (6-14)*	8 (6-14)*	10.50 (5.75-15)*	0.664[b]
Length of ICU Stay (day)	9 (7-11)*	11 (6-15)*	9 (5.75-11)*	0.370[b]
Thorax CT score	0 (0-3)*	0 (0-4.50)*	0 (0-0.75)*	0.001[b]
Symptomatic				0.001[a]
Yes	309 (69.4%)	174 (76.4%)	134 (62%)	
No	136 (30.6%)	54 (23.6%)	82 (38%)	
Result				1.000[c]
Healed	431 (96.9%)	222 (96.9%)	209 (96.8%)	
Exitus	14 (3.1%)	7 (3.1%)	7 (3.2%)	

*Median (IQR), a Chi-Square Testi, b Mann-Whitney U Test, c Fisher's Exact Test, ICU:Intensive care unit, CT:Computerized tomography

DISCUSSION

COVID-19 continues to be a widespread public health problem all over the world and causes serious social and economic consequences. The Omicron variant is currently the pre-dominant variant circulating globally (11,12). Although it is stated that the Omicron variant has a milder clinical course than other variants, there are very few clinical studies on this subject. In the current study, we examined the clinical courses of Omicron and Delta variant COVID-19 patients over 65 years old and compared them. We also accomplished comparisons according to the vaccination status and vaccine types of the patients.

We found that the hospitalization rate, the ICU admission rate, and the mortality rate were significantly higher in the Delta variant group than the Omicron variant group. In the Omicron variant group, the hospitalization rate was 31.7%, the ICU admission rate was 18.3%, the rate of symptomatic patients was 21% and the mortality rate was 12% lower than the delta variant group. These indicators suggest that the Delta variant causes a more severe clinical course than the Omicron variant. Although there are not enough studies on this subject, the results of some studies are similar to ours. After the Omicron variant was reported, in the early studies conducted on the general population in England, Scotland, and South Africa, it has been reported that patients infected with the Omicron variant of SARS-CoV-2 are 50-70% less likely to be admitted to the hospital, 31%-45% less likely to be admitted to the ICU and 76% less likely to be death than patients infected with the delta variant (13-15). A Swedish study comparing the Omicron and Delta variant periods found that the risk of severe disease was 40% lower in unvaccinated patients and 71% lower in vaccinated patients in the Omicron variant period (16).

We found that the median chest CT severity score was significantly higher in the Delta variant group than in the Omicron variant group. Accordingly, pulmonary involvement due to COVID-19 was milder in the Omicron variant group. In a study of mice infected with the Delta and Omicron variant of SARS-CoV-2, it was found that mice infected with the Omicron variant resulted in less severe clinical signs, lower levels of inflammation, and less injury to the lungs than mice infected with Delta variant viruses (17). In our study, the rate of asymptomatic patients was significantly higher in the Omicron variant group than in the Delta variant group. Although there are not many studies on this subject, the rate of asymptomatic Omicron infections is estimated to be between 25-54%. In a study conducted on healthcare workers, the rate of asymptomatic patients was found to be 50% in patients infected with the Omicron variant (18).

The reason why the Omicron variant causes a milder clinical course may be the high number of mutations in the virus compared to other variants. Immune response induced by vaccination or reinfections may also be causes of mild clinical course. However, since our study is in the group of patients over 65 years of age, different results are likely to occur compared to the general population. Patients over 65 years of age are expected to have a more severe clinical course than the general population.

When we compared the vaccination status of the patients, we found that the hospitalization rate, the ICU admission rate, the mortality rate, and the asymptomatic patient rate were significantly higher in the unvaccinated patient group. The hospitalization rate was 49.8%, the ICU admission rate was 37.2%, the symptomatic patient rate was 22.5%, and the mortality rate was (34.7%) higher in the unvaccinated patient group than in the vaccinated patient group. In unvaccinated patients, these findings were expected results. Many studies have shown that vaccines improve prognosis in COVID-19 patients (19,20). The median thorax CT score was significantly higher in the unvaccinated patient group than in the vaccinated patient group. Thorax CT involvement was more severe in the unvaccinated patient group. Similarly, other studies have shown that the CT severity score is lower in vaccinated patients (20).

When we compare vaccinated patients according to inactivated SARS-CoV-2 and SARS-CoV-2 mRNA vaccine types, we found that the hospitalization rate, the asymptomatic patient rate, and the median thorax CT score were significantly higher in the inactivated SARS-CoV-2 vaccinated group than in mRNA vaccine group. There was no significant difference in the mortality rates and the ICU admission rates between the inactivated SARS-CoV-2 vaccinated group and the SARS-CoV-2 mRNA vaccinated group. As is well known, two vaccines reduce the severe course of the disease and the risk of death in COVID-19. Similar to our results, various studies have shown that these two types of vaccines are effective in COVID-19 (19-21). Although two vaccines similarly prevented a very severe course, we found that the mRNA vaccine provided better clinical outcomes in terms of hospitalization rate, asymptomatic patient rate, and CT involvement, which are some indicators of severe disease. Different types of vaccines can induce different immune responses in the same individual. In addition, different individuals may produce different levels of antibodies from the same vaccine due to different characteristics such as race, gender, age, and medical conditions. Like another RNA viruses, Coronaviruses and as well as COVID-19 evolving some mutations and this fact also affects the vaccine success (22). Comprehensive clinical studies are needed on this subject. There are some studies

on the ability of vaccines to produce antibodies. Recently, Lim et al. (23) found that two doses of mRNA vaccine produced more antibodies against SARS-CoV-2 than the inactivated vaccine.

In our study, we compared the clinical course of Omicron variant and Delta variant, vaccinated and unvaccinated patients in COVID-19 patients over 65 years old. We found that the Omicron variant compared to the Delta variant and the vaccinated patients compared to the unvaccinated patients had a milder clinical course and less mortality.

Study Limitations

There are some limitations of our study. An important limitation is that the SARS-Cov-2 variants of the patients were not confirmed by PCR testing, the patients were grouped according to the period in which the dominant variants were present. Another limitation is that vaccinated and unvaccinated patient groups cannot be compared according to SARS-Cov-2 variants.

CONCLUSION

Our study showed that the SARS-CoV-2 Omicron variant compared to the Delta variant and the SARS-CoV-2 vaccinated patients compared to the unvaccinated patients had a milder clinical course and less mortality in COVID-19 patients over 65 years old. Although we found that the Omicron variant which is the pre-dominant variant of COVID-19 today, causes a milder clinical course, especially in the elderly, COVID-19 is still a very serious health problem. In other words, vaccination is very important on the prognosis in geriatric patients.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Sancaktepe Şehit Prof. Dr. İlhan Varank Training and Research Hospital Scientific Researches Ethics Committee (Date:12.01.2022, Decision No: 2022/259).

Informed Consent: Written informed consent was obtained from the patients. However, written consent was not obtained because the patients in September 2021 were analyzed retrospectively.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Effects of DPP-4 inhibitors on brain natriuretic peptide, neuropeptide Y, glucagon like peptide-1, substance P levels and global longitudinal strain measurements in type 2 diabetes mellitus patients

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ABSTRACT

Introduction: Previously, a significant relationship between saxagliptin treatment and increased rate of hospitalization for congestive heart failure was reported. We aimed to investigate effects of vildagliptin and saxagliptin on brain natriuretic peptide (BNP), neuropeptide Y (NPY), substance P (SP), glucagon like peptide-1 (GLP-1) levels and left ventricular global longitudinal strain (GLS), assessed by 3-dimensional speckle tracking echocardiography in uncontrolled type 2 Diabetes mellitus (T2DM).

Material and method: Thirty seven uncontrolled T2DM (HbA1c>7,5%) patients who were recently prescribed to either vildagliptin 50 mg BID (n=21) or saxagliptin 5 mg QD (n=16) were included in this study. Levels of BNP, NPY, SP, GLP-1 levels were measured at admission, first and third months of treatment. GLS was measured at admission and third month.

Results: In whole group, BNP and NPY values increased significantly at third month of treatment ($p< 0.001$, 0.004; respectively). In the vildagliptin group, BNP and NPY values increased significantly at third month of treatment ($p=0.02$ and $p=0.04$, respectively). In the saxagliptin group only BNP levels increased significantly ($p=0.015$). In both groups; SP, GLP-1 levels and GLS measurements did not change significantly during follow-up period.

Conclusion: The current study demonstrated that treatment with saxagliptin and vildagliptin, was associated with increased levels of BNP and NPY levels. No evidence of subclinical myocardial damage or cardiac dysfunction could be detected by GLS measurements. Since our study population had no previous clinical cardiac disorders, increases in BNP and NPY levels with these two DPP4 inhibitors can be considered as a safety signal.

Keywords: DPP-4 inhibitors, brain natriuretic peptide, neuropeptide Y, substance P, glucagon like peptide-1, 3 dimensional echocardiography

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INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a major risk factor for cardiovascular diseases, and cardiovascular complications are the major cause of mortality in T2DM (1). Chronic heart failure (HF) is a frequent diabetes related cardiac complication that may also be related with glucose lowering medications (2-4). Cardiovascular safety is essential for approval of new glucose lowering drugs (5).

The risk of HF has been a controversial issue for DPP-4 inhibitors. "Saxagliptin Assessment of Vascular Outcomes Recorded in Patients with Diabetes Mellitus (SAVOR-TIMI 53)" trial revealed a significantly rising risk for HF hospitalizations in saxagliptin arm when compared to placebo (6). The "Examination of Cardiovascular Outcomes with Alogliptin versus Standard of Care (EXAMINE) " study also revealed an increased trend of HF hospitalizations, although this finding was not statistically significant (7). FDA added safety warnings about the risk of HF hospitalizations to labels of saxagliptin and alogliptin in 2016. Results from CVOTs of other DPP-4 inhibitors; omarigliptin (OMNEON),

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linagliptin (CARMELINA) and sitagliptin (TECOS-Trial Evaluating Cardiovascular) did not show an increased risk for HF (8-10). The only randomized-controlled trial for HF risk, "Effects of Vildagliptin on Ventricular Function in Patients With Type 2 Diabetes Mellitus and Heart Failure (VIVIDD)" study demonstrated an increase in left ventricular volumes, despite it did not have significant effect on left ventricular ejection fraction (11).

The causes of increased risk for HF hospitalizations in SAVOR-TIMI 53 and EXAMINE trials have not been explained yet. In SAVOR-TIMI 53 trial, HF hospitalizations were more frequent in patients with highest NT-proB-type natriuretic peptide (BNP) levels, so subclinical cardiac dysfunction was suggested as a risk factor for saxagliptin related worsening of HF (12). Also, DPP-4 enzyme has many substrates including neuropeptide Y (NPY), substance P, peptide YY, BNP, and stromal-derived factor 1 alpha (SDF-1 aka CXCL12), besides incretin hormones. All these molecules have several effects on cardiovascular system (13-20). Accumulation of these substrates by DPP-4 inhibition was suggested to cause alterations on cardiovascular system (13-20). Also, chance effect or statistical methodology errors were suggested as possible explanations for increased risk of HF in SAVOR-TIMI 53 and EXAMINE trials (21,22).

In the previous study, we purposed to study acute effects of vildagliptin and saxagliptin on brain natriuretic peptide (BNP), substance P (SP), neuropeptide Y and glucagon like peptide-1 (GLP-1) levels in patients with uncontrolled T2DM. Also left ventricular global longitudinal strain (GLS), assessed by 3-dimensional speckle tracking echocardiography.

MATERIAL AND METHOD

The study was carried out with the permission of Ankara University Faculty of Medicine Clinical Researches Ethics Committee (Date: 09.02.2015, Decision no: 02-74-15) and written informed consent was obtained from all individual participants. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Patients

Thirty seven uncontrolled insulin naïve type 2 DM (HbA1c >7.5%) patients who were started to use either vildagliptin 50 mg BID (n=21) or saxagliptin 5 mg QD (n=16) and completed the study period were enrolled in this study. All patients had metformin treatment for at least six months. A history of heart failure and coronary artery disease, previous treatment with insulin and DPP-4 inhibitors, stage 3-5 chronic kidney disease and active genitourinary infection were exclusion criteria. This study is a prospective study.

Biochemical parameters

Plasma NPY and BNP levels were measured with enzyme-linked immunosorbent assay (ELISA) method (Sunred Biological Technology. Shanghai, China). Substance P and GLP-1 were measured with ELISA method (Cloud-Clone Corp. Houston, USA). Biochemistry parameters were measured with ADVIA 2400 chemistry system assay (Erlangen, Germany) by Beckman Coulter DXC 800 device. Glycosylated hemoglobin (HbA1C) was measured with high-performance liquid chromatography (CinQ. Erlangen, Germany).

Levels of BNP, NPY, SP and GLP-1 were measured at admission, 1st - 3rd months of treatment.

Global Longitudinal Strain Measurements

GLS was measured at admission and 3rd month. Global longitudinal strain index measurements were performed by same experienced cardiologist. Standard echocardiography was performed with 4V multiphase array probe and Vivid E9 device (GE Vingmed, Horten, Norway). Basal echocardiographic measurements were performed according to the American Society of Echocardiography recommendations. Apical four chamber, three chamber and two chamber views were recorded at 60-100 frames/seconds rate for global longitudinal peak systolic strain measurements. Three cardiac cycles were archived from all views. Manual tracings of endocardium was performed by operator in all views and controlled during the systole. Global longitudinal strain was calculated with 18 segments model by dividing each view into six segments. Strain measurements were performed with the program on the device (EchoInsight, Epsilon, Ann Arbor, MI, USA).

Statistical Analyses

Statistical analysis was performed by using SPSS version 15 (IBM Corp, NY, US) for windows. Results were expressed as percentage of the patients or mean±SD where appropriate. The chi-square Fisher exact test used for categorical data. Student t test used for group data, and the Mann-Whitney U or Kruskal-Wallis tests used for nonparametric data. Correlations between continuous variables with and without normal distribution were assessed with Pearson and Spearman correlation tests, respectively. The changes of continuous variables over the follow-up period were assessed with paired test if data was normally distributed and with Wilcoxon test if data was not normally distributed. Friedman test or analyses of variance were performed for multiple comparisons. Changes in categorical variables over follow-up period were compared with McNemar test. Multiple comparisons were performed with Cochran Q test. $p<0.05$ was considered statistically significant.

RESULTS

Thirty-seven patients [18 male (48.6%) and 19 female (51.4%)] completed the study. Twenty-one patients were in vildagliptin and sixteen were in saxagliptin treatment arms. Baseline characteristics of patients in both groups were summarized in **Table 1**. There was no statistical significant difference between the treatment arms regarding to basal patient characteristics and biochemical parameters at the beginning of the DPP-4 treatment.

All patients had metformin treatment for at least six months prior to DPP-4 prescription. Eleven patients (52.3%) in vildagliptin arm and nine patients (56.2%) in saxagliptin arm had concomitant sulphonylurea administration.

Twenty-one patients (56%) had essential hypertension, seven (18.9%) had hyperlipidemia, and seven (18.9%) had hypertriglyceridemia. Seven patients were ex/current smoker.

Serum HbA1c, ALT and AST decreased significantly at the third month of DPP-4 treatment when compared to basal levels. The changes in laboratory parameters are summarized in **Table 2**.

BNP and NPY levels increased significantly at the 12th week of DPP-4 treatment when compared to basal measurements, in whole group. The levels of substance P, GLP-1 and global longitudinal strain measurements were similar to the basal at the end of the 12th week (**Table 3**).

Table 1. Basal characteristics and laboratory parameters of patients in two treatment arms.

Parameters	Vildagliptin arm	Saxagliptin arm	P value
Age, years	54.81±8.903	53.13±8.016	NS
BMI, kg/m²	29.13±3.11	28.93±3.97	NS
Body weight, kg	80.5±12.4	79.6±11.3	NS
Gender, F/M	11/10	8/8	NS
FPG, mg/dl	149.57±48.82	139.94±29.73	NS
Hemoglobin, g/dl	13.6±2.16	13.5±2.11	NS
HbA1c, %	8.17±1.33	7.88±1.25	NS
Creatinine, mg/dl	0.82±0.21	0.92±0.27	NS
ALT, UI/L	35.29±11.09	28.56±7.25	NS
AST, UI/L	25.90±6.74	20.94±4.04	NS
LDL, mg/dl	118.81±29.34	108.63±38.12	NS
HDL, mg/dl	43.14±8.32	41.94±11.16	NS
Triglyceride, mg/dl	188.29±115.42	179.56±92.32	NS
TSH, mIU/L	1.92±1.35	1.87±1.12	NS

ALT: alanine aminotransferase, AST: aspartate aminotransferase , LDL: Low density lipoprotein, HDL: high density lipoprotein,BMI: Body mass index; F/M: Female/Male; FPG: Fasting plasma glucose; HbA1C: Glycosylated hemoglobin; NS: non–significant; TSH: thyroid stimulating hormone.

Table 2. Changes in laboratory parameters during follow–up period in whole group.

Parameters	Basal	12th week	P value
FPG, (mg/dL)	145.41±41.42	134.89±32.22	NS
BMI, (kg/m²)	28.98±3.49	28.3±3.46	NS
Body weight, (kg)	80.2±12.6	79.5±12.7	NS
Hemoglobin, g/dl	14.19±1.75	14.16±1.49	NS
HbA1c, (%)	8.05±1.28	7.3±1.1	0.004
Creatinine, mg/dl	0.84±0.26	0.85±0.21	NS
ALT, UI/L	32.28±12.69	27.0±10.04	0.002
AST, U/L	23.76±6.9	19.89±4.8	<0.001
LDL, mg/dL	114.41±33.32	101.81±26.17	NS
HDL, mg/dL	42.62±9.52	44.14±9.29	NS
TG, mg/dL	181.51±104.75	185.11±88.51	NS
TSH, mIU/mL	1.90±0.74	1.71±0.82	NS

ALT: alanine aminotransferase, AST: aspartate aminotransferase , LDL: Low density lipoprotein, HDL: high density lipoprotein,BMI: Body mass index; F/M: Female/Male; FPG: Fasting plasma glucose; HbA1C: Glycosylated hemoglobin; NS: non–significant; TG: triglyceride TSH: thyroid stimulating hormone.

Table 3. Changes in BNP, NPY, GLP–1, SP levels and GLS measurements at the 12th week of treatment.

Parameter	Whole group	P value*	Vildagliptin	Saxagliptin	p
BNP (ng/L)					
Basal	11.0 (3.71–44.0)	P<0.001	10.1 (5.2 – 36.0)	8.5 (3.7 – 44.0)	0.020/0.015
4th week	19.0 (2.38– 37.0)		17.7 (5.8 – 36.0)	14.0 (2.4 – 37.0)	
12th week	21.7 (2.36–60.89)		20.5 (6.4 - 60.9)	18.4 (2.4 – 50.0)	
NPY (ng/L)					
Basal	8.1 (2.0– 44.0)	p=0.004	9.6 (4.3 - 27.7)	7.5 (2.0 – 44.0)	0.047/NS
4th week	12.9 (4.4–32.9)		14.0 (4.4 - 32.9)	12.4 (4.6 – 23.0)	
12th week	15.0 (2.6–42.1)		17.0 (3.92 - 42.1)	14.0 (2.6 – 37.0)	
GLP–1 (pg/mL)					
Basal	21.0 (8.9–33.0)	NS	23.5 (12.1 – 33.0)	19.9 (8.9 - 31.0)	NS/NS
4th week	21.0 (10.0–35.8)		22.0 (10.0 - 35.8)	20.2 (10.0 - 34.2)	
12th week	19.1 (9.0–33.2)		19.1 (10.5 - 33.2)	20.5 (9.0 – 31.0)	
SP (pg/mL)					
Basal	22.5 (5.3–46.5)	NS	17.4 (5.3 - 46.5)	27.5 (10.6 - 39.3)	NS/NS
4th week	22.0 (7.4–47.9)		24 (7.4 - 47.9)	18.0 (8.0 - 40.5)	
12th week	28.4 (8.0–46.0)		29.4 (9.4 – 39.0)	27.5 (8.0 – 46.0)	
GLS (%)					
Basal	–16.3 (–10.3 /–21.6)	NS	-16.34±2.83	-16.41±2.80	NS/NS
12th week	–16.2 (–11.1 /–21.7)		-15.82±2.40	-16.72±2.83	

BNP: Brain natriuretic peptide; GLP–1: Glucagon like peptide–1; GLS: Global longitudinal strain; NPY: Neuropeptide Y; SP: Substance P, NS: Non–significant.
*p values belong to comparison of 12th week vs. basal measurements.

Serum BNP level increased significantly at the end of the treatment when compared to basal in vildagliptin and saxagliptin treatment groups (p=0.02 and p=0.015, respectively). Serum NPY level increased significantly in only vildagliptin group at the 12th week when compared to basal measurement (p=0.047). Serum SP, GLP-1 levels and GLS measurements were similar to the basal values at the 12th week of treatment in both groups (**Table 3**).

Serum BNP levels of 16 patients (76.2%) in vildagliptin arm and 13 patients (81.3%) in saxagliptin arm increased during the follow-up period. Serum NPY levels of 17 patients (80.9%) in vildagliptin arm and 12 patients (75%) in saxagliptin arm increased during the follow-up period.

DISCUSSION

Our study showed that the treatment with saxagliptin and vildagliptin, were associated with increased levels of BNP. Substance P and GLP-1 levels were not changed during the follow-up period. Vildagliptin treatment was also associated with increased NPY levels. Nevertheless, GLS measurements were stable during the treatment period and patients did not need hospitalization for heart failure.

The CVOTs of DPP-4 inhibitors have not showed increased risk for MACE, whereas their effects on the risk of HF hospitalizations have been conflicting (6,8-11,23,24). A significant increase in the risk of hospitalization for HF was observed with saxagliptin treatment in SAVOR-TIMI 53 trial (6). A secondary analysis of the EXAMINE trial also demonstrated an increased trend towards heart failure hospitalizations with alogliptin among patients without prior HF (25). Cardiovascular trials of omarigliptin, sitagliptin and linagliptin showed similar safety profile with placebo with regard to MACE and heart failure hospitalizations (8-10). Some meta-analyses of CVOTs showed significant increase of heart failure with DPP-4 inhibitors, but their results were attributed to large cohort of SAVOR-TIMI 53 trial (26-27). Consistent with this opinion, a meta-analysis by Kongwatcharapong et al. (28) included 54 randomized controlled studies and suggested that use of saxagliptin was associated with heart failure, whereas other DPP-4 inhibitors were not. Otherwise, a large number of studies reported at least non-inferiority of DDP-4 versus placebo or other antidiabetics with regard to HF risk (3,29-32). The data from these meta-analyses were obtained by indirect comparisons, as head to head comparisons were not available. In the present study, patients enrolled the study without prior HF and observed that vildagliptin and saxagliptin treatments did not cause HF in a three months follow-up period.

The cause of increased risk for HF hospitalizations with saxagliptin treatment has not been understood yet. One hypothesize was a chance effect or a statistical error in SAVOR-TIMI trial. The inappropriate statistical methods were suggested as the cause of increased HF hospitalization risk (22). In the study of Kaneko et al. (21), authors re-evaluated the TECOS, EXAMINE and SAVOR-TIMI study groups for cardiovascular risk including hospitalization for HF risk with a different method and did not find any significant risk. Deterioration of endothelial function by DPP-4 inhibitors was suggested as a potential cause for HF previously (33). In the study of Ayaori et al. (33), it was found that flow mediated dilatation was significantly reduced with sitagliptin and alogliptin treatments. Authors emphasized that reduced GLP-1 metabolite by DPP-4 inhibition may be the cause of endothelial dysfunction (16,33). However, this result was not supported by van Poppel's study as they showed that endothelial function was improved with vildagliptin treatment (34). A class effect caused by accumulation of enzyme substrates has been widely suggested as a possible mechanism by which DPP-4 inhibitors may increase the risk of HF. The DPP-4 enzyme is expressed by many tissues and organs, and it is responsible for cleavage of many peptide hormones and cytokines (35,36). Substance P and NPY are the substrates of DPP-4 and they stimulate the sympathetic activity (14,20). Both SP and NPY were suggested to be related with myocardial fibrosis and adverse remodelling (17,37). In the study of Hubers et al. (19), sitagliptin treatment potentiated the norepinephrine release and NPY induced vasoconstriction. Consistently, a clinical study by Wilson et al. (38), showed that DPP-4 inhibition by sitagliptin increased NPY and norepinephrine levels. The increase in norepinephrine concentration was through a substance P receptor-dependent mechanism and under renin-angiotensin system blockage (38). The subgroup analysis of beta-blocker users in SAVOR-TIMI 53 trial demonstrated that hospitalization rates for HF were not increased in this group (24). This finding was suggested as an evidence of relation between HF and increased sympathetic activation by DPP-4 substrates (13,24). Treatment of patients with HF was not optimized with beta blockers and this may be another evidence for the impact of increased sympathetic activity by saxagliptin (13). The increase in HF hospitalization rate was associated with prior chronic kidney disease, HF and high basal BNP levels in SAVOR-TIMI 53 trial. Subclinical cardiac dysfunction was suggested as a risk factor for saxagliptin related worsening of HF (12). In our study, we did not observe change in substance P level over the treatment period but NPY level was increased in both groups. However, this finding was not associated with an evidence of increased subclinical cardiac dysfunction, as GLS measurements did not change over the treatment period.

The GLP-1 is the incretin substrate of DPP-4 and not only intact GLP-1 but also its metabolite were suggested to be biologically active and decrease the myocardial ischemia (16,18). Conversely, GLP-1 potentiation by DPP-4 inhibition is related with increased cAMP in myocardium and this alterations were suggested as a possible cause of heart failure exacerbation (39). Increased GLP-1 levels were also suggested to be associated with inflammation (40). Thus, impact of increased GLP-1 and decreased GLP-1 metabolite on cardiovascular system by DPP-4 enzyme inhibition has not been fully understood yet (41). In the present study, there was no significant changes in GLP-1 levels with neither saxagliptin, nor vildagliptin over a 12 weeks follow-up period.

Brain natriuretic peptide is another substrate for DPP-4 enzyme and a marker for heart failure (15). A study by Fadini et al. (42), reported that linagliptin treatment was not associated with acute effects on BNP and NT-proBNP levels. The change in BNP levels during DPP-4 treatment was evaluated in SAVOR-TIMI and EXAMINE study groups, but the treatment period was long, so the results did not reflect acute changes (7,24). Results of SAVOR-TIMI and EXAMINE trials were controversial, as both increased and decreased NT-proBNP levels were reported (6,7). A recent meta-analysis evaluated 9 randomized controlled trials with 3056 patients, by Mu et al. (43). They reported that DPP-4 inhibitors show no significant effect on BNP or NT-pro-BNP. Also they demonstrared that these agents shows no stronger effect than traditional antidiabetic agents in T2DM.

In our study, we observed that vildagliptin and saxagliptin treatments were both related with elevated levels of BNP, which may be a signal for HF.

The CVOTs of DPP-4 inhibitors were not designed to evaluate the risk of HF specifically and "hospitalization for HF" was a secondary outcome (6,8–10). Effects of DPP-4 inhibitors on echocardiographic parameters were evaluated in a limited number of studies (11,44,45). The only randomized-controlled trial for HF risk was performed with vildagliptin (VIVIDD study) and demonstrated an increase in left ventricular volumes when compared to placebo, despite it did not have significant effect on left ventricular ejection fraction (11). The baseline BNP levels and end-diastolic volumes were higher in vildagliptin arm. However, the significance or cause of this change could not be explained, and adverse cardiac remodeling could not be excluded. A randomized study evaluated changes in echocardiographic parameters over a 6 months treatment period with sitagliptin and showed that sitagliptin was not associated with improvement in left ventricle diastolic functions (44). On the other hand, in a pilot study by Leung et al. (45), DPP-4 inhibition was related with improved left ventricular function and GLS measurements at 12 months treatment period. Some of the previous studies, revealed favorable effects of DPP-4 inhibitors on diastolic functions (45,46). On the contrary, a recent meta-analysis by Zhang et al. (47), revealed that DPP-4 inhibitors had a negative impact on left ventricular end diastolic volume without increased ejection fraction. In our study, we evaluated GLS to assess any acute subclinical changes over a three months treatment period. Neither saxagliptin, nor vildagliptin were related with acute changes in GLS measurements.

The major limitations of the current study were the small sample size and lack of inclusion of patients with prior HF.

CONCLUSION

Our study showed that treatment with saxagliptin and vildagliptin was related with increased levels of BNP and NPY levels. However, subclinical myocardial damage or cardiac dysfunction could not be detected by GLS measurements assessed by 3-dimensional speckle tracking echocardiography over a 12 weeks period. There was no history of clinical cardiac disorder in our study population. Therefore, increases in BNP and NPY levels with vildagliptin and saxagliptin can be considered a safety signal. Patients with known heart problems are needed to clarify this.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Ankara University Faculty of Medicine Clinical Researches Ethics Committee (Date: 09.02.2015, Decision no: 02-74-15).

Informed Consent: Written informed consent was obtained from all participants who participated in this study.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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The effects of body mass index on postoperative pain in patients undergoing thoracic paravertebral block after video-assisted thoracoscopic surgery: A retrospective analysis

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ABSTRACT

Aim: Postoperative pain is an important problem in patients undergoing video-assisted thoracic surgery (VATS). Thoracic paravertebral block (TPVB) is among the commonly used techniques for pain control after VATS. Despite the analgesic methods applied, the desired level of pain control can not be achieved in all patients. Therefore, clinicians and researchers are interested in factors affecting postoperative pain. One factor is the relationship between postoperative pain and body mass index (BMI). Although it has been reported that acute or chronic pain is more common in the general population with a BMI, the relationship between postoperative pain and BMI is still controversial. This study aims to investigate the effects of BMI on postoperative pain in patients who underwent TPVB in the treatment of pain after VATS.

Material and Method: Patients who had elective VATS and TPVB were included in the study. Patients who underwent TPVB with ultrasonography (USG) and postoperative intravenous (iv) morphine patient-control-analgesia (PCA) for postoperative analgesia were divided into three groups according to BMI. Group-I BMI: 18-24.99 kg/m², Group-II BMI: 25-29.9 kg/m², Group-III BMI: 30-40 kg/m².

Results: 146 patients were included in the study. There was no significant difference between the postoperative 30th minute, 1st hour, 6th hour, 12th hour, and 24th-hour VAS values of the patients in Group-I, Group-II, and Group-III. There was no statistically significant difference in terms of morphine consumption, additional analgesic requirement, and complications in all three groups.

Conclusion: It was determined that there was no relationship between BMI and postoperative pain scores in the first 24 hours in patients who underwent TPVB after VATS. In addition, it was determined that postoperative morphine consumption and additional analgesic needs were not associated with BMI. Effective pain control can be achieved in all patients, regardless of BMI, with effective peripheral nerve blocks and analgesics using practical imaging techniques such as USG.

Keywords: Body mass index, postoperative pain, thoracic paravertebral block, visual analog scale, video-assisted thoracic surgery

INTRODUCTION

Video-assisted thoracic surgery (VATS), a less invasive technique, has become a popular method of choice in thoracic surgery (1). Although not as much as thoracotomy, postoperative pain is an important problem in patients undergoing VATS. Postoperative pain arises from the nociceptive stimulation induced on tissues by surgery and is at its highest intensity in the first 24 hours (2,3). Especially in the first 24-hour period, patients who underwent VATS are tried to provide postoperative pain control by applying different techniques. Thoracic paravertebral block (TPVB) is a common technique for pain control after VATS (4). Despite the analgesic methods applied, the desired level of pain control can not be achieved in all patients. However, prevention of postoperative pain is very important in terms of patient comfort, patient satisfaction and in preventing postoperative complications. Therefore, clinicians and researchers are always interested in the topics such as the factors affecting postoperative pain. One of these factors is the relationship between postoperative pain and BMI. Although it has been reported that acute or chronic pain is more common in the general population with a high body mass index (BMI), the relationship between postoperative

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pain and BMI is still not clear (2,5-8). However, the studies in the literature state that adipose tissue and BMI affect the distribution of the analgesic drug in the area where the local anesthetic agent is applied, and the effect differs according to the age, gender, and weight of the patients (9-14). Therefore, the effectiveness of the analgesia method applied after thoracic surgery may vary in patients with different BMIs, regardless of the success of the method. This may enable us to predict the postoperative pain level in patients based on BMI and to intervene early, before pain occurs. Thus, patient satisfaction can be increased, hospital stays can be shortened, and medication expenditures can be reduced by enabling personal postoperative pain management (15,16).

This study aims to investigate the relationship between BMI and postoperative pain in patients who underwent TPVB during the treatment of pain after VATS.

MATERIAL AND METHOD

Our study was carried out in a 3rd-level thoracic surgery center after the approval of the Ankara Keçiören Training and Research Hospital Ethics Committee (Date: 12.04.2022, No: 2012-KEAK-15/2493). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

The operating room receipts and postoperative pain forms of the patients who underwent elective VATS and TPVB between June 2021 and December 2021 were reviewed retrospectively. It was checked that informed consent was obtained from the patients. Patients who underwent TPVB with ultrasonography (USG) and postoperative intravenous (iv) morphine patient-control-analgesia (PCA) for postoperative analgesia were divided into three groups according to BMI. Group-I BMI: 18-24.99 kg/m², Group-II BMI: 25-29.9 kg/m², Group-III BMI: 30-40 kg/m².

Following patients are included in our study: Aged between 18 and 80 years, in the American Society of Anesthesiologists (ASA) I-II-III risk group, BMI between 18 and 40 kg/m², had elective VATS and TPVB and postoperative iv PCA with morphine for analgesia treatment.

Following patients were excluded from the study: Under the age of 18 and over the age of 80, ASA score IV and above, BMI below 18 kg/m², BMI over 40 kg/m², operated under emergency conditions, had chronic pain before the operation, were treated with analgesics other than TPVB and PCA with iv morphine.

Patients' age, height, body weight, BMI, gender, applied surgery, complications (such as hypotension, bradycardia, nausea, vomiting, sweating, itching) if any, postoperative 30th minutes, 1st hour, 6th hours, and 24th hours visual analog scale (VAS) values, postoperative 30th minutes, 1st hour, 6th hours, and 24th hours hearth rate, postoperative 30th minutes, 1st hour, 6th hours, and 24th hours mean arterial pressures (MAP), morphine consumption during the postoperative 24 hours were recorded.

Thoracic Paravertebral Block Analgesia Protocol

TPVB application in our clinic is as follows. After cleaning and covering the skin in the lateral decubitus position, following the rules of antisepsis, in the operating room, TPVB was applied using 20 ml of bupivacaine 0.25% 2-3 cm lateral to the T5 spinous process with USG. Intravenous morphine PCA was administered to these patients for 24 hours postoperatively. The PCA pump was limited to administering a bolus dose of 1 mg/2ml of morphine and delivering a maximum dose of 12mg/24ml of morphine over 4 hours with 15-minute lock-in intervals.

In addition to the routine clinical practice, a different analgesic protocol can be applied to patients who have problems in their renal and hepatic functions, have allergies to the drugs, and do not accept the analgesia method to be applied. For analgesia, 20 minutes before the end of the operation 50 mg iv dexketoprofen and 100 mg iv tramadol are administered and, 10 mg iv metoclopramide is administered for antiemetic purposes.

All patients receive paracetamol 1 g every 8 hours and 50 mg dexketoprofen every 12 hours for multimodal analgesia.

Statistical Analysis

Data analyses were performed with SPSS for Windows, version 22.0 (SPSS Inc., Chicago, IL, United States). Whether the distribution of continuous variable was normal was determined by the Kolmogorov Smirnov test. Levene test was used for the evaluation of homogeneity of variances. Unless specified otherwise, continuous data were described as mean±SD and median (interquartile range). Categorical data were described as the number of cases (%). The chi-square test or Fisher's exact test was used to compare qualitative data between groups. Statistical analysis differences in normally distributed variables between three independent groups were compared by the One-Way ANOVA test. Kruskal Wallis test was applied for comparisons of not normally distributed data. When a difference was detected, the post-hoc Tukey HSD test or Conover Inman test was used to identify the origin of the difference. p-value <0.05 is accepted as a significant level in all statistical analyses.

RESULTS

A total of 146 patients were included in the study. The demographic data of the patients, their ASA scores, applied surgery, and the duration of the anesthesia are given in **Table 1**.

Table 1. The demographic data of the patients, their ASA scores, applied surgery, and the duration of the anesthesia

	Mean±SD	Median (IQR)
Gender		
Female	46	31.5%
Male	100	68.5%
Age	47.11±15.74	49.50 (26)
BMI	26.02±5.29	25.33 (7.08)
Group-I: BMI: 18-24.99 kg/m²	71	48.6%
Group-II: BMI: 25-29.9 kg/m²	48	32.9%
Group-III: BMI: 30-40 kg/m²	27	18.5%
ASA		
ASA II	87	59.6%
ASA III	59	40.4%
Operation		
Wedge Resection	71	48.6%
Segmentectomy / Lobectomy	39	26.7%
Decortication	36	24.7%
Duration of the anesthesia/ minute	192.79±78.25	180 (105)

SD: Standart deviation, IQR: interquartile range, BMI: body mass index, ASA: American Society of Anesthesiologists

1st hour, 12th hour, and 24th hour MAP values of Group-III were statistically significantly higher than Group-I and Group-II. There was no significant difference between the groups in terms of postoperative heart rates.

VAS values at 30 minutes, 1 hour, 6 hours, 12 hours, and 24 hours postoperatively did not differ significantly between Group-I, Group-II, and Group-III patients. (**Table 2**). The change in VAS according to BMI is shown in **Figure 1**.

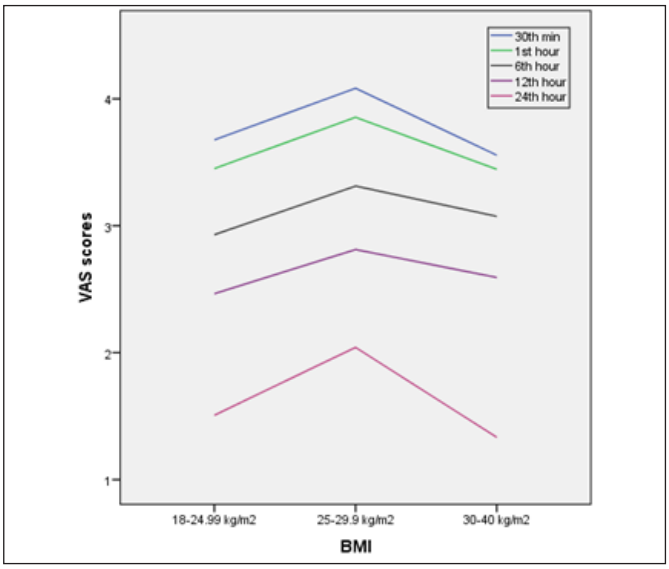

Figure. VAS Changes according to BMI

There was no statistically significant difference in terms of morphine consumption, additional analgesic requirement, and complications in all three groups (**Table 3**).

Table 2. VAS values of groups

	Group-I Mean±SD	Group-II Mean±SD	Group-III Mean±SD	p
VAS 30th min	3.68±1.71	4.08±1.80	3.56±2.08	0.320 β
VAS 1st hour	3.45±1.73	3.85±1.58	3.44±2.01	0.409 β
VAS 6th hour	2.93±1.57	3.31±1.64	3.07±1.71	0.442 β
VAS 12th hour	2.46±1.27	2.81±1.41	2.59±1.19	0.384 β
VAS 24th hour	1.51±1.22	2.04±1.53	1.33±0.96	0.099 β

Kruskal Wallis β. VAS: visual analog scale, SD: Standart deviation, min: minute

Table 3. Morphine consumption, additional analgesic need, and complications

	Group-I (n:71)	Group-II (n:48)	Group-III (n:27)	p
Morphine consumption, ml*	43.51±26.92	41.40±21.40	37.33±24.01	0.542
Additional analgesia				0.194
No	33 (46.5%)	15 (31.3%)	13 (48.1%)	
Yes	38 (53.5%)	33 (68.8%)	14 (51.9%)	
Complications				
Nausea/vomiting	1 (1.4%)	3 (6.3%)	-	0.313
Hypotension	1 (1.4%)	-	1 (3.7%)	0.449
Bradycardia	-	-	-	-
Sweating	1 (1.4%)	-	-	0.999
Itching	-	-	-	-

One-Way ANOVA*, Post hoc Tukey HSD or Conover-Inman test was performed for the binary comparisons among the groups and the p value was set at 0.05. ml: milliliter

DISCUSSION

According to our findings, there was no significant relationship between BMI and postoperative 30th minute, 1st hour, 6th hour, 12th hour, and 24th hour pain scores in patients who underwent TPVB after VATS. There was no difference between the groups in terms of postoperative morphine consumption and additional analgesic needs.

Pain after VATS is a common condition and adequate control of postoperative pain in patients undergoing VATS is very important for the prevention of pulmonary complications (17). Adequate management of postoperative pain provides early mobilization, lowers hospital stay, lowers costs, and increases patient satisfaction (18-21). It is also important to protect the lung, which has already suffered tissue damage after thoracic surgery, from complications such as postoperative pneumonia and atelectasis. For the early mobilization of patients after surgery, coughing should be ensured so that lung secretions can be cleared. This can only be possible by providing adequate analgesia to the patient (17,22). However, with using more opioids than necessary while providing postoperative pain control, side effects, such as respiratory depression and

low saturation, may be encountered (23). Here, it may be necessary to provide patients with invasive mechanical ventilator support again. This may cause additional complications, such as ventilator-associated pneumonia, barotrauma, and volutrauma in patients (24). Therefore, providing analgesia with adequate doses of agents is especially important in thoracic surgery. In short, predicting pain is important to prevent unnecessary drug administration and the side effects of these drugs while providing effective control of pain (25).

Postoperative pain can be affected by behavioral and emotional changes as well as nerve damage, tissue inflammation, genetic factors and, physiological factors (26). Many factors that can affect pain have been the area of interest of researchers. One of them is BMI. However, the relationship between postoperative pain and BMI has not been identified. There are studies that highlight different relationships between postoperative pain and BMI in different surgeries.

In a study conducted on patients who underwent thoracotomy and underwent epidural catheterization, it was stated that an increase in BMI increased VAS (27). Again, it has been reported in the literature that patients with higher BMI have higher pain scores and more narcotic requirements (28,29). Unlike the results obtained in these studies, it was revealed that there is no relationship between BMI and postoperative pain in a study conducted on patients who underwent ankle fracture surgery (30). Similarly, in another study conducted with patients undergoing general surgery, it was found that high BMI was not associated with a higher pain score (2). A positive relationship between BMI and postoperative pain was not seen in another study of breast cancer patients (31). Similarly, in a study conducted on patients who underwent tubular gastrectomy, no relationship was found between BMI and postoperative pain (7). In our study, no correlation was found between BMI and VAS in patients who underwent VATS and TPVB. This result can be considered as the result of effective analgesia applied to the patients. Because when we look at the VAS values of our patients, it was determined that the VAS averages in all groups (Group-I, Group-II, and Group-III) were below 4 in the first 12 hours postoperatively, and below 3 after the 12th hour. Only the mean VAS at the 30th minute in Group-II was found to be 4.08. However, this is not statistically significant.

There are studies showing that obese patients have different opioid needs in the postoperative period (28,29). However, in our study, it was determined that there was no difference between the groups in terms of morphine consumption amounts and additional analgesic needs.

In a study, it was stated that the VAS value of the patients increased as the BMI increased in patients who underwent thoracotomy and provided analgesia with an epidural catheter (27). Because BMI affects the adipose tissue in the epidural region, it may reduce the effectiveness of medications or result in epidural catheter application failure (32). Often, epidural catheterization is not performed using imaging techniques. Therefore, besides to the failure of catheter application, situations such as lateralization or displacement of the catheter to different levels cannot be noticed. This may cause insufficient analgesia in patients with high BMI. However, since the TPVB was performed with USG in our study, we can say that the success rate was high and effective analgesia was provided in all patients. Studies have reported that peripheral nerve blocks are technically more difficult and have lower success rates in patients with a BMI greater than 25 kg/m² (32,33). The paravertebral block has high failure rates according to other blocks (33). However, with introducing USG in peripheral block applications, the failure rates have decreased considerably (34,35). In our study, TPPV was performed under the guidance of USG. We can state that the applied block is effective and provides effective analgesia. However, studies have shown that the fat ratio in the area where the local anesthetic is administered may affect the distribution and pharmacokinetics of the agent (27,11-14). As a result, the transport of drugs to target tissues and the duration of anesthesia are affected (27, 36). These studies are mostly focused on the epidural area. In our study, a local anesthetic agent applied to the paravertebral area. Unlike these studies, similar analgesic effects were observed in all groups in our study and the VAS values of all groups were similar regardless of BMI, which may indicate a difference in adipose tissue between the epidural area and the paravertebral area. However, we think this situation should be supported by different studies.

The limitation of our study is that the study is retrospective and single-centered. More comprehensive information about the results of the study can be obtained with prospective and large series.

CONCLUSION

In conclusion, we found that there was no relationship between BMI and postoperative pain scores in the first 24 hours in patients who underwent TPVB after VATS. Postoperative morphine consumption and additional analgesic need were not associated with BMI. We think that effective pain control can be achieved in all patients, regardless of BMI, with analgesics and effective peripheral nerve blocks using practical imaging techniques such as USG.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was initiated with the approval of the Ankara Keçiören Training and Research Hospital Ethics Committee (Date: 12.04.2022, No: 2012-KEAK-15/2493).

Informed Consent: All patients were informed about the application and their informed consent was obtained.

Referee Evaluation Process: Externally peer-reviewed.

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

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Original Article

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The adaptation to Turkish of the caregiver contributions to selfcare of heart failure index: a validity and reliability study

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ABSTRACT

Objective: Although heart failure is a chronic and progressive disease, it is also a disease that requires the patient and caregivers who are not healthcare professionals to spend many years together in the process of follow-up, treatment, and care. Correct evaluation of the patient and caregiver in this process is one of the most important points that will guide the process. The aim of this study was to conduct a validity and reliability study of the Turkish version of the Caregiver Contributions to Self-Care of Heart Failure Index v.2- (CC-SCHFI) and to determine the contributions of caregivers of patients with heart failure.

Material and Method: The study sample was formed of the caregivers of patients who presented at a training and research hospital with a diagnosis of heart failure, who voluntarily agreed to participate in the research. Data were collected using a Personal Information Form and the CC-SCHFI. For the reliability study of the language adaptation of the CC-SCHFI, the internal consistency coefficient and the item-total points reliability coefficient were used, and to determine structure validity, Explanatory Factor Analysis (EFA) and Confirmatory Factor Analysis (CFA) were applied.

Results: The cultural adaptation to Turkish of the CC-SCHFI was found to be high. In the validity and reliability study, the structure validity and internal consistency were high and it was concluded that the scale could be used under the sub-dimension headings of "Recommendations for Protection", "The Role of the Caregiver in Treatment Compliance", and "Caregiver Practices".

Keywords: Caregiver, selfcare, heart failure, validity, reliability

INTRODUCTION

Despite continuous developments in science and technology in the field of healthcare, heart failure is one of the most important causes of morbidity and high mortality with an increasing prevalence and incidence worldwide. According to the 2015 data of the American Heart Association, there were approximately 6.2 million heart failure (HF) patients aged >20 years in the USA, and when 870,00 new diagnoses per year are added, it is estimated that the rate of diagnosed cases will increase by 46% by the year 2030 (1,2). According to the HAPPY study, HF prevalence in Turkey is 6.9% and there are 2,000,424 adult HF patients (3). As heart failure is a chronic and progressive disease, it requires many years of follow up, treatment, and care.

The primary aims of HF treatment are to reduce mortality and hospital admissions, increase functional capacity, correct symptoms and findings, and improve quality of life. In addition to the medical treatment of patients with HF, to provide compliance with the recommendations related to the management of signs and symptoms which cause mild -severe impairments in daily life because of fatigue, shortness of breath, and other cardiac findings, it is necessary to record and strengthen self-care practices (4). Heart failure self-care is defined as the process of health care and disease management in which stability is preserved in decisions and behaviors, changes in the patient's condition are identified and correct practices are provided (5).

In the management processes of diseases, patients with HF are usually supported by their spouse, family members, or friends. Caregiver is defined in literature as a person supporting the self-care of the patient in the management of the disease but they are also important in many other respects such as preventing symptoms, observations, keeping records, and treatment compliance (6). The presence of caregivers is associated with a positive prognosis and less use of hospital services (7,8).

Clinicians have always needed valid and reliable measurement tools to be able to develop and support self-care, and studies have been conducted in this field. One of the most widely used tools throughout the world is the

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Caregiver Contributions to Self Care of Heart Failure Index (CC-SCHFI). Version 7.2 of the CC-SCHFI is formed of 3 sections of self-care (10 items), self-care management (8 items), and symptom perception (11 items) (9).

The aim of this study was to conduct a validity and reliability study of the Turkish version of the Caregiver Contributions to Self-Care of Heart Failure Index v.2- CC-SCHFI and to determine the contributions of caregivers of patients with heart failure.

MATERIAL AND METHOD

The study was carried out with the permission of İstanbul Başakşehir Çam and Sakura City Hospital Clinical Researchs Ethics Committee (Date: 07.07.2022, Decision No: KAEK/2022.07.230). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Universe-Sample

The recommended sample size for a scale to be adapted to a different culture is in the range of 5-10-fold more than the number of items in the scale (10). Thus the minimum sample size required for the validity and reliability study of the CC-SCHFI-2, which is formed of 29 items, was calculated to be 145 individuals. The sample group of volunteeers for this research was formed of 246 caregivers of patients who presented at a training and research hospital with a diagnosis of heart failure.

Data Collection

The first section was applied as a sociodemographic information form to elicit general information of age, gender, marital status, children, educational level, occupation, current employment status, economic status, and people living in the same home. The second section was applied as the Turkish version of the CC-SCHFI, formed of the 3 sections of 1) HF Self-Care Recommendations (10 items), 2) Symptom Management (11 items), and 3) Care Practices (8 items).

Language Validity of the Scale

The Turkish translation of the CC-SCHFI (version 2) was made by 3 specialists proficient in both Turkish and English languages. Two of these 3 specialists were healthcare professionals and one was a language specialist not in the field of healthcare. The translated scales were collated and examined in respect of language compatibility by a different language specialist. The corrected form was back-translated into English by a language specialist, then compared in respect of compatibility with the CC-SCHFI-2, and the translation to Turkish was completed (10,12).

Statistical Analysis

Data obtained in the study analyses were evaluated using IBM SPSS (Statistical Package for Social Sciences) and 20 LISREL software. Descriptive statistics were calculated for all the variables and stated as number (n), percentage (%), mean±standard deviation (SD) values, skewness and kurtosis. To evaluate the knowledge of data factors, the Kaiser-Meyer-Olkin (KMO) test, sample sufficiency measurement, and the Bartlett sphericity test were used. Significance of the Bartlett sphericity test ($p<0.000$) and $1.00 \leq KMO \leq 0.90$ showed that there was a sufficient sample to support factor analysis. To determine the structure validity of the scale, Explanatory Factor Analysis (EFA) and then Confirmatory Factor Analysis (CFA) were applied. Internal consistency coefficients (Cronbach alpha) were calculated to examine reliability.

RESULTS

The sociodemographic characteristics of the caregivers of the HF patients are shown in **Table 1**. As seen in **Table 1**, the study participants comprised 142 (57.7%) males and 104 (42.3%) females with a mean age of 57 years, 186 (75.6%) were married, 198 (80.5%) had children, 90 (36.6%) had an educational level of primary school, 70 (28.5%) were housewives, 185 (75.2%) had an average economic status, 152 (61.8%) were unemployed, 235 (95.5%) had social insurance, and 224 (91.1%) lived together with family.

Table 1. Sociodemographic characteristics of the caregivers

Age (years)	57.8049±15.09408	
Gender		
Female	104	42.3
Male	142	57.7
Marital status		
Married	186	75.6
Single	42	17.1
Divorced/Widowed	18	7.3
Children		
Yes	198	80.5
No	48	19.5
Education level		
Literate	35	14.2
Primary school	90	36.6
High school	64	26.0
University	57	23.2
Occupation		
Housewife	70	28.5
Retired	62	25.2
Self-employed	65	26.4
Clerk	35	14.2
Student	7	2.8
Manual worker	7	2.8
Economic status		
Poor	27	11.0
Average	185	75.2
Good	34	13.8
Current employment status		
Employed	94	38.2
Unemployed	152	61.8
Social Insurance		
Present	235	95.5
Absent	11	4.5
Other people with whom currently living		
Living alone	22	8.8
Living with family	224	91.1

In the descriptive analysis of the Caregiver Contributions to Self Care of Heart Failure scale, the skewness and kurtosis values were seen to be between -3 and +3, showing normal distribution (**Table 2**).

To be able to determine whether or not the data were suitable for EFA, first the KMO and Bartlett tests were applied. The results of the KMO and Bartlett tests are shown in **Table 3**.

As a result of the analysis, the KMO value of 0.92 and the Bartlett test (x2 =5163.009; p=0.000) were found to be significant. The results obtained showed that the data set was suitable for EFA. A Scree Plot obtained as a result of EFA is shown in **Figure 1**.

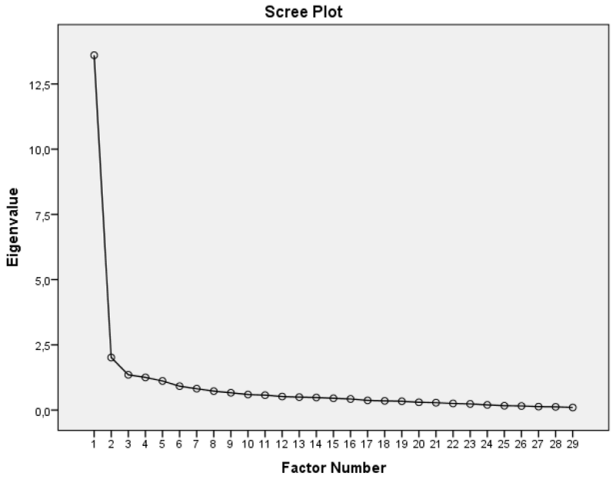

Figure 1. EFA ScreePlot Grafiği

Table 3. Suitability of the sample for factor analysis	
Kaiser-Meyer-Olkin Measure of Sampling Adequacy	.928
Bartlett's Test of Sphericity	
Approx. Chi-Square	5163.009
Df	406
Sig.	.000

When the graph is examined, it can be said that the scale has 3 sub-dimensions. The 3-factor cumulative values in the EFA were found to be >40%, with Factor 1 determining 46.90%, Factor 2 determining 6.94%, and Factor 3 determining 5.41% variance. After determining the factor numbers, the common variances and factor loading of the items were determined, and are presented in **Table 4**. When **Table 4** is examined, the CC-SCHFI was seen to be formed of 3 sub-dimensions, which explained 59.25% of the total variance. The factor load values of the items collected under 3 sub-dimensions varied between 0.54 and 0.90, and as the difference between the factor loads was >1, there was not seen to be a need to remove any items.

To be able to confirm the 3-dimensional structure obtained with EFA, CFA was performed with the LISREL program and this is presented in **Figure 2**. The scale items were given t values. In accordance with the analyses performed, the level representing the implicit variable of all the items (observed oblique) of all the factors was significant at 0.05.

Table 2. Descriptive statistic of the caregiver contributions to self care of heart failure index

The caregiver contributions to self care of heart failure index	**Mean**	**(±) SD**	**Skewness**	**Kurtosis**
1.Try to avoid getting sick (e.g., wash your hands)?	3.9878	1.15522	-1.177	.695
2. Get some exercise (e.g., take a brisk walk, use the stairs)?	3.7236	1.27648	-.763	-.486
3. Eat a low salt diet?	4.0366	1.09297	-1.113	.604
4. See the health care provider for routine health care?	3.9797	1.02797	-.891	.151
5. Take prescribed medicines without missing a dose?	4.1301	1.04557	-.933	-.155
6. Order low salt items when eating out?	3.7886	1.15530	-.620	-.573
7. Make sure to get a flu shot annually?	3.1138	1.51834	-.004	-1.500
8. Ask for low salt foods when visiting family and friends?	4.1138	1.07440	-1.005	.146
9. Use a system or method to help remember to take medicines?	3.9431	1.37203	-1.063	-.160
10. Ask your health care provider about medicines?	4.2154	1.13134	-1.285	.585
11. Monitor weight daily?	3.7114	1.27856	-.804	-.370
12. Pay attention to changes in how he/she feels?	4.0000	.88985	-.911	1.139
13. Look for medicine side-effects?	4.1423	1.08048	-1.109	.405
14. Notice whether he/she tires more than usual doing normal activities?	4.2886	.99490	-1.257	.786
15. Ask the health care provider how he/she is doing?	4.3984	.94967	-1.622	2.051
16. Monitor closely for symptoms?	4.3211	.99310	-1.462	1.391
17. Check ankles for swelling?	4.3699	.95477	-1.566	2.013
18. Check for shortness of breath with activity such as bathing and dressing?	4.1626	1.12394	-1.125	.274
19. Keep a record of symptoms?	3.9268	1.37423	-1.028	-.256
20. How quickly did you recognize that he/she had symptoms?	3.9512	1.05253	-.876	.247
21. How quickly did you know that the symptom was due to heart failure?	2.9146	1.36308	-.039	-1.267
22. Further limit the salt he/she eats that day?	4.1220	1.03479	-1.049	.452
23. Reduce fluid intake?	4.0854	1.07515	-1.085	.495
24. Take a medicine?	3.9106	1.25525	-1.016	.061
25. Call the health care provider for guidance?	4.2967	1.02088	-1.341	.982
26. Ask a family member or friend for advice?	4.1545	1.12146	-1.044	-.089
27. Try to figure out why he/she has symptoms?	3.5854	1.18098	-.813	-.094
28. Suggest that he/she limit activity until he/she feels better?	4.1545	1.12146	-1.044	-.089
29. Did the treatment you used make him/her feel better?	3.5854	1.18098	-.813	-.094

The goodness of fit index (GFI) values of the CFA were found to be Chi-square (x2)914.70, Degree of Freedom (df) 360, x2 / df 2.54, and Root Mean Square Error of Approximation (RMSEA) 0.079. The Normalised Fit Index (NFI)=0.95, Non-Normalised Fit Index (NNFI)=0.96, and GFI=0.64. The values of the defined fitness indexes were seen to be above the acceptable values, and the first level CFA model of the CC-SCHFI was determined to generally show good fit (**Table 5**).

In the CFA of the CC-SCHFI, items 3,4,5,12,14, 15, 16, 17, 20, 21,22, 24,25, 27, 28, and 29 were in Factor 1, and explained 46.90% of variance, and these items were seen to be questions related to caregiver practices. Items 1, 2, 9, 10, 11, 13, 19, and 26 in Factor 2 explained 6.94% of variance, and these items were related to the role of the caregiver in treatment compliance. Items 6,7,8, and 23 in Factor 3 explained 5.41% of variance and were related to patient self-care protection recommended behaviours.

Table 4. Factor structure of the CC-SCHFI

CC-SCHFI	Factor 1	Factor 2	Factor 3	Item Total Correlation
Item 1		.347		.693
Item 2		.592		.519
Item 3	.635		.471	.792
Item 4	.684		.427	.656
Item 5	.616		.476	.644
Item 6	.351		.717	.690
Item 7			.427	.698
Item 8	.357		.557	.653
Item 9		.723		.526
Item 10	.553	.683		.669
Item 11		.744		.746
Item 12	.758			.744
Item 13	.594	.709		.718
Item 14	.713	.344		.621
Item 15	.792			.494
Item 16	.794			.610
Item 17	.746			.673
Item 18	.657	.431		.614
Item 19		.709		.740
Item 20	.743			.688
Item 21	.744	.316		.659
Item 22	.647	.329		.797
Item 23			.328	.690
Item 24	.755			.557
Item 25	.666	.350		.645
Item 26		.657		.797
Item 27	.829			.690
Item 28	.683	.350		.557
Item 29	.673			.645
Variance Source	**Factor 1**	**Factor 2**	**Factor 3**	**Total**
Explained variance	46.90	6.94	5.41	59.25%

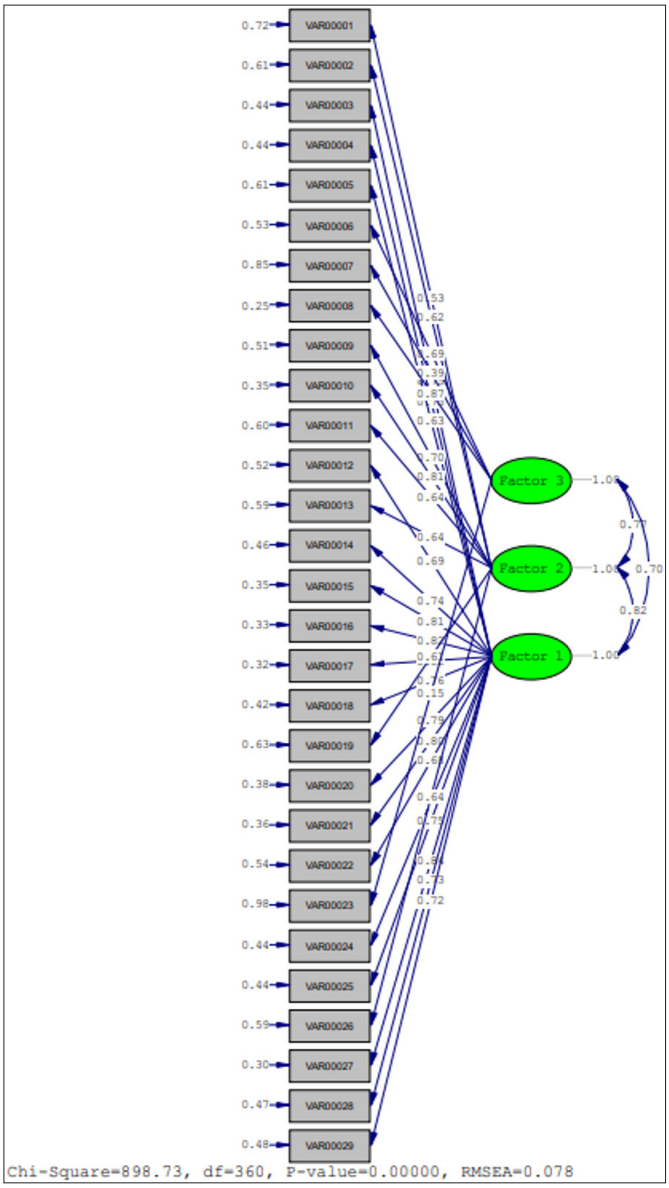

Figure 2. CFA Model of the CC-SCHFI

Table 6. Reliability coefficients of the CC-SCHFI and sub-dimensions

	Cronbach Alpha		Item Number	
Total scale	0.952	0.952	29	29
Recommendation	0.762	0.624	7	4
Symptom management	0.923	0.856	8	8
Carer role	0.916	0.958	14	17

Table 5. CFA Fit Indexes of the CC-SCHFI

Fitness measurements	Good fit	Acceptable fit	Measurement value	Fit
X2/df	0≤χ2 /df≤2	2≤χ2 /df≤3	2.49	Acceptable fit
RMSEA	0≤RMSEA≤0.05	0.05≤RMSEA≤0	0.078	Acceptable fit
NFI	0.95≤NFI≤1.00	0.90≤NFI≤0.95	0.96	Good fit
NNFI	0.97≤NNFI≤1.00	0.95≤NNFI≤0.97	0.97	Good fit
CFI	0.97≤CFI≤1.00	0.95≤NNFI≤0.97	0.97	Good fit
GFI	0.95≤GFI≤1.00	0.90≤GFI≤0.95	0.78	Poor fit
AGFI	0.90≤AGFI≤1.00	0.85≤AGFI≤0.90	0.84	Poor fit

Reliability

When the reliability coefficients calculated of the CC-SCHFI, formed of 29 items, are examined in Table 6, the total reliability coefficent was 0.952 and the reliability coefficients of the sub-dimensions varied between 0.762 and 0.923. According to these findings, the internal consistency of this scale is high.

DISCUSSION

Self-care of patients with heart failure and the disease management processes generally include the management of more than one drug, the follow-up of recommended diet and fluid restrictions, the performing of daily exercise, daily monitoring of symptoms and weight, managing changes in symptoms (eg., when taking an extra diuretic or experiencing early fluid overload seeking a healthcare provider for guidance) and navigating the healthcare system. Self-care of HF patients, which is defined in literature as behaviours to protect and maintain health, is focussed on the processes of self-care, observation and management of symptoms and treatment compliance. The management process of HF patients is made together with caregivers who are not professional healthcare workers in the majority of cases (13,14).

In Turkey, the validation of the Turkish version 6.2 of the CC-SCHFI was performed by Akbıyık and Enç (14) in 2016. Validation studies of the CC-SCHFI in Spain and Thailand found a structure of one dimension, whereas the Brazilian version and the current study showed a structure with 3 dimensions, similar to the original (15,17). As in the original study, the analyses showed generally high factor loading in all 3 sub-dimensions of the Turkish version of the CC-SCHFI, and caregiver practices was seen to have the highest factor loading. It is noteworthy that the caregiver practices focus on being aware and preventing the development of symptoms, and managing the process. In recent years, specific scale studies related to the effect of symptom management on both patient and caregiver have shown the importance of symptom management (18,19). Protection, treatment compliance, and symptom management are subjects in the education given to patients and their families by healthcare professionals (20,21).

The ability of healthcare professionals to measure the contribution of both the patient and caregivers to the process of management of HF will be of guidance in the treatment and care process to be able to maintain quality of life and continuity of life without disability. Previous studies have shown that awareness, behaviours, and levels of knowledge are important in the disease management process for caregivers and patients with HF (22,23).

CONCLUSION

The CC-SCHFI evaluates the process in three dimensions and can help caregivers identify deficient areas of self-care for HF patients, and it is an easy-to-manage tool allowing the design of individual plans which aim to expand knowledge to improve skills. In this validity and reliability study of the adaptation between cultures of the CC-SCHFI to Turkish, the structure validity and internal consistency were determined to be high. It was concluded that the scale can be used under the sub-dimension headings of "Recommendations for protection", The Role of the Caregiver in Treatment Compliance", and "Caregiver Practices".

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of İstanbul Başakşehir Çam and Sakura City Hospital Clinical Researchs Ethics Committee (Date: 07.07.2022, Decision No: KAEK/2022.07.230).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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The progress of chronic renal disease patients followed by the diagnosis of COVID-19 in ICU

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ABSTRACT

Aim: The mortality and morbidity of COVID-19 disease are higher in patients with comorbidities. In this study, we staged patients with chronic renal failure hospitalized in the intensive care unit (ICU) and aimed to evaluate the process of the disease according to the stage of failure.

Material and Method: The medical records of 249 patients followed in Ankara City Hospital MH3 ICU were reviewed retrospectively. The patients were divided into three stages according to their estimated glomerular filtration rate (e-GFR) value (stage 1: e-GFR≥90 ml/min/1.73 m², stage-2: e-GFR: 15-89 ml/min/1.73 m², stage- 3: e-GFR≤15 ml/min/1.73 m²). Data such as age, gender, comorbidity status, length of stay in the ICU, duration of mechanical ventilation, and mortality rate of the patients were recorded. Patients who were evaluated as stage-2 were also classified into 3 stages (stage-2a: e-GFR: 60-89 ml/min/1.73 m², stage-2b: e-GFR:30-59 ml/min/1.73 m², stage-2c: e-GFR: 15-29 ml/min/1.73 m²) and evaluated with the same parameters.

Results: The mean age of all patients was 71 years. It was found that the intubation rate was higher (p=0.012) and the mortality rate was higher (p=0.003) in patients evaluated as stage-3. APACHE II and SOFA scores were higher than the other groups (p=<0.001, p=<0.01). In addition, IL-6, procalcitonin, D-dimer, and ferritin levels were also found to be significantly higher in these patients. Hemoglobin and thrombocyte levels were significantly lower than the other groups. When stage-2 patients were divided into subgroups, it was found that APACHE II and SOFA scores, mechanical ventilation rate, and mortality rate were higher in stage-2c than the other subgroups. While CRP, procalcitonin, and D-dimer values were significantly higher in this group, hemoglobin, thrombocyte, and lymphocyte values were found to be low.

Conclusion: The mortality rate is high in COVID-19 patients with chronic kidney disease(CKD) in ICU. As the stage of the disease increases, the mechanical ventilation rate and mortality rate of the patients increase gradually. For this reason, it may be recommended to be more careful in terms of preventive measures in cases of CKD.

Keywords: COVID-19, chronic kidney disease, mortality, intensive care unit, glomerular filtration rate

INTRODUCTION

Coronavirus disease 2019 (COVID-19) disease started in December 2019 in Wuhan, China. Then, it spread all over the world and was declared a pandemic by the World Health Organization (WHO) as of March 2020 (1-3). One of the targets of COVID-19, which affects many systems, is the kidneys. The effects of COVID-19 disease on kidney functions are thought to be multifactorial. First, it was thought that it might have a direct cytopathic effect on the kidney since SARS-COV-2 RNA could be detected in the urine (4). In addition, it is thought that the virus acts on ACE-2 receptors and that these receptors are found more in the kidneys, which strengthens this thesis (5). Secondly, it was thought that immune complexes might accumulate in the kidney, especially through a T-lymphocyte-mediated mechanism, but this could not be proven by electron microscopy (6). Thirdly, it is thought that viral-derived cytokines may have indirect effects on kidney tissue such as shock, rhabdomyolysis, and hypoxia (7).

In a study by Richardson et al. (8), it was reported that 20% of patients hospitalized with the diagnosis of COVID-19 developed acute kidney injury(AKI) and that 3.2% of these patients required renal replacement therapy(RRT). There are data showing that COVID-19 disease is more severe and the mortality rate is higher in pre-existing chronic

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kidney damage (9). Based on these data, it has been reported that the mortality of patients with signs of renal failure (such as hematuria, proteinuria) is high even after other causes have been excluded (9). For this reason, it has been emphasized that it may be important to monitor kidney function tests and detect abnormalities early, even in patients presenting with mild flu symptoms (9).

Research on COVID-19 continues to increase in all areas and is noticed in the literature. (10-13). Studies on the course of COVID-19 are also ongoing in patients with chronic kidney damage. In this study, we aimed to examine the course and characteristics of the disease in the current population hospitalized in the intensive care unit (ICU).

MATERIAL AND METHOD

The study was carried out with the permission of Ankara City Hospital No 1 Clinical Researches Ethics Committee (Date: 15.06.2022, Decision No: E1-22-2617). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Patients Criteria

249 patients diagnosed with COVID-19 in Ankara City Hospital Intensive Care Unit between 01.03.2020-01.01.2022 were included in the study. Patients with pregnancy status and a history of renal transplant were not included in the study.

Study Design

Patients' data, files, and follow-up forms were reviewed retrospectively. The estimated glomerular filtration rate (e-GFR) was calculated and recorded with the modification of diet in renal disease (MDRD) formula. Afterward, the data of these patients such as age, gender, comorbidity, length of stay in the intensive care unit, duration of mechanical ventilation, and mortality rate were recorded. Patients were first classified into three stages (stage 1: e-GFR >90 ml/min/1.73 m^2, stage-2: e-GFR: 15-89 ml/min/1.73 m^2, stage-3: e-GFR ≤ 15 ml/min./1.73 m^2) were separated. Then, the patients who were evaluated as stage-2 were also divided into 3 stages themselves (stage-2a: e-GFR: 60-89 ml/min/1.73 m^2, stage-2b: e-GFR:30-59 ml/min/1.73 m^2, stage-2c: e-GFR: 15-29 ml/min/1.73 m^2) were separated and evaluated with the same parameters. Demographic data and laboratory data of all groups were compared.

Statistical Analysis

Shapiro Wilk test was used for assessing whether the variables follow normal distribution or not. Continuous variables were presented as median (minimum: maximum) and mean±standard deviation values. Categorical variables were reported as n (%). According to the normality test results, the Kruskal Wallis test or ANOVA test was used if the number of groups were more than two. Multiple comparison procedures were performed using the Dunn Bonferroni approach to identify different group or groups after the Kruskal Wallis test. Pearson Chi-square, Fisher's exact test, and Fisher Freeman-Halton test were used for comparing categorical variables. SPSS (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0, Armonk, NY-IBM Corp.) was used for statistical analysis, and a p-value; 0.05 was considered statistically significant.

RESULTS

The data of 249 patients were scanned in the study. Of these patients, 63 (25%) were evaluated as Stage-1, 167 (67%) as Stage-2, and 19 (7.6%) as Stage-3. Demographic data, comorbidities, and laboratory data of these groups are shown in **Table 1**.

The patients in stage 2 were also divided into 3 groups and their demographic data, comorbidities, and laboratory data were compared in **Table 2**.

DISCUSSION

It is known that COVID-19 disease has a worse prognosis and is more mortal in patients with comorbidities. In this study, we aimed to show the characteristics of patients admitted to the ICU with chronic kidney disease(CKD) diagnosed with COVID-19 and the effect of CKD on mortality.

Cheng et al. (9) compared patients who have normal serum creatinine (SCr) at admission to patients who have high SCr values at admission in a prospective analysis of 701 patients with COVID-19. In patients presenting with high SCr, higher leukocytes, lower lymphocytes, lower platelets, prolonged partial thromboplastin time, higher D-dimer levels, increased procalcitonin, and increased lactose dehydrogenase (LDH) were found. The incidence of AKI was significantly higher in patients with elevated baseline (9). In addition, admission to the ICU and mechanical ventilation showed a higher prevalence in patients with COVID-19 and high baseline SCr (14). High baseline SCr nearly tripled the risk of in-hospital death (9). It was thought that a more pronounced cytokine storm might develop in patients with chronic renal failure and COVID-19, and more severe systemic inflammation and hypercoagulability could be seen in these patients (9). In our study, patients admitted to the ICU were staged according to their e-GFR levels. It was observed that the median procalcitonin, ferritin, and cytokine storm values such as IL-6, and D-dimer of the patients in the stage-3 group were significantly higher than the other groups. There was no significant difference in LDH, Lymphocyte, and WBC values. A

Tablo 1. Comparison of demographic and laboratory data of stages 1, 2, and 3

	Total (n=249)	Stage 1 (e-GFR≥90 ml/ dk/1.73 m²) (n=63)	Stage 2 (e-GFR:15-89 ml/ dk/1.73 m²) (n=167)	Stage 3 (e-GFR≤15 ml/ dk/1.73 m²) (n=19)	p-value
Age (year)	71(19-97)	57 (19-86)	76 (28-97)	70 (47-93)	<0.001[a]
Length of stay (day)	9 (1-49)	10 (1-49)	9 (1-43)	7 (2-47)	0.583[a]
Duration of intubation (day)	1 (0-29)	0 (0-28)	1 (0-29)	4 (0-23)	0.081[a]
Gender (Male)	166 (66.67%)	47 (74.60%)	105 (62.87%)	14 (73.68%)	0.193
Co-infection (yes)	123 (49.40%)	28 (44.44%)	84 (50.30%)	11 (57.89%)	0.543[b]
Outcome (exitus)	118 (47.39%)	22 (34.92%)	81 (48.50%)	15 (78.95%)	0.003[b]
HFNO (yes)	105 (42.17%)	29 (46.03%)	73 (43.71%)	3 (15.79%)	0.051[b]
NIMV(yes)	81 (32.53%)	19 (30.16%)	57 (34.13%)	5 (26.32%)	0.708[b]
IMV (yes)	132 (53.01%)	26 (41.27%)	91 (54.49%)	15 (78.95%)	0.012[b]
DM (yes)	82 (32.93%)	16 (25.40%)	59 (35.33%)	7 (36.84%)	0.335[b]
HT (yes)	154 (61.85%)	25 (39.68%)	118 (70.66%)	11 (57.89%)	<0.001[b]
CAD (yes)	122 (49%)	22 (34.92%)	85 (50.90%)	15 (78.95%)	0.002[b]
COPD (yes)	42 (16.87%)	10 (15.87%)	30 (17.96%)	2 (10.53%)	0.693[b]
Cancer (yes)	26 (10.48%)	8 (12.70%)	17 (10.24%)	1 (5.26%)	0.640[b]
Neurological disease (yes)	47 (18.95%)	10 (15.87%)	30 (17.96%)	8 (42.11%)	0.027[b]
IVIG (yes)	3 (1.21%)	2 (3.17%)	1 (0.60%)	0	0.357[c]
Anakinra (yes)	28 (11.24%)	12 (19.05%)	16 (9.58%)	0	0.035[b]
Inotropic agents (yes)	117 (46.99%)	24 (38.10%)	77 (46.11%)	16 (84.21%)	0.002[b]
Corticosteroid					
No	78 (31.33%)	16 (25.40%)	52 (31.14%)	10 (52.63%)	
<250 mg Methylprednisolone	95 (38.15%)	17 (26.98%)	70 (41.92%)	8 (42.11%)	0.002[b]
≥250 mg Methylprednisolone	76 (30.52%)	30 (47.62%)	45 (26.95%)	1 (5.26%)	
APACHE II	14 (2-57)	10 (2-36)	14 (3-57)	32 (3-54)	<0.001[a]
SOFA	5 (3-41)	4 (3-13)	5 (3-41)	11 (6-18)	<0.001[a]
Ferritin (µg/L)	500 (1.55-62904)	524 (7-5195)	455 (1.55-62904)	883 (209-40284)	0.003[a]
CRP (mg/L)	0.11 (0-0.80)	0.11 (0-0.80)	0.10 (0-0.43)	0.16 (0.01-0.33)	0.258[a]
Procalcitonin (µg/L)	0.20 (0-397)	0.08 (0-397)	0.20 (0.02-57.32)	6.90 (0.21-195.53)	<0.001[a]
IL-6 (pg/mL)	47 (2.72-14585)	45 (3.45-14585)	43.60 (2.72-5066)	159 (6.09-2582)	0.004[a]
Fibrinogen (g/L)	5.16 (1-678)	5.13 (2.45-678)	5.44 (1.06-10.10)	4.53 (1-7.68)	0.353[a]
D-dimer (mg/L)	1.60 (0.19-135)	1.26 (0.19-135)	1.60 (0.20-56.36)	3.82 (0.87-104)	0.007[a]
LDH (U/L)	500 (37-4897)	500 (166-4897)	492 (37-3800)	520 (189-1255)	0.744[a]
ALT (U/L)	34 (1-1684)	41 (6-126)	33 (1-1684)	30 (3-435)	0.252[a]
AST (U/L)	46 (4-4088)	44 (12-332)	48 (4-4088)	47 (14-1413)	0.486[a]
WBC (×10⁹/L)	9.8 (2.40-88.0)	10.2 (24.0-37.6)	9.7 (1.08-8.8)	9.1 (1.7-27.0)	0.857[a]
Neutrophil (×10⁹/L)	8.6 (1-14)	8.7 (3.7-14)	8.7 (1-28.2)	7.9 (14.8-25.3)	0.957[a]
Lymphocyte (×10⁹/L)	0.6 (0.1-10.1)	0.72 (0.1-3.6)	0.62 (0.14-10.1)	0.66 (0.19-3)	0.896[a]
NLR	13.20 (0.05-238)	13.32 (0.05-238)	13.20 (1.25-89.36)	12.30 (3.16-44.34)	0.944[a]
Hemoglobin (g/dL)	12.60 (6.20-92)	13.20 (7.30-92)	12.60 (6.20-17.50)	10.70 (7.60-16.40)	0.013[a]
Platelet (×10⁹/L)	231 (9-672)	283 (9-582)	225 (318-672)	162 (32-381)	0.001[a]

HFNO: High flow nasal oxygen; NIMV: noninvasive mechanical ventilation; IMV: invasive mechanical ventilation; DM: diabetes mellitus; HT: hypertension; CAD: coronary artery disease; COPD: chronic obstructive pulmonary disease; IVIG: intravenous immunoglobulin; CRP: C-reactive protein; IL: interleukin; LDH: lactate dehydrogenase; ALT: alanine amino transferase; AST: aspartate amino transferase; WBC: white blood cell; NLR: neutrophil lymphocyte ratio.

significant decrease in Hb value was observed. However, this may be due to the fact that this group of patients in end-stage renal disease patients. The platelet count was significantly higher than the other groups. In addition, mechanical ventilation and mortality rates were found to be higher. In our study, the stage-2 patient group was also evaluated by dividing it into 3 groups. While the median CRP, procalcitonin, d-dimer values, and N/L ratio were found to be high in the stage 2c group, the lymphocyte count and Hb values were found to be low. Mechanical ventilation and mortality rates were also higher. Although the mechanical ventilation rate of stage 2c patients was higher than stage 2b, no significant difference was found in terms of mortality rate.

In a study, it was suggested that the development of kidney damage during hospitalization and patients with pre-existing CKD are independent risk factors for poor prognosis (14). Scoring systems such as APACHE II and SOFA are used to evaluate mortality in ICU patients (15). In our study, it was determined that APACHE II and

SOFA scores increased as the stage grade increased, as e-GFR decreased, the mechanical ventilation rates and inotropic needs of these patients were higher, and the mortality rates were higher.

In a study conducted on 3,319 patients in New York, it was found that patients with CKD increased mortality up to 7 times in SARS-COV-2 infection. Specifically, when we look at this group, it has been reported that the mortality rate is higher in patients with atrial fibrillation, coronary artery disease(CAD), and ischemic heart failure associated with CKD (16). In our study, the incidence of CAD was found to be significantly higher in the stage-3 group than in the stage-1 group, but its relation to mortality was not evaluated. In addition, it was observed that the group with the neurological disease was more common in this stage. However, when compared with the stage-2 group, no significant difference was found in terms of CAD association.

Tablo 2. Demographic data, comorbidities, and laboratory data of the patients in stage 2

	Stage 2a (e-GFR: 60-89 ml/ dk/1.73 m²) (n=83)	**Stage 2b (e-GFR: 30-59 ml/dk/1.73 m²) (n=60)**	**Stage 2c (e-GFR: 15-29 ml/ dk/1.73 m²) (n=24)**	***p*-value**
Age (year)	73 (28-93)	78.50 (45-97)	80.50 (30-93)	0.114[a]
Length of stay (day)	10 (1:40)	8 (1:43)	6 (1:32)	0.108[a]
Duration of intubation (day)	0 (0:29)	1 (0:25)	2 (0:16)	0.167[a]
Gender (Male)	52 (62.65%)	37 (61.67%)	16 (66.67%)	
Outcome (exitus)	34 (40.96%)	30 (50%)	17 (70.83%)	0.034[b]
HFN (yes)	37 (44.58%)	26 (43.33%)	10 (41.67%)	0.966[b]
NIMV (yes)	27 (32.53%)	23 (38.33%)	7 (29.17%)	0.661[b]
IMV (yes)	40 (48.19%)	31 (51.67%)	20 (83.33%)	0.008[b]
DM (yes)	32 (38.55%)	19 (31.67%)	8 (33.33%)	0.680[b]
HT (yes)	53 (63.86%)	50 (83.33%)	15 (62.50%)	0.026[b]
CAD (yes)	38 (45.78%)	32 (53.33%)	15 (62.50%)	0.316[b]
COPD (yes)	14 (16.87%)	12 (20%)	4 (16.67%)	0.876[b]
Canser (yes)	9 (10.84%)	8 (11.86%)	1 (4.17%)	0.558[b]
Neurological Disease (yes)	15 (18.07%)	13 (20.34%)	2 (8.33%)	0.417[b]
IVIG (yes)	1 (1.20%)	0	0	>0.99[c]
Anakinra (yes)	9 (10.84%)	5 (8.33%)	2 (8.33%)	0.859[b]
Inotropic agents (yes)	30 (36.14%)	27 (45%)	20 (83.33%)	<0.001[b]
Corticosteroid				
No	26 (31.33%)	14 (23.33%)	12 (50%)	
<250 Mg Methylprednizolone	31 (37.35%)	30 (50%)	9 (37.50%)	0.098[b]
≥250 Mg Methylprednizolone	26 (31.33%)	16 (26.67%)	3 (12.50%)	
APACHE II	11 (3-36)	14.50 (3-38)	26 (5-57)	<0.001[a]
SOFA	4 (3-12)	5 (4-41)	8 (4-14)	<0.001[a]
Ferritin (µg/L)	488 (7-62904)	384 (1.55-19215)	472.50 (15-3440)	0.479[a]
CRP (mg/L)	0.09 (0.01-0.37)	0.11 (0-0.43)	0.17 (0.01-0.38)	0.043[a]
Procalcitonin (µg/L)	0.16 (0.02-12.34)	0.21 (0.03-28.13)	1.29 (0.06-57.32)	<0.001[a]
IL-6 (pg/mL)	33 (2.72-2718)	55.35 (5.40-2972)	62.90 (7-5066)	0.051[a]
Fibrinogen (g/L)	5.38±1.54	5.65±1.85	5.01±1.99	0.293[d]
D-dimer (mg/L)	1.55 (0.30-35.20)	1.27 (0.20-53)	4.02 (0.27-56.36)	0.013[a]
LDH (U/L)	505 (37-3800)	470.50 (129-2972)	459 (217-1507)	0.612[a]
ALT (U/L)	39 (2-1270)	29 (1-1684)	37 (8-440)	0.055[a]
AST (U/L)	52 (4-2871)	42.50 (8-4088)	66.50 (17-603)	0.062[a]
WBC (×10⁹/L)	8.8 (10.8-23.2)	9.9 (16.1-88.0)	9.7 (3.5-30.9)	0.358[a]
Neutrophil (×10⁹/L)	8.2 (1.0:21.8)	8.8 (27.8-27.3)	8.4 (10.8-28.2)	0.295[a]
Lymphocyte (×10⁹/L)	0.67 (0.14-1.01)	0.64 (0.18-2.9)	0.48 (0.15-0.97)	0.026[a]
NLR	11.74 (1.25-89.36)	12.50 (1.38-79)	15.62 (4.39-75.85)	0.033[a]
Hb (g/dL)	12.50±2.35	12.95±1.95	10.47±2.64	<0.001[d]
Platelet (×10⁹/L)	259 (62-672)	205 (72-491)	182 (31-448)	0.002[a]

Data are expressed as n(%), median(minimum: maximum), and mean±standard deviation.
a:Kruskal-Wallis Test, b: Pearson Chi-Square Test, c: Fisher Freeman-Halton Test, d: ANOVA Test.
HFNO: High flow nasal oxygen; NIMV: noninvasive mechanical ventilation; IMV: invasive mechanical ventilation; DM: diabetes mellitus; HT: hypertension; CAD: coronary artery disease; COPD: chronic obstructive pulmonary disease; IVIG: intravenous immunoglobulin; CRP: C-reactive protein; IL: interleukin; LDH: lactate dehydrogenase; ALT: alanine amino transferase; AST: aspartate amino transferase; WBC: white blood cell; NLR: neutrophil lymphocyte ratio.

In a retrospective study conducted on 1210 patients in Turkey, the mortality rates of stage 3-5 CKD patients and routine hemodialysis (HD) patients were found to be similar, but higher than the normal population (17). It has been stated that it is difficult to obtain meaningful data due to the small number of renal transplantation patients (17). Renal transplantation patients were not included in our study. According to our discrimination scale, all stage-3 patients were in HD, and mortality rates were higher than the other groups. Although the mortality rates of stage 2c patients were higher than stage 2a, no significant difference was found with stage 2b.

In meta-analyses performed on ICU patients followed up with COVID-19, the incidence of AKI was reported as approximately 23%, and it was shown that the patients in need of RRT were 5% (18). In another study, it was reported that 42 (42.9%) of 99 COVID-19 patients developed AKI, and 13 (13.4%) needed RRT (19). In our study, according to Kidney Disease Improving Global Outcomes (KDIGO) criteria, 19 (30%) of stage-1 patients and 108 (64%) of stage-2 patients developed AKI. We found that 4 (21%) of stage-1 patients with AKI and 28 (25%) of stage-2 patients with AKI needed HD. The emergence of such different results regarding the incidence of AKI may be due to the different designs of the studies and the lack of a clear AKI protocol.

There are some limitations of this study. First of all, our study is a retrospective study and is single-centered. The second is to calculate the kidney functions of the patients with the MDRD formula. For this reason, there may be uncertainty about whether the errors will show the kidney functions effectively or not because the parameters other than age and weight are not taken into account. Finally, these data were not recorded because it was thought that the evaluation of hematuria and proteinuria in the ICU would not be accurate.

CONCLUSION

It has been determined that COVID-19 disease is more severe and mortal in CKD patients. In addition, it can be predicted that more severe inflammation values are observed in this group and hypercoagulopathy may be more common due to D-dimer elevation. For this reason, it should be questioned whether there is a known history of CKD in every patient hospitalized with COVID-19 disease and care should be taken about protective measures.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Ankara City Hospital No 1 Clinical Researches Ethics Committee (Date: 15.06.2022, Decision No: E1-22-2617).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution and analysis of the paper and that they have approved the final versio.

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Evaluation of cardio-pulmonary functions of previously healthy adults with moderate-severe COVID-19 pneumonia after discharge

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ABSTRACT

Aim: Persistent dyspnea is one of the most frequent post-COVID symptoms. We aimed to evaluate the cardiopulmonary functions of COVID-19 survivors with moderate to severe COVID-19 pneumonia without comorbidity, during the first wave of pandemics.

Material and Method: The study was conducted retrospectively in a single center. The electronic data of patients applied with dyspnea one month after hospital discharge, without any comorbidities, and who were evaluated with pulmonary function test (PFT) and echocardiography were included in the study. A total of adult 88 patients who suffered from COVID-19 pneumonia (46 moderate and 42 severe) were enrolled. Results of biochemical, hematological and radiological examinations, PFT parameters and echocardiography were recorded and compared between moderate and severe cases.

Results: The mean age of 88 patients included in the study was 48±13 years. Sixty-seven (74.4%) of the patients were male. Pulmonary thromboembolism was not detected in both groups. PFT parameters performed were similar in the two groups and there was no statistically significant difference**.** Pulmonary function test of the patients with moderate COVID-19 pneumonia revealed mild restriction in 21.7% and moderate restriction in 2% of the patients. In the severe group, 38.1% of the patients had mild and 4.8% of the patients had a moderate restrictive pattern. Small airway obstruction was detected in 37% of the moderate group and in 38.1% of the severe group. Conventional echocardiographic parameters of the two groups were normal. Pulmonary arterial pressures were 22.6±8.3 vs 22.1±6.8; p=0.8 was found. Tricuspid annular plane systolic excursion were within normal limits.

Conclusıon: The persistent dyspnea following COVID-19 pneumonia may be related to disturbances in PFT even in patients without comorbidities. We concluded that; the detailed evaluation of the patients with prolonged respiratory symptoms might help to detect the cardiopulmonary functional disturbances.

Keywords: COVID-19, pneumonia, comorbidity, echocardiography, pulmonary function test

INTRODUCTION

SARS-CoV-2 has affected millions of people to date. It caused mortality and morbidity (1). SARS-CoV-2 is an RNA virus from the same family as severe acute respiratory syndrome (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV). Although SARS-CoV-2 has a lower mortality (1-7%) than the other two viruses, it is more contagious. The virus can cause asymptomatic infection as well as severe respiratory failure, dysfunction in other organs and death (1-3).

The target organ of the virus is the lung. Apart from the lungs, it can also affect many organs, especially the heart. Male gender, older age, obesity and comorbidities (coronary artery disease, hypertension, diabetes and lung disease) is related to increased morbidity and mortality. But the virus can cause severe pneumonia and death in some patients, even without comorbidity (4,5).

COVID-19 infection can cause cardiovascular complications such as myocarditis, heart failure, cardiac arrest, cardiogenic shock, acute myocardial infarction, arrhythmia, Takotsubo cardiomyopathy and venous thromboembolic disease. The disease can cause complications directly or indirectly (5,6).

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After COVID-19 pneumonia, respiratory physiology deterioration, pulmonary fibrosis and vascular complications may occur (7). Of patients who were treated for COVID-19 pneumonia, impairment of diffusion capacity in PFT was found to be the most common abnormality at the time of discharge (8).

It has been determined that the permanent deterioration in lung function and in the exercise capacity in patients with SARS and MERS infection continues for months or even years (9-11). Long-term studies with SARS and MERS viruses will guide our understanding of the long-term complications of the COVID-19 virus.

In this study, we selected the patients who suffered from COVID-19 pneumonia during the first wave of the pandemic as the morbidity and mortality were more in the first few months of pandemics. The use of intensive corticosteroids and vaccination against SARS CoV-2 resulted in a better prognosis recently (12,13). But the evaluation of patients affected formerly may help us to understand the consequences of the disease in naive cases, as there are still many people against vaccination.

There are few studies addressing cardiac and respiratory functions together after discharge of patients with moderate-to-severe COVID-19 pneumonia. These studies were conducted mainly on patients who have comorbidity. The group we studied, consisted of patients without comorbidities who were unvaccinated and treated with the first treatment protocol in the first wave of the pandemic.

Our study is the first to date in patients with moderate to severe COVID-19 pneumonia without comorbidities.

MATERIAL AND METHOD

The study was approved by the Ministry of Health's Scientific Research Platform, and the study was initiated with the approval of the Ümraniye Training and Research Hospital Clinical Researches Ethics Committee (Date: 11.06.2020, Decision No: B.10.1. TKH.4.34.H.GP.0.01/217). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Study Population

The medical records of the adult patients who applied post-COVID outpatient clinic were evaluated retrospectively in a single center between 15 June and 15 August 2020. There were 384 patients who complained of persistent dyspnea on the effort who suffered from moderate and severe COVID-19 pneumonia and applied one month after discharge. The diagnosis of COVID-19 pneumonia was made according to the clinical and radiological definition of the WHO (14). The COVID PCR tests of the patients were positive on admission. All of the patients were evaluated with routine hematological tests and chest X-rays and/or chest computed tomography. The PFT was performed following a negative SARS CoV-2 PCR test. The study was completed with 88 cases with a history of 46 moderate and 42 severe COVID-19 pneumonia.

Patients with severe COVID-19 pneumonia were defined as cases who had one of the following criteria: 1) Peripheral oxygen saturation at rest <93%, 2) Respiratory distress and respiratory rate >30/minute, 3) Partial arterial oxygen pressure/fraction of inspired oxygen < 300 mmHg. Patients who did not undergo invasive mechanical ventilation were included in the study.

Patients with a history of chronic lung disease, coronary artery disease, hypertension, heart failure, atrial fibrillation, right and left bundle branch block, moderate-severe valve pathology, diabetes mellitus, anemia, chronic renal failure, thyroid dysfunction, pulmonary embolism, cancer, rheumatic valve disease, BMI>30 kg/m2 and acute coronary syndrome during hospitalization, myocarditis, patients with pregnancy, chronic infectious and autoimmune diseases, upper airway obstruction, neuromuscular disease alcohol/drug abuse were excluded from the study (**Diagram**).

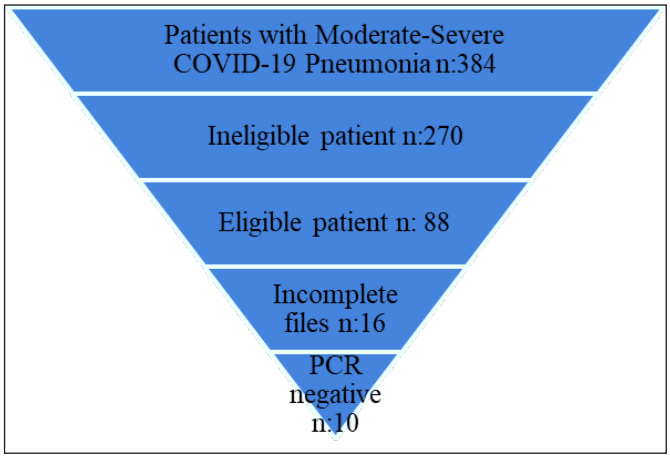

Diagram. Flow-chart of the patient selection

Methods

Routine biochemical examinations, hemograms, D-dimers, troponin values, inflammatory markers, radiological examinations, medications and oxygen treatments given to the patients during hospitalization and one month after discharge were examined. One month after discharge, thorax computed tomography (CT), echocardiography and PFT results were obtained from the system.

The duration of stay in the service and intensive care unit was recorded. The Modified Medical Research Council (mMRC) scale was calculated at the Borg rating of the

perceived exertion scale. Forced vital capacity (FVC), forced expiratory volume in one second (FEV1), FEV1/FVC, peak expiratory flow (PEF) and forced expiratory flow (25-75%) were recorded from pulmonary function test parameters.

Among the transthoracic echocardiography parameters, left ventricular end-diastolic and end-systolic diameter, interventricular septum, posterior wall and left atrial diameter, right ventricle diastolic diameter, mitral early diastolic and late diastolic maximal flow rates, left ventricular ejection fraction, pulmonary artery systolic pressure, tricuspid S wave, tricuspid annular plane systolic excursions (TAPSE's) were recorded.

Statistical Analysis

SPSS (Statistical Package for Social Sciences) for Windows 26 package program was used for statistical analysis (IBM SPSS 26, New Orchard Road Armonk, Newyork 10504-12722 United States). While evaluating the data, in addition to descriptive statistical methods, the student-t test was used for comparison of quantitative data, the comparison of normally distributed parameters between groups and the Mann Whitney-U test for intergroup comparisons of non-normally distributed parameters. The paired sample-t test was used for in-group comparisons of parameters and Chi-square test was used for the comparison of qualitative data. The results were accepted at the 95% confidence interval and the significance was accepted at the $p<0.05$ level.

RESULTS

Among the 88 patients (46 moderate and 42 severe) included in this study, 67 (74.4%) were male and the mean age was 48±13 years. Their average saturation was 90% during their hospitalization and an average of 98% after discharge from the control saturation. The mean lowest saturation at admission of patients with severe pneumonia was 88% [88 (86-90) vs. 92 (89.7-93.2); p 0.001]. The patients were admitted to the hospital at an average of 4±2 days after the first symptom onset. The time before the admission to the hospital was longer in the severe group, 5±3 days (**Table 1**).

Biochemical tests, hemograms and inflammatory markers of all patients were within normal limits after discharge. The lymphocyte count was statistically significantly lower on the first, third and fifth days of hospitalization of severe patients. CRP level was statistically higher in the severe group on the 1st and 3rd days. LDH value was found to be higher in severe patients. While there was no statistical difference between the two groups in terms of troponin levels during their hospitalization, they were higher in the severe group. The severe group had higher proBNP and D-dimer levels, but there was no statistical difference between the two groups. **Table 2** shows the laboratory findings of the patients.

Patients were treated according to the national COVID-19 treatment guidelines in the first wave of the pandemic. In the severe group, significantly more favipiravir [29 (63%) vs. 37 (88.1%); p 0.007] and tocilizumab [2 (4.3%) vs. 11 (26.2%); p 0.004] was used. The administration of plasma and intravenous steroid in the treatment was low in both groups and there was no statistically significant difference. All patients received oxygen therapy during their hospitalization. 12 patients from the severe group and 4 patients from the moderate group had intensive care admissions. The duration of intensive care and hospital stay was significantly higher in the severe group (**Table 3**).

Table 1. Basic characteristics and vital parameters of the study population

	N (%) / Mean±SD / Median (%25-75)			p
	Total (n:88)	Moderate (n:46)	Severe (n:42)	
Age, years	48±13	48±13	47±10	0.91
Sex, n (%)				0.04
male	67 (74.4%)	31 (67.4%)	36 (85.7%)	
female	21 (23.3%)	15 (32.6%)	6 (14.3%)	
BMI, kg/m^2	28 (26.1-29.3)	28,3 (26,7-29,3)	25.5 (25.9-29.3)	0.12
Tobacco exposure, n (%)				0.67
Never used	59 (67%)	30 (65.2%)	29 (69%)	
Active	3 (3.4%)	1 (2.2%)	2 (4.8%)	
Quit smoking	26 (29.5%)	15 (32.6%)	11 (26.2%)	
SBP, mmHg	120 (110-120)	120 (110-121)	115 (110-120)	0.07
DBP, mmHg	70 (70-80)	70 (70-80)	70 (70-80)	0.30
Heart Rate, beats/min	90.1±13.8	90±14.1	90.2±13.7	0.96
Control Oxygen , Saturation %	98 (96-98)	98 (96-98)	97 (96-98)	0.3
Lowest Oxygen, Saturation %	90 (87-93)	92±(89.7-93.2)	88 (86-90.2)	0.001

*Independent Samples T-Test, chi-square Test, Fisher's Exact Test *p<0.05 statistically significant. Continues variables are reported mean±SD. Categorical variables are reported n(%). Abbreviations: BMI: Body mass index; SBP: systolic blood pressure; DBP: diastolic blood pressure.

Table 2. Laboratory findings of the study population

	N (%) / Mean±SD / Median (%25-75)		p
	Moderate (n:46)	Severe (n:42)	
WBC	5600 (4475-6850)	5850 (4435-7412)	0.46
Neutrophile, count	3350 (2475-4325)	4200 (2982-6025)	0.008
Lymphoctye,count (1st day)	1500 (1200-2100)	1150 (880-1425)	0.000
Lymphoctye, count (3rd day)	1440 (1160-2000)	1100 (830-1380)	0.000
Lymphoctye, count (5th day)	1400 (1000-2100)	1200 (795-1400)	0.015
Hemoglobin, g/dL	14±1.7	13,7±1.2	0.39
Hematocrite	41±5.8	40.3±3.6	0.5
Platelet, count	191500 (170500-219500)	175000 (156500-224000)	0.22
CRP, mg/dL (1st day)	1.85 (0.40-3.95)	4.95 (1.20-10.25)	0.001
CRP mg/dL (3rd day)	4 (1.45-7.00)	6.80 (3.05-12.45)	0.003
CRP, mg/dL (5th day)	5.72±5.70	7.54±5.40	0.13
LDH, U/L (1st day)	280 (228.7-334.5)	332.5 (226.7-444.5)	0.15
LDH, U/L (3rd day)	307.3±88.8	369.9±143	0.019
LDH, U/L (5th day)	339.6±105.6	385.4±194.2	0.21
Troponin, ng/mL (1st day)	0.002 (0.001-0.005)	0.003 (0.002-0.008)	0.54
Troponin, ng/mL (3rd day)	0.003 (0.001-0.007)	0.004 (0.002-0.009)	0.22
Troponin, ng/mL (5th day)	0.002(0.0008-0.0045)	0.002 (0.001-0.006)	0.92
proBNP, pg/ml (1st day)	10.00 (10.00-10.00)	29.00 (29.00-29.00)	0.2
D-dimer, ng/mL (1st day)	550.00 (352-741)	759.50 (467.5-1015)	0.01
D-dimer, ng/mL (3rd day)	582.50 (413.7-922.2)	825.00 (543.5-1417)	0.59
D-dimer, ng/mL (5th day)	860.50 (448-1234.7)	1010.00 (594-1830)	0.2

*Independent Samples T-Test, Mann Whitney-U Test *p<0.05 statistically significant. Continues variables are reported mean±SD. Categorical variables are reported n (%). Abbreviations: WBC: White blood cells; CRP: C-reactive protein; LDH:lactate dehydrogenase; proBNP: pro-brain natriuretic peptide.

Table 3. Inpatient treatment and follow-up.

	N (%) / Mean±SD / Median (%25-75)		p
	Moderate (n:46)	Severe (n:42)	
Plaquenyl, n (%)			0.19
Plaquenyl	3 (6.5%)	8 (19%)	
Plaquenyl+azithromycin	21 (45.7%)	15 (35.7%)	
Plaquenyl+azithromycin+ oseltamivir	22 (47.8%)	19 (45.2%)	
Favipiravir, n (%)	29 (63%)	37 (88.1%)	0.007
Tocilizumab, n (%)	2 (4.3%)	11 (26.2%)	0.004
Plasma, n (%)	3 (6.5%)	6 (14.3%)	0.23
Steroid, n (%)	3 (6.5%)	8 (19%)	0.07
Inhaler usage at discharge, n (%)	1 (2.2%)	0 (0%)	0.33
Oxygen concentrator at discharge, n (%)	0 (0%)	2 (4.8%)	0.13
ICU stay, n	4	12	0.016
ICU duration, days	11.7±7.6	12.3±7.2	0.016
Total time of stay, days	10.3±6.3	15.7±7.5	0.000
mMRC Score	0.24±0.52	0.47±0.74	0.08
Borg Score	0.53±1.10	0.95±1.69	0.17

*Independent Samples T-Test, chi-square Test, Fisher's Exact Test *p<0.05 statistically significant. Continues variables are reported mean±SD. Categorical variables are reported n (%). Abbreviations: ICU: intensive care unit.

Pulmonary thromboembolism was not detected in both groups. Subcutaneous heparin was given to the patients during their hospitalization and discharge. Among the thorax CT findings at admission, consolidation and interstitial septal thickening were seen significantly more frequently in the severe group (**Table 4**).

13 patients from the moderate group and 11 patients from the severe group had a control thorax CT taken 1 month after discharge. It was determined that the ground glass appearance persisted in 8 patients from the severe group and 9 patients from the moderate group. There was nodular appearance in the lungs of 1 patient from the moderate group and 2 patients from the severe group. Reticular/fibrotic appearance was detected in the control CT of 5 patients from the moderate group and 8 patients from the severe group. Interstitial septal thickening and cobblestone appearance were present in 2 patients from the severe group.

88 patients had pulmonary function test results. One patient from the moderate and severe groups each had

obstructive pathology in PFT. In the moderate group, restrictive pattern was mild in 10 patients (21.7%) and moderate in 2 patients (2%). In the severe group, mild restrictive pattern was found in 16 patients (38.1%) and moderate in 2 patients (4.8%). Small airway obstruction was detected in 17 patients (37%) in the moderate group and 16 patients (38.1%) in the severe group (**Table 4**).

33 patients from the severe group and 28 patients from the moderate group had echocardiography in the post-discharge system. Conventional echocardiography parameters of the two groups were normal, there was no statistically significant difference. Left ventricular and right ventricular functions were normal. Right atrial and ventricular sizes were within normal limits, but were larger in the severe group than in the moderate group. Tissue Doppler tricuspid S wave was detected as 13.8 cm/sec in the moderate group and 14.05 cm/sec in the severe group. Pulmonary arterial pressures were 22.6±8.3 vs 22.1±6.8; p=0.8 was detected. TAPSE's were within normal limits, with no statistically significant difference (**Table 5**).

Table 5. Comparison of echocardiographic findings between the groups

	N (%) / Mean±SD / Median (%25-75)		p
	Moderate (n:28)	Severe (n:33)	
LVEDD, cm	4.8 (4.6-5.1)	4.9 (4.6-5.2)	0.97
LVESD, cm	3 (2.7-3.1)	3 (2.8-3.1)	0.8
LVEF %	65.7±2.4	64.5±2.8	0.09
Left Atrium, cm	3.6 (3.5-3.8)	3.7 (3.5-3.8)	0.69
IVS thickness, cm	1 (1-1.1)	1 (1-1.1)	0.4
PW thickness, cm	1 (0.9-1.07)	1	0.4
Aortic root, cm	2.5±0.2	2.4±0.2	0.79
RA Area, mm^2	10.8±3.1	11.9±2.2	0.15
RA mediolateral diameter, cm	3.3±0.2	3.4±0.2	0.16
RA apicobasal diameter, cm	3.6±0.2	3.7±0.2	0.06
RVEDD basal, cm	2.5 (2.4-2.6)	2.6 (2.4-2.7)	0.22
Mitral Inflow E	75.1±12.2	75±16.5	0.98
Mitral Inflow A	72.9±17	67.9±12.9	0.21
Tricuspid Annular S, cm/sn	13.8 (13.3-15.1)	14 (12.7-16.2)	0.67
SPAB, mmHg	22.1±6.8	22.6±8.3	0.8
TAPSE, cm	2.2±0.2	2.3±0.2	0.21

*Independent Samples T-Test, chi-square Test, Fisher's Exact Test, Mann Whitney-U Test *p<0.05 statistically significant. Continues variables are reported mean±SD. Categorical variables are reported n (%). Abbreviations: LVEDD: Left Ventricular End-diastolic Diameter; LVESD; Left Ventricular End-systolic Diameter; LVEF: Left Ventricular Ejection Fraction; IVS; Interventricular Septum; PW: Posterior Wall; RA; Right Atrium; RV: Right Ventricle; RVEDD: Right Ventricular End–diastolic Diameter; SPAB: Systolic Pulmonary Arterial Pressure; TAPSE: Tricuspid Annular Plane Systolic Excursion.

Table 4. Comparison of thorax computed tomography and pulmonary function tests findings between the groups

	N (%) / Mean±SD / Median (%25-75)		p
	Moderate	Severe	
Consolidation, n (%)	18 (39%)	26 (61%)	0.033
Infiltration, n (%)	15 (32%)	12 (28%)	0.68
Reticular fibrosis, n (%)	13 (28%)	9 (21%)	0.46
Pleural effusion, n (%)	1 (2 %)	1 (2.4%)	0.26
Honeycombing, n (%)	45 (97%)	40 (95%)	0.5
Nodule, n (%)	4 (8 %)	2 (4%)	0.46
Interstitial septal thickening, n (%)	7 (15 %)	14 (33%)	0.046
Cobble stone appearance, n (%)	8 (17 %)	11 (26%)	0.31
Pericardial effusion, n (%)	0 (0%)	1 (2%)	0.29
Pulmonary thromboembolism, n (%)	0	0	-
Air bronchogram, n (%)	4 (8%)	4 (9%)	0.89
FVC LT	3±0.8	3.5±0.8	0.41
FVC (%)	87.0±18.4	85.1±16	0.62
FEV 1 LT	3 (2.2-3.7)	3.2 (2.7-3.5)	0.54
FEV 1 (%)	99.5 (86-103.7)	90.5 (82-104)	0.24
FEV 1/FVC (%)	93 (87-96.7)	91.6 (84-96.1)	0.35
PEF LT	6.8±2.5	7±2.6	0.74
PEF (%)	84.8±23.7	85.2±25.8	0.94
FEF 25-75 (%)	117 (94.5-126)	105 (85-123)	0.29
Obstruction on PFT, n (%)	1 (2.2%)	1 (2.4%)	0.94
Restriction on PFT, n (%)			0.21
Mild	10 (21.7%)	16 (38.1%)	
Moderate	2 (4.3%)	2 (4.8%)	
Small Airway Disease on PFT, n (%)	17 (37%)	16 (38.1%)	0.89

*Chi-square Test, Fisher's Exact Test *p<0.05 statistically significant. Continues variables are reported mean±SD). Categorical variables are reported n (%).
Abbreviations: FVC: Forced Vital Capacity; FEV1: Forced Expiratory Volume on 1st Second; PEF: Peak Expiratory Flow; FEF 25-75: Maximal mid expiratory flow between 25-75%; PFT: pulmonary function test.

DISCUSSION

The patients included in our study had no comorbidities and were treated for moderate to severe COVID-19 pneumonia. The patients were treated with the treatment protocol in the first wave of the pandemic and they did not have any vaccines. Conventional echocardiography and PFT parameters were similar in the two groups and there was no statistically significant difference. In pulmonary function tests, obstructive type pathology was found in 2 patients, restrictive type pathology was found in 26.4% patients and small airway obstruction was detected in 29% patients.

In the first wave, high mortality rates were seen in the elderly population. With the increase in vaccination studies, the spread of the virus has shifted from the elderly patient group to the younger patient group. COVID-19 vaccines protect against serious illness, hospitalization and death (15,16). Our study has demonstrated the possible level of cardiopulmonary function in unvaccinated healthy individuals with moderate to severe COVID-19 pneumonia in the first month after discharge.

Echocardiography is a cost-effective device used to evaluate cardiac structure and functions. Studies have shown that patients with COVID-19 pneumonia may have right and left ventricular dysfunction and an increase in pulmonary artery pressure (17). In our study, we found that the results of conventional echocardiography of our patients were within normal limits.

COVID-19 infection causes damage to alveolar epithelial cells and endothelium together with fibroproliferation. As a result, it may cause lung fibrosis and pulmonary hypertension in the long term (18,19). In studys conducted in patients with community-acquired pneumonia, it was found that the risk of active cardiovascular disease was significantly increased for several years after hospitalization (20,21). There are still healthy individuals in our society who are not vaccinated. With the removal of pandemic measures, these complications may develop as a result of serious infection in this patient group.

SARS-CoV-2 causes systemic inflammation and coagulopathy (22). In autopsy studies performed on patients who died due to COVID-19 infection, the incidence of micro and macrothrombus was found to be 58% (23). Although some patients received anticoagulant therapy, thromboembolic complications were observed (24,25). Our patients received subcutaneous heparin treatment at admission and at discharge. We did not detect pulmonary thromboembolism in any of the patients but microthrombi may still have formed.

In PFTs performed on patients with COVID-19 pneumonia during their discharge, it was determined that the most common anomaly in lung functions was deterioration in diffusion capacity (8,26). In another study conducted one month after discharge, patients' PFTs also found slight changes in lung function (27). Long follow-up studies of patients with SARS have shown that lung diffusion capacity may remain abnormal within 3 years after recovery in some patients (28). We did not have the data for lung diffusion capacity but we found mild obstructive and restrictive abnormalities in PFTs on first month after discharge. We suggest that the long term evaluations of patients with abnormal PFTs may enlighten whether COVID-19 has a permanent negative effect on pulmonary functions or not.

In the pulmonary function test performed in the 3rd year of 71 patients with SARS pneumonia who received high-dose steroids, restrictive-type pulmonary function test impairment was detected in 22%, while restrictive-type pulmonary dysfunction was not detected in any patient at the 15th year. Mild diffusion capacity deterioration was detected in 1/3 of the patients at 15 years. It was thought that less fibrosis was seen in the patients due to the use of high-dose steroids (29). Our patients did not receive intensive steroid treatment and at the end of the first month, we found restrictive type disorder in 26.4% of their PFTs.

Limitations

The number of patients in our study was small since most hospitalized patients had comorbidities. If the number of patients was more, statistically more significant results could have been obtained. Most of the patients did not have baseline echocardiograms. If they had baseline echocardiograms, we could have the opportunity to compare them with echocardiograms done at one month post-discharge. In addition, if the patients had high-level echocardiographic analyzes and cardiac MRIs, the cardiac involvement of COVID-19 could have been revealed more clearly.

CONCLUSION

The long-term effects of COVID-19 infection are not fully known. We think that especially in the first wave of the pandemic, those who have had a severe illness may be at greater risk for long term efects of COVID-19. Detailed evaluation of the patients with prolonged respiratory symptoms might help to detect the cardiopulmonary functional disturbances. Vaccines are necessary to prevent serious COVID-19 infection, but due to decreased efficacy of vaccines and increased virus variants, effective treatment study must continue.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was approved by the Ministry of Health's Scientific Research Platform, and the study was initiated with the approval of the Umraniye Training and Research Hospital Clinical Researches Ethics Committee (Date: 11.06.2020, Decision No: B.10 .1.TKH.4.34.H.GP.0.01/217).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Original Article

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Comparison of BK virus nephropathy risk between double-J-stent with anti-reflux mechanism and standart double-J-stent: single-center experience

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ABSTRACT

Aim: Ureteral stend use is a risk factor for BK nephropathy (BKVN). In 2015, we compared the patients with anti-reflux mechanism DJS (ARD-DJS) and those used standard DJS (st-DJS) in terms of BKV and BKVN frequency in 90 kidney transplant patients in two centers. With the increase in the number of our patients over time and lengthening of the follow-up duration, we needed to re-evaluate the data in one center.

Material and Method: We retrospectively evaluated 211 patients who underwent kidney transplantation at Gazi Yaşargil Training and Research Hospital between September 2012 and September 2019. The following parameters were recorded, demographic data, immunosuppression protocols, presence of rejection, graft loss, plasma BKV levels, and presence of BKVN.

Median and IQR follow-up time for ARD-DJS and St-DJS patients was 72 months (62,5-80,3 months) and 27,8 months (17,4-39,6 months) respectively.

Results: Thirteen patients (6,1%) had BKV viremia. BKVN was revealed by kidney biopsy in 3 of 13 patients. However, graft loss due to BKVN was observed in only one patient. ARD-DJS was used in 4 of these cases and standard DJS was used in 9 of these cases. Patients in whom BKV revealed in the first 3 months were compared in the aspect of DJS technique, BKV was significantly less observed in the ARD-DJS group (ARD-DJS: 2 patients; St-DJS:9 patients), (p=0,046).

Conclusion: In our study, BKV was observed less in patients with ARD-DJS that were clinically significant but not statistically significant. Therefore, prospective randomized studies with high patient numbers are needed to determine the effectiveness of ARD-DJS.

Keywords: BKV, kidney transplantation, ureteral stend

INTRODUCTION

BK virus (BKV) is a member of the polyomavirus family, which was first isolated in 1971 from the urine sample of a renal transplant patient, with clinical observations made over the years, it was understood that BKV is responsible for BKV nephropathy (BKVN) (1-3). The exact prevalence of BKVN is not known, however, it is estimated to be in the range of 1-15 %. BKVN is an important clinical problem causing graft loss in 15-50 % of transplant patients (4). Risk factors for BKV are aggressive immunosuppression, high human leukocyte antigen (HLA) mismatch, ureteral stent use, rejection therapy, prolonged ischemia duration, advanced age, male recipient, diabetes mellitus (DM), lymphocyte depleting induction therapies, tacrolimus, and mycophenolate-based regimens (5-10). It has been shown that the use of double-J-Stent (DJS) in experimental animal models causes superficial epithelial destruction, ulceration, and inflammatory changes on the transitional epithelium in rat ureters, as well as increasing the incidence of BKVN by 4 times (2,11-13). Studies have shown that conditions that cause persistent hydronephroses, such as posttransplant ureter flexion and ureteral stenosis, may play a dynamic role in the pathogenesis of polyoma virus-associated nephropathy by causing ureteral reflux. Vesicoureteral reflux (VUR) may occur with normal bladder contraction while using DJS (14,15). In 2015, we compared the patients with anti-reflux mechanism DJS (ARD-DJS) and those used standard DJS (st-DJS) in terms of BKV and BKVN frequency in 90 kidney transplant patients in two centers (**Figure 1**). In this study, BKV was observed with significantly less frequency in

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univariate analyzes in patients with ARD-DJS. However, no significant difference was observed in the multivariate analyzes. In this study, the small number of cases and the short follow-up duration were the factors limiting our study (13). With the increase in the number of our patients over time and lengthening of the follow-up duration, we needed to re-evaluate the data in one center.

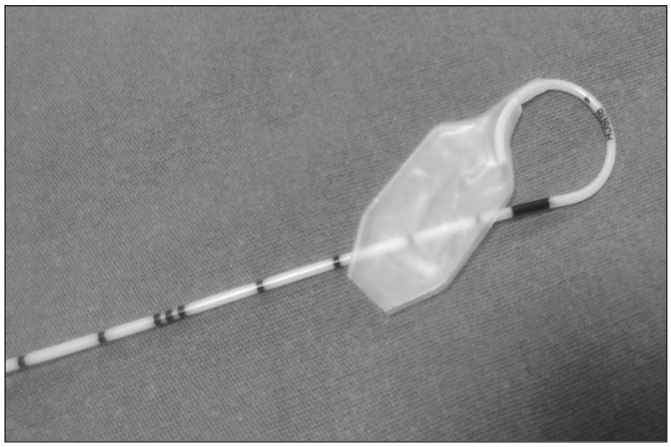

Figure. Anti-reflux mechanism double J stent

MATERIAL AND METHOD

We retrospectively evaluated 211 patients who underwent kidney transplantation at Gazi Yaşargil Training and Research Hospital between September 2012 and September 2019. One hundred seventy of the patients had a living donor and 41 of them had deceased donor kidney transplantation. The following parameters were recorded, demographic data, immunosuppression protocols, presence of rejection, graft loss, plasma BKV levels, and presence of BKVN (**Table 1,2**).

The study was carried out with the permission of Gazi Yaşargil Training and Research Hospital Clinical Researches Ethics Committee (Date: 26.03.2021, Decision No: 743). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Immunosuppression and Prophylaxis

Basiliximab (20 mg at days 0 and 4 of operation) or anti-thymocyte globulin (ATG; for high-risk patients, 3 mg/kg during operation and 1,5 mg/kg at postoperative days 1 and 2) were used as induction therapy. Methylprednisolone 1000 mg was given intra-operatively. Methylprednisolone dose was decreased by half every day and 20 mg oral prednisolone was started on the 6th postoperative day for daily use. Oral prednisolone dosage was reduced gradually to reach 5 mg a day in the first year after transplantation. Calcineurin inhibitors (tacrolimus or cyclosporin) and mycophenolate mofetil (MMF; 2 g /day in two divided doses) or mycophenolate sodium (MMF; 1440 mg/day, in two divided doses) were used as maintenance immunosuppression therapy. MMF was used as 600 mg/m2 in two divided doses in children. We considered both mycophenolate mofetil and mycophenolate sodium in doses described above as the same drugs in our study. Everolimus was used in only one case (plasma level of the drug was targeted as 8-10 mg/dl). Trimethoprim/sulfamethoxazole and valganciclovir (450 mg a day) was prescribed for Pneumocystis jirovecii and cytomegalovirus (CMV) prophylaxis for 6 months after the transplantation. Acute rejection was diagnosed by kidney biopsy. The acute cellular rejection was treated with intravenous pulse methylprednisolone or ATG depending on the severity of the rejection. Plasmapheresis was added for acute humoral rejection. Delayed graft function (DGF) was described as a need for hemodialysis in the first week of kidney transplantation.

Table 1. Comparison of the cases with ARD-DJS and non ARD-DJS use during kidney transplantation

	St-DJS n=111	ARD-DJS n=100	P value
Sex F/M	46/65 (41.4%/58.6%)	42/58 (42%/58)	0,935
Age (years)	30.99±13.4 (9-72)	35.06±12.8 (14-68)	0.018
Med / IQR follow up (m)	27.8 [17.4-39.6]	72 [62.5-80.3]	0.00
Living / deceased Donor	80/31 (72%/28%)	90/10 (90%/10%)	0.002
Relationship			
Parent to child	37 (33.3%)	32 (32%)	
Child to parent	0 (0%)	6 (6%)	
Sibling	11 (9.9%)	18 (18%)	
Between husband/wife	26 (23.4%)	29 (29%)	
3rd/4th degree relative	5 (4.5%)	6 (6%)	
donors			
Cadaveric	31 (28%)	10 (10%)	0.59
Preemptive	35 (31.2%)	35 (35%)	
Induction			
No	4 (3.6%)	11 (11%)	0.00
Basiliximab	9 (8.1%)	57 (57%)	0.00
ATG	98 (88.2%)	32 (32%)	0.00
Number of Rejection			
Cellular Rejection	7 (6,3%)	6 (6%)	1
Humoral Rejection	1 (0,9%)	2 (1.8%)	
CR + HR	2 (1.8%)	1 (0,9%)	
Graft loss	6 (5.4%)	6 (6%)	1
DGF	6 (5,4%)	3 (3%)	0.68
BKV	9 (8.1%)	4 (4%)	
500-5000	2 (1.8%)	2 (2%)	
5000-10000	1 (0.9%)	1 (1%)	
>10000	6 (5.4%)	1 (1%)	0.122
BKVAN	3	0	0.248
Last Cr level (mg/dl) med and IQR*	1.19 [0.96-1.47]	1.03 [0.86-1.25]	0,002

ARD-DJS: anti-reflux mechanism double J stenting; st-DJS: standart-DJS; ATG: anti thmocyte globülin DGF: delayed graft function; BKV: BK viremia; BKVAN: BKV-associated nephropathy; Cr: creatine.

Table 2. Comparison of BKV positive and negative cases

	BKV + (>500)	BKV – (<500)	p
Donor			
Cadaveric	1 (0.05%)	40 (19%)	0.470
Alive	12 (5.6%)	158 (74.8%)	
Preemptive	6 (2.8%)	64 (30.3%)	0.365
Dialysis patient	7 (3.3%)	134 (63.5%)	
DGF (+)	0 (0%)	6 (2.8%)	1
DGF (-)	13(6.2%)	192 (90.2%)	
Mismatch			0,694
0 MM	0 (0%)	23 (10.9%)	
1MM	1 (0.005%)	6 (2.8%)	
2MM	2 (0.009%)	23 (10.9%)	
3 MM	5 (0.024%)	63 (29.9%)	
4 MM	1 (0.005%)	21 (9.9%)	
5 MM	1 (0.005%)	27 (12.8%)	
6 M	2 (0.009%	15 (7.1%)	

DGF: delayed graft function; BKV: BK viremia

Surgical Technique

An open or laparoscopic nephrectomy technique was applied for living donors. An extravesical technique (Lich-Gregoir) was applied to all transplanted patients for ureteroneocystostomy (UNS). Types of DJS used were dependent on the choice of surgeons. Cases were grouped later by whether their DJS had an anti-reflux mechanism or not (St-DJS vs ARD-DJS). Surgical drains were placed to all patients to the operation side. Urinary catheters were removed on the 5th postoperative day. Surgical drains were removed if there were no urinary leak and after urinary catheters were removed. All DJSs were removed by cystoscopy under local anesthesia at the 4th postoperative week. Urinary complication was described as the presence of urinary leak and stenosis at UNS.

Follow-up

All patients were followed up closely for renal functions, BK viremia, and BKVN after kidney transplantation. First BKV tests were done during 1st postoperative month. All transplanted patients were tested for BKV from their serum by polymerase chain reaction (PCR) monthly in the first year after transplantation. All the patients were followed up at the posttransplant period for at least 12 months. Cases with >500 copies/ml by two or more consecutive measurements were accepted as having viremia. The immunosuppression dose was reduced when there was a higher viremia load (>5,000 copies/ml). Firstly MMF was discontinued and the prednisolone dose was reduced. If viremia continued, the calcineurin dose was reduced. Leflunomide was used for patients with BKVN. Kidney biopsy was performed to all cases with viremia for excluding BKVN.

Statistical Analysis

Statistical analyses were performed using the SPSS software version 16. The variables were investigated using (histograms, probability plots) and analytical methods (Kolmogorov-Smirnov/Shapiro-Wilk's test) to determine whether or not they were normally distributed. Descriptive analyses were presented using medians and interquartile range (IQR) for the non-normally distributed and ordinal variables. The proportions of patients with ATG use, preemptive transplantation, and BKVN were grouped by types of DJS using cross-tabulations. The Chi-square test or Fisher's exact test, where appropriate, were used to compare these proportions in different groups. Blood creatinine measurements were not normally distributed; nonparametric tests were conducted to compare these parameters. The Mann-Whitney U test was used to compare the relationship between blood creatinine levels and the use of the ARD-DJS.

RESULTS

In this study, the data of 211 patients who underwent kidney transplantation between September 2012 and September 2019 were retrospectively evaluated. The median and interquartile range (IQR) of age and time from kidney transplantation of patients was 32 years (22-42 years) and 48,9 months (26,2-71 months) respectively. Median and IQR follow-up time for ARD-DJS and St-DJS patients was 72 months (62,5-80,3 months) and 27,8 months (17,4-39,6 months) respectively.

Seventy patients (33,1%) underwent preemptive kidney transplantation. Basiliximab was used in 66 patients (31,3%) and ATG was used in 130 patients (61,6%) as induction therapy. Fifteen patients (7,1%) were not given induction therapy. Three cases used cyclosporin+MMF+ prednisolone, eight cases used everolimus+tacrolimus+prednisolone, and all other patients used tacrolimus+MMF+prednisolone for maintenance immunosuppression. Acute rejection was observed in 13 patients, humoral rejection in 3 patients, humoral, and cellular rejection in 3 patients. In 6 patients, graft loss due to rejection was observed (CR:3 HR:2 CR+HR: 1 patient). In postoperative duration DGF was observed in 9 patients (4,2%), graft loss was observed in 12 patients (5,7%). DJS was used in all patients, ARD-DJS was used in 100 patients (47,4). Thirteen patients (6,1%) had BKV viremia. BKV was revealed by kidney biopsy in 3 of 13 patients. However, graft loss due to BKVN was observed in only one patient. Except for 2 patients viremia was observed in the first 3 months in these patients, while in the other 2 patients, viremia was observed at 12 and 84 months after transplantation respectively. These 2 patients were in the group of ARD-DJS, in 4 of 13 patients with BKV, ARD-DJS, and in 9

patients st-DJS was used. Patients in whom BKV revealed in the first 3 months were compared in the aspect of DJS technique, BKV was significantly less observed in the ARD-DJS group (ARD-DJS: 2 patients; St-DJS:9 patients), (p=0,046). St-DJS was used in 6 of 7 patients with viremia level>10,000, however, this finding was not statistically significant. When the patients were compared according to their final blood creatinine levels, the creatinine levels in patients with st-DJS and ARD-DJS was 1,19 (range 0,96-1,47) and 1,03 (range 0,86-1,27) respectively which was statistically significant (p=0,002). None of the patient groups had urinary leakage in the postoperative period. However, in one patient in each group, urinary stenosis was observed in the postoperative follow-up period and revision operation was performed.

DISCUSSION

Ureteral stent use is considered as an independent risk factor for BKV viruria, BKV viremia, and BKVN. In a study conducted on 1147 patients, a ureteral stent was used in 443 (38,6%) of the patients, and BKV was observed in 17,2% of these patients, on the other hand, this ratio was 13,5% in patients without stent (16). Moreno et al. (17) detected BKV in 11 patients (6%) in their study conducted on 184 patients. In this study, they explained the low BKV rates by the different ureteral stent techniques. At the same time in this study, ureteral anastomosis was performed using the modified Taguchi extravesical reimplantation technique and, the stent was externalized from the skin with a 6 F radiopaque infant nasogastric tube, and the stent was removed on the 5th day (17). Gupta et al. (18) evaluated 402 transplant kidney biopsies between 2013 and 2016, BKVN was detected in 6 (1,49) patients. They attributed the lower rate of BKVN than the literature to the low immunosuppression dose and the high match in transplants from live relative donors. In our study, BKV was observed in 13 (6,1%) patients, which is lower than the rates of BKV in the literature. Another remarkable inference in our study is that BKV was observed in 9 (8,1%) of 111 patients in which st-DJS was used and in 4% of the patients used ARD-DJS, although these rates are not statistically significant among themselves, they show that ureteral stenting technique and stent type may also have aclinical meaning. BKVN was observed in 3 patients in our study, one of these patients resulted in graft loss, and also diffuse deep vein thrombosis occurred in the same patient. This patient died due to diffuse pulmonary embolism during follow-up. Considering that acute venous thrombosis was observed in a patient diagnosed with BKVN in a case report published in 2015, it is important to pay attention to the relationship between BKVN and DVT despite case-level studies (19).

In our study, we also reached some results that we could not interpret, when we compared the final blood creatinine levels in both groups we revealed that the creatinine level was lower in the patients with ARD-DJS, additionally, although the patients with ARD-DJS had a much longer follow-up period, the number of graft loss was similar to the patients with st-DJS.

The limitations of this study were its retrospective nature, the absence of a control group without DJS, and the need for much higher patient numbers, although the number of patients increased than our previous study. However, to the best of our knowledge, there are not enough studies in the literature evaluating the relationship between ARD-DJS and BKV.

CONCLUSION

In our study, BKV was observed less in patients with ARD-DJS that were clinically significant but not statistically significant. Also, it was observed that the majority of patients with early developed BKV (first 3 months) were patients with st-DJS. Although close monitoring and reduction of immunosuppression prevent BKVN to a great extent, all arguments that can reduce the risk of BKV deserve to be investigated in detail. Therefore, prospective randomized studies with high patient numbers are needed to determine the effectiveness of ARD-DJS.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Gazi Yaşargil Training and Research Hospital Clinical Researches Ethics Committee (Date: 26.03.2021, Decision No: 743).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The author has no conflicts of interest to declare.

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Original Article

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Donor and recipient characteristics associated with rebubbling rate, endothelial cell loss, and graft failure in primary descemet membrane endothelial keratoplasty

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ABSTRACT

Aim: To determine whether donor and recipient characteristics are associated with rebubbling rate, endothelial cell loss (ECL), and graft failure 3 years after primary Descemet membrane endothelial keratoplasty (DMEK).

Material and Method: Records of 295 consecutive DMEK surgery and match with corresponding donor data were reviewed at a tertiary referral clinic. Recipients with intraoperative complications and coexisting ocular pathologies were excluded. Age, sex of donor and recipient, cause of donor death, death-to-preservation time (DtPT), storage time, donor endothelial cell density (ECD), and indications for surgery were analyzed for correlation with rebubbling rate, postoperative ECL, and graft failure. Further, subgroup analyses of the cause of death, donor sex, DtPT (median value, 3.5 h), and indications were performed. Multiple regression and receiver operating characteristics (ROC) analysis were used to determine the independent risk factors for graft failure.

Results: This study included 114 eyes that underwent DMEK for bullous keratopathy (BK; 64%) and for Fuchs' endothelial corneal dystrophy (FECD; 36%). The graft failure percentage was the only parameter that was higher in patients with DtPT > 3.5 h (p=0.047) than those with shorter DtPT. The probability of graft failure was seven times higher in eyes with DtPT > 3.5 h than with shorter DtPT (odds ratio 7.36, 95% confidence interval CI 1.34-40.53) and 10 times higher in eyes with BK than those with FECD (odds ratio 10.29, 95% CI 1.01-104.54).

Conclusion:. DtPT and recipients with BK diagnosis were found to be independent risk factors for graft failure. Therefore, surgeons should consider DtPT for DMEK in eyes with BK.

Keywords: descemet membrane endothelial keratoplasty, death-to-preservation time, graft failure, bullous keratopathy, Fuchs endothelial corneal dystrophy

INTRODUCTION

Over the past two decades, anterior and posterior lamellar keratoplasties, such as deep anterior lamellar keratoplasty, Descemet stripping automated endothelial keratoplasty (DSAEK), or Descemet membrane endothelial keratoplasty (DMEK), have supplanted penetrating keratoplasty (PK) in selective replacement of diseased corneal stroma or endothelium[1]. Globally, DMEK has become the standard surgery for pseudophakic bullous keratopathy (BK) and Fuchs' endothelial corneal dystrophy (FECD) due to better visual outcomes and rapid visual rehabilitation (1).

Donor and recipient characteristics can help determine the success of keratoplasty. The Cornea Donor Study (CDS) and Corneal Protection Time Study (CPTS) results highlighted the evidence-based donor selection criteria for PK or DSAEK procedures (2, 3). However, clinical trials similar to CDS and CPTS studies, have not yet been conducted in DMEK. Therefore, it is not yet clear to what extent specific donor and graft characteristics affect the success of DMEK surgery.

The purpose of this study is to investigate the effects of donor and recipient characteristics on rebubbling rates, endothelial cell loss (ECL), and graft failure 3 years after primary DMEK. Secondary to those analyses, we hoped to provide DMEK surgeons with clues that will allow them to evaluate donor tissue and recipient characteristics together for donor selection.

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MATERIAL AND METHOD

The study was carried out with the permission of University of Health Sciences Dr. Lütfi Kırdar Kartal City Hospital Noninvasive/ Clinical Researches Ethics Committee (Date: 29.04.2020, Decision No: 2020/514/176/1). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Study Design

Data from the medical records were retrospectively reviewed for DMEK procedures performed between January 1, 2014, and March 30, 2019, at a tertiary referral center. Furthermore, the database of the local eye bank was reviewed for corresponding corneal donor tissue parameters. BK and FECD recipients were followed up at least 3 years after primary DMEK were included in the study. Recipients with intraoperative complications or coexistence of other ocular pathologies, such as retinal disorder or glaucoma, vitrectomized and aphakic eyes, or regrafts, were excluded to achieve homogeneity and avoid misleading results. Further, the first 25 cases of DMEK representing the learning curve of this technique were excluded from this study.

Collection of Donor Data

Before postmortem excision, serological and microbiological tests were done and seronegative donor corneas were used. University of Health Sciences Dr. Lütfi Kırdar Kartal City Eye Bank provided all donor corneal buttons, which were stored in a short-term storage solution (Eusol-C®, Corneal Chamber, Alchimia, Ponte San Nicolo, Italy) at 4°C. Eligible donor corneas were obtained from individuals aged 10–75 years who had endothelial cell density (ECD) values of 2300–3300 cells/mm^2 in line with the standard criteria of the Eye Bank Association of America (EBAA) (4), measured using a specular microscope (Konan Eye Bank KeratoAnalyzer, EKA-04, Japan). Donor and recipient age, sex of donor (male/female), cause of death, death-to-preservation time (DtPT), and storage time (ST) until grafting were recorded. ECL was calculated as the difference between preoperative and 36-month ECD values, expressed as a percentage of preoperative ECD. Death-to-preservation time was divided into two times intervals according to median average of 3.5 hours (<3.5 h and >3.5 h) for statistical analyses.

Surgical Technique and Postoperative Treatment

The donor graft preparation and DMEK procedures were all performed by one experienced surgeon (BK) according to the techniques described in the literature (1, 5). The graft was prepared on the same day as the DMEK surgery and used without delay. Descemet membrane were detached and cut using a 8.00-mm punch. Asymmetric triangle marking was used in all DMEK graft preparations to ensure placement of the graft in the correct position, as described previously (6). In all phakic cases, standard phacoemulsification and intraocular lens implantation were performed prior to DMEK surgery.

Following the DMEK surgery, all eyes were treated with a topical antibiotic (0.5% moxifloxacin hydrochloride; Vigamox, Alcon Pharma GmbH, Freiburg, Germany) and a corticosteroid (0.1% dexamethasone; Maxidex, Alcon Pharma GmbH) five times daily. The antibiotic was discontinued after 10 days. Dexamethasone was replaced with 0.5% loteprednol etabonate (Lotemax, Bausch + Lomb, Bridgewater, NJ, USA) four times daily 3 months after the surgery. According to the patient's clinical outcomes, the local steroid treatment was then gradually decreased to a maintenance dose of once daily.

Collection of Recipient Data

The standardized eye examinations included best corrected visual acuity (BCVA) assessment by Snellen chart (means and medians BCVA were converted to logarithm of the minimum angle of resolution (logMAR) units), slit lamp examination, tonometry, funduscopy, subjective refractometry, corneal topography (Sirius Scheimpflug Placido topographer, Costruzione Strumenti Oftalmici, Florence, Italy), and corneal pachymetry (Optikon pacline, Rome, Italy) for central corneal thickness (CCT). ECD (Topcon Corporation, Tokyo, Japan) measurement was carried out both preoperatively and at 3, 6, and 12 months and then annually for up to at least 3 years postoperatively. Graft-attachment/detachment was evaluated with anterior segment optical coherence tomography (Optos PLC, Dunfermline, United Kingdom) at each follow-up visit. Rebubbling (air reinjection after partial graft detachment) rates were also recorded. If the endothelial graft was more than one-third detached, rebubbling was performed 24–36 hours after surgery. If the endothelial graft was less than one-third detached with presence of corneal edema, patients were followed up for 2–3 weeks. If edema persisted, rebubbling was performed.

Postoperative complications were also recorded. Graft failure was defined as corneal edema and haze due to endothelial decompensation (7). We classified cases as primary graft failure, which is defined as a cornea that failed to be clear in the presence of an attached graft, whereas secondary graft failure was defined as corneal decompensation after an initial period of a functional graft after DMEK (7). In cases of graft failure, DMEK was repeated or PK was performed.

Statistical Analysis

The Number Cruncher Statistical System 2007 (Kaysville, Utah, USA) program was used for statistical analysis. Homogeneity tests were performed with the Shapiro–Wilk test and graphical analysis. An independent *t*-test was used to compare the preoperative and postoperative BCVA, ECD, and CCT values. One-way variance analysis and dependent groups *t*-test were also used where appropriate. Pearson chi-square, Fisher's exact, and Fisher–Freeman–Halton exact tests were used to compare qualitative data. Statistical significance was assigned if $p < 0.05$.

Pearson correlation analysis was used to evaluate associations between patient age, donor age, DtPT, ST, and donor ECD with graft failure 36 months after DMEK. In addition, logistic regression and receiver operating characteristics (ROC) analysis were performed to determine the independent risk factors for graft failure. Based on logistic regression analysis, sensitivity, specificity, PPV, NPV, and overall accuracy percentages were calculated for DtPT and graft failure indications of recipients.

RESULTS

In total, 295 DMEK cases were reviewed retrospectively. Overall, 114 eyes that met the inclusion criteria with a minimum 3 years of follow-up time were included in the study, which included 73 (64.0%) eyes with BK and 41 (36%) eyes with FECD. The mean follow-up time was 41.7 (±4.5, range 36–72) months. Patients' and donors' data and visual and clinical outcomes are provided in **Table 1**.

Postoperatively, 11 eyes (9.6%) required rebubbling. There was no association between patient or donor characteristics on the rebubbling rate, 36-month ECL values, and graft failure (**Table 2, Figure 1**). Only the percentage of graft failure was greater in patients with DtPT > 3.5 h (p=0.047) (**Table 2**).

Mean DtPT was 4.8 (±4.05) hours. According to logistic regression analysis, the probability of graft failure for grafts from donors with DtPT > 3.5 h was 7.3 times higher than for those with shorter DtPTs (**Table 3**). The probability of graft failure was 10.3 times higher in eyes with BK than those with FECD (**Table 3**). Further, 9 (90%) of 10 graft failures occurred in eyes with BK (**Table 3**).

ROC analysis was performed using predicted probability values (DtPT and BK) obtained as a result of the model performed for graft failure. For the probability of graft failure, the area under the ROC curve was 0.861 (95% CI=0.748, 0.973, $p < 0.001$; **Figure 1**).

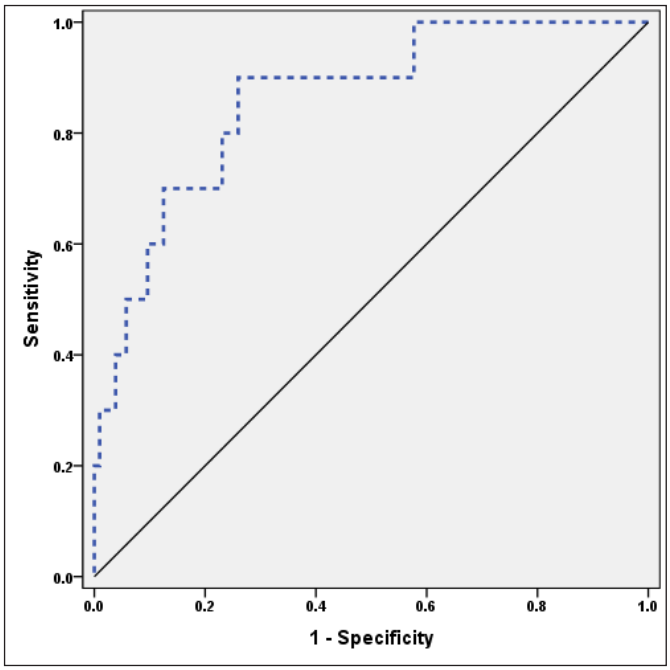

Figure 1. Receiver operating characteristics curve of predicted probabilities obtained as a result of regression analysis for graft failure

Table 1. Patients' and donors' data and visual and clinical outcomes.

	Range (Min–Max)	Mean±SD	p
Recipient age (year)	30–93	69.56±11.78	
Preoperative BCVA (LogMAR)	0.3–2	1.56±0.47	**0.001**
36-month BCVA (LogMAR)	0–1.3	0.48±0.55	
Preoperative CCT (µ)	609–898	734.01±62.65	**0.001**
36-month CCT (µ)	453–742	567.4±71.41	
Donor ECD (cells/mm²)	2087–3236	2628.54±266.98	**0.001**
36-month ECD (cells/mm²)	715–2550	1602.64±431.57	
ECL (%)	0.04–0.72	0.39±0.16	
Donor age (year)	38–70	58.56±7.69	
DtPT (hours)	0.33–18.42	4.8±4.05	
ST (day)	1–17	4.78±2.96	
	N	**%**	
Recipient diagnosis			
BK	73	64.0	
FECD	41	36.0	
Eye			
Right	60	52.6	
Left	54	47.4	
Donor sex			
Female	42	36.8	
Male	72	63.2	
Cause of death			
Cardiopulmonary arrest	75	65.8	
Multiple trauma	12	10.5	
Cancer	13	11.4	
Others	14	12.3	
Postoperative complications	18	15.8	
Graft failure	10	8.8	

[a]Pearson correlation coefficient, [b]Independent samples t-test, [c]One-way analysis of variance. Bold p values indicate statistically significant (*p < 000.1) Abbreviations: PBK: Pseudophakic bullous keratopathy; FECD: Fuchs endothelial corneal dystrophia, BCVA: best corrected visual acuity, CCT: central corneal thickness, ECD: endothelial cell density, ECL: endothelial cell loss, DtPT: Death-to-preservation time; ST: Storage time, SD: standard deviation (In order to make the groups, sub-analyses, pre-, and post-DMEK values more understandable and organized, gray shading was drawn in all tables).

Table 2. Analyses of the relationship between donor, recipient risk factors, and their subgroups and rebubbled or non-rebubbled grafts, ECL, graft failure, or without graft failure.

	Rebubbled Graft		p	ECL ar	p	Graft Failure		p
	Yes Mean±SD	No Mean±SD				Yes Mean±SD	No Mean±SD	
Recipient age (year)	65.09±13.58	70.04±11.54	[b]0.187	0.133	0.157	64.4±18.7	70.06±10.9	[b]0.370
Donor age (year)	59.36±7.34	58.48±7.75	[b]0.718	0.065	0.493	61.3±5.14	58.3±7.86	[b]0.240
DtPT (hour)	4.14±3.38	4.87±4.13	[b]0.569	0.107	0.259	5.83±3.77	4.7±4.08	[b]0.402
ST (day)	5.05±4.02	4.75±2.85	[b]0.756	0.067	0.476	4.9±1.85	4.77±3.05	[b]0.894
DonorECD (cell/mm²)	2655.18±254.2	2625.7±269.35	[b]0.729	0.092	0.332	2531.7±217.34	2637.86±270.33	[b]0.231
	n (%)	n (%)	p	Mean±SD	p	n (%)	n (%)	p
DtPT			[d]0.751		[b]0.504			**[d]0.047***
≤3.5 h	6 (10.5)	51 (89.5)		0.38±0.16		2 (3.5)	55 (96.5)	
>3.5 h	5 (8.8)	52 (91.2)		0.4±0.16		8 (14)	49 (86)	
Recipient diagnosis			[c]0.323		[b]0.065			[c]0.092
BK	9 (12.3)	64 (87.7)		0.41±0.17		9 (12.3)	64 (87.7)	
FECD	2 (4.9)	39 (95.1)		0.35±0.13		1 (2.4)	40 (97.6)	
Donor sex			[c]0.324		[b]0.436			[c]0.743
Female	6 (14.3)	36 (85.7)		0.4±0.16		3 (7.1)	39 (92.9)	
Male	5 (6.9)	67 (93.1)		0.38±0.16		7 (9.7)	65 (90.3)	
Cause of death			[f]0.744	0.39±0.15	[c]0.639			[f]0.715
Cardiopulmonary arrest	9 (12)	66 (88)		0.35±0.19		8 (10.7)	67 (89.3)	
Multiple trauma	0 (0)	12 (100)		0.4±0.18		0 (0)	12 (100)	
Cancer	1 (10)	9 (90)		0.42±0.19		0 (0)	10 (100)	
Other	1 (5.9)	16 (94.1)				2 (11.8)	15 (88.2)	

[a]Pearson correlation coefficient, [b]Independent samples t-test, [c]One-way ANOVA, [d]Pearson chi-square test, [e]Fisher's exact test, [f]Fisher–Freeman–Halton exact test. Bold p values indicate statistically significant. *p < 0.05. Abbreviations: DtPT: Death-to-preservation time; ST: Storage time ECD: Endothelial cell density; BK: Bullous Keratopathy FECD: Fuchs endothelial corneal dystrophy; SD: standard deviation; h: hour

Table 3. Association between donor, recipient risk factors on rebubbling graft, ECL, and graft failure after primary DMEK at 3 years.

	Rebubbled Grafts	p	ECL	p	Graft Failure	p
	OR (95% CI)		Beta (95% CI)		OR (95% CI)	
Donor age (year)	0.994 (0.896, 1.104)	0.915	0.001 (−0.003, 0.006)	0.554	1.020 (0.903, 1.151)	0.752
Donor ECD(cell/mm²)	1.001 (0.998, 1.003)	0.622	6.97E−5 (−4.84E−5, 1.88E−4)	0.245	0.999 (0.996, 1.002)	0.534
DtPT >3.5 hour	1.01 (0.27, 3.783)	0.988	0.02 (−0.041, 0.082)	0.519	**7.365** (1.338, 40.530)	**0.022***
ST (day)	1.066 (0.862, 1.319)	0.554	0.006 (−0.004, 0.017)	0.228	1.151 (0.866, 1.531)	0.332
Recipient diagnosis BK	3.615 (0.693,18.857)	0.127	0.069 (−0.004, −0.134)	0.039*	**10.295** (1.014, 104.536)	**0.049***
Cause of death						0.808
Cardiopulmonary arrest	2.311 (0.264,20.224)					
Multiple trauma	-	0.449	−0.027 (−0.113, 0.058)	0.531	0.620 (0.097, 3.979)	
Cancer	1.597 (0.083,30.733)	-	−0.088 (−0.213, 0.036)	0.163	-	-
Others		0.756	−0.025 (−0.152, 0.102)	0.698	-	-

Linear regression analysis for ECL, logistic regression analysis for graft failure, and rebubbling were performed. OR: Odds Ratio, CI: Confidence Interval Bold p values indicate statistically significant. *p < 0.05. Abbreviations: DtPT: Death-to-preservation time; ST: Storage time; ECD: Endothelial cell density; BK: Bullous keratopathy; FECD: Fuchs endothelial corneal dystrophy; DMEK: Descemet membrane endothelial keratoplasty; SD: standard deviation

Based on logistic regression analysis, the sensitivity, specificity, PPV, NPV, and overall accuracy are presented for DtPT and recipients' indications of graft failure. According to this, the specificity was highest (100%) among recipients with BK and with a graft DtPT > 3.5 h (**Table 4**).

Complications developed in 18 (15.8%) eyes. Primary and secondary graft failures were observed in 3 (2.6%) and 7 (6.1%) of 114 eyes, respectively. Other complications were postoperative high intraocular pressure resistant to medical treatment in four eyes (3.5%), severe keratitis in two eyes (1.7%), graft rejection in one eye (0.8%), and intraocular lens deposit in one eye (0.8%). Repeated keratoplasty was required in 10 (8.8 %) eyes (four eyes re-DMEK and six eyes PK).

Table 4. Based logistic regression analysis for DtPT and diagnosis on graft failure

Factors	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)
DtPT < 3.5 h. BK-	100 (69.2, 100)	19.2 (12.2, 28.1)	10.6 (5.2, 18.7)	100 (83.2, 100)
Only DtPT > 3.5 h	90 (55.5, 99.7)	38.5 (29.1, 48.5)	12.3 (5.8, 22.1)	97.6 (87.1, 99.9)
Only BK +	70 (34.8, 93.3)	72.1 (62.5, 80.5)	19.4 (8.2, 36)	96.2 (89.2, 99.2)
DtPT > 3.5 h; BK +	0 (0, 30.8)	100 (96.5, 100)	-	91.2 (84.5, 95.7)

Abbreviations: DtPT: Death-to-preservation time, BK: Bullous keratopathy, PPV: positive predictive value, NPV: negative predictive value, h: hours

DISCUSSION

In this study, we evaluated donor age, cause of death, preoperative ECD, DtPT, ST, and recipients' diagnosis and age to determine whether these factors influenced the rate of rebubbling, ECL, and graft failure 3 years after primary DMEK while excluding cases with intraoperative complications or coexistence of other ocular pathologies. Donor age, cause of death, preoperative ECD, ST, and recipients' age were not independent risk factors for rate of rebubbling, ECL, or graft failure. However, DtPT and eyes with BK were revealed as two independent risk factors for graft failure according to multivariable regression analysis.

Several studies have been conducted to determine possible correlations between donor characteristics and the results of DMEK surgery. Donor age is one of the most debated donor characteristics in the keratoplasty literature. We found that donor age did not affect the rebubbling rate, 36-month ECL, and graft failure after DMEK. This finding is consistent with the prospective, large, multicenter, and long-term results for PK from the CDS and for DSEK/DSAEK from the CPTS (2, 3). The CPTS also pointed out that donor age will not be as important for DMEK graft success as for DSAEK graft success (3). However, donors >55 years are generally recommended for DMEK surgery because the Descemet membranes of younger donors tend to be more fragile and adherent to the stroma (8). Heinzelmann and associates performed an in-vitro study of 28 prepared DMEK grafts to investigate how donor characteristics might affect DMEK surgical outcomes (9). They found that donor age affects the duration of surgery because the Descemet membrane is more tightly scrolled in younger than in older donors, and this can complicate the process of opening the scroll in recipient eyes. Thus, they highlighted that increased unfolding times resulted in higher ECL (9). We did not find a similar effect of donor age. The reason for this result may be that there were only 17 donor grafts under the age of 55 in our study.

We concluded that donor ECD is not an independent risk factor for postoperative rebubbling, ECL, or graft failure 36 months after DMEK. Our conclusions are similar to those reached by Straiko et al. (10) and are found in several other studies as well (2,3,10,11).

The role of cause of death has previously been assessed in relation to DMEK outcomes. Boydoun et al. (12) reported that noncancer donor death causes were associated with higher ECL 8 years after surgery Oellerich et al. (13) found that donors with causes of death other than cancer were associated with lower ECD values In our study, cause of donor death was not an independent risk factor for rebubbling rate, 36-month ECL, or graft failure. However, we had smaller samples per cause of death than the previous studies mentioned above.

ST did not affect post-DMEK rebubbling rates, ECL, or graft failure, which is similar with reports from Straiko et al. (10) and Patel et al. (14). However, a 5-year follow-up of 500 DMEK cases with an average graft ST of 13.5 days showed an association between graft ST and ECD decline after DMEK (8, 15). Rosenwasser et al. (16) highlighted that the effect of ST on graft success rate in DSAEK is as low as 11 days and that surgeons recommend accepting corneas stored for 12–14 days or less. Therefore, our DMEK study results agree with the results for DSAEK and do not adversely affect the DMEK results of longer protection periods (up to 13 days in our series).

We have achieved two results with DtPT in primary DMEK cases. First, we found that probability of graft failure was 7.3 times higher in donors with DtPT longer than 3.5 hours than in those with shorter DtPT. Whether DtPT affects graft success of keratoplasties has been debated (3, 10, 17-23). In the CPTS, a maximum DtPT of 11 hours was an eligibility criterion for DSAEK donors (3). In some studies, DtPT has not been found to affect graft failure (2, 10, 22). Our mean and median DtPT values were lower than the times in some previous studies. In traditional Turkish culture, the dead are buried as soon as possible (24). However, similar to our DtPT results, Gavrilov et al. (25) suggested that DtPT should be <6 hours for corneal suitability for use (25). Secondarily, when we evaluated the eyes with DtPT and eyes with BK together according to logistic regression analyses for graft failure, we found that these characteristics were two independent risk factors for graft failure at 3 years after primary DMEK. Additionally, when we examined the predicted probability values for DtPT and BK in recipients obtained from the graft failure model, our results showed that specificity was highest (100%) among recipients with BK and with a graft DtPT > 3.5 h. Therefore, the use of grafts with donor

DtPT longer than 3.5 hours in recipients with BK may increase the likelihood of endothelial decompensation. In support of this hypothesis, Armitage et al. (19) used donors with DtPT of 19–24 hours in eyes with BK and found an increased risk of endothelial failure at 5 years after surgery.

It is important to consider the distribution of indications for keratoplasty in studies evaluating graft survival. For many years, BK has been shown to be the most common indication for PK and DSAEK, and it has been reported that graft survival rates are lower in recipients with BK than in recipients with other indications (2, 3, 15, 19). Some other studies, including CDS and CPTS, have found that graft failure rates are higher in BK recipients than in FECD recipients (2, 3, 26-28). Additionally, the indications of recipients for DMEK are different between the United States (US), Europe (where FECD is the most frequent indication), and Asia (where BK is the most frequent indication) (29-32). Studies conducted in Europe and US for graft failure in DMEK found that the recipient's indication is not an independent risk factor, whereas in Asia, the recipient indication has been reported as a risk factor (13, 15, 33). In our study, 64% of graft recipients had BK and 36% had FECD, and our results highlighted that BK is an independent risk factor for graft failure in DMEK.

Mechanisms whereby eyes with BK rather than FECD reduce the rate of graft success after DMEK should also be discussed in detail. There is a large reserve of peripheral endothelial cells in FECD, and a healthy peripheral endothelium could fill damaged areas of the endothelium in the graft (34). This theory is only supported by success in FECD-diagnosed eyes treated with descemetorhexis (35). Whereas in BK, there are few healthy endothelial cells in the periphery. Additionally, in some studies, abnormal immune responses established in the anterior chamber of the eyes in recipients with BK (36, 37). These results support our finding that the probability of graft failure is 10 times higher in eyes with BK than in those with FECD.

The limitations of this study are that it was retrospective and had a small sample size. However, the strengths of our study are the exclusion of other ocular pathologies to ensure homogeneity, use of a standardized DMEK technique, and the follow-up of cases for at least 3 years.

CONCLUSION

Donor characteristics, including age of donor, cause of donor death, DtPT, preoperative ECD, graft ST, and recipient age had no significant effect on the rate of rebubbling, ECL, and graft failure at 3 years after primary DMEK. DtPT and BK in recipients were considered valuable independent factors in predicting the risk of graft failure in DMEK. In this aspect, the current study can provide clues about how donor tissue selection can maximize the success and how efficiency can be increased in using the existing corneal donor tissue pool for future studies.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of University of Health Sciences Dr. Lütfi Kırdar Kartal City Hospital Noninvasive/ Clinical Researches Ethics Committee (Date: 29.04.2020, Decision No: 2020/514/176/1).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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Original Article

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The relationship of nailfold capillaroscopy patterns with clinical features, functional status, pain and fatigue in patients with systemic sclerosis

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ABSTRACT

Aim: To identify the frequency of scleroderma-type capillaroscopic patterns and evaluate the association of capillaroscopic patterns with clinical parameters, functional status, fatigue, and pain in systemic sclerosis (SSc).

Material and Method: This cross-sectional study included SSc patients consecutively between January 2017 and January 2019. Cutaneous involvement was evaluated with the modified Rodnan skin score (mRSS). The presence of digital ulcers, Raynaud phenomenon, interstitial lung disease, pulmonary hypertension, cardiac, gastrointestinal system (GIS), renal, joint and muscle involvement were recorded. The severity of the Raynaud phenomenon, fatigue, pain, and patient global assessment (PGA) was assessed on the Visual Analogue Scale (VAS). The Health Assessment Questionnaire (HAQ) and the Duruoz Hand Index (DHI) were used to assess physical disability and hand function, respectively. Nailfold videocapillaroscopic examinations of the patients were performed, and they were classified into four groups, including normal/non-specific, early, active, and late scleroderma patterns.

Results: The mean age of 32 patients with SSc (31 female, one male) was 48.93±12.77. Anormal capillaroscopic examination findings were detected in 93.7% of the patients, and the most common capillaroscopic pattern was the active pattern. The comparison of scleroderma pattern groups revealed no difference in age (p=0.224), but disease duration was shorter in the early pattern group (p=0.005). The duration and severity of the Raynaud phenomenon, and mean mRSS were lower in the early pattern group (p=0.004, p=0.009, and p=0.001, respectively). The digital ulcer (p=0.011) and diffuse cutaneous SSc (p=0.016) were more common in the late pattern group. The percentage of pulmonary hypertension (p=0.011), GIS involvement (p<0.001), and arthralgia (p=0.027) were higher in the late pattern group. Fatigue (p=0.575), pain (p=0.536), PGA (p=0.861), HAQ (p=0.164) and DHI (p=0.064) scores were not different between the capillaroscopy pattern groups.

Conclusion: Digital ulcer, pulmonary hypertension, and GIS involvement were more common in SSc patients with the late pattern. The fatigue, pain, physical disability, and hand function were similar between capillaroscopy pattern groups.

Keywords: Scleroderma, nailfold capillaroscopy, functional status

INTRODUCTION

Systemic sclerosis (SSc) is an autoimmune disease with microvascular changes followed by progressive tissue fibrosis. Characteristic microvascular changes in the nailfold area occur early and can be assessed with simple, non-invasive methods that allow early diagnosis of SSc (1-4).

The gold standard method for the evaluation of nailfold capillaries is videocapillaroscopy (2,4). It enables to differentiate primary Raynaud phenomenon and SSc. Capillaroscopy findings that occur in the majority of patients are specific for SSc (4). These capillaroscopy findings were described by Maricq et al. (5-7) as "scleroderma patterns". Cutolo et al. (8) classified the "scleroderma" type capillaroscopic patterns into three phases including "early" phase (few enlarged or giant

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capillaries, few capillary haemorrhages, no capillary loss), "active" phase (frequent giant capillaries and capillary haemorrhages, mild disorganization, moderate capillary loss) and "late" phase (few or no giant capillaries and haemorrhages, extensive avascular areas, disorganization, ramified/bushy capillaries). Findings of "scleroderma pattern" on capillaroscopy were included in the criteria for (very) early diagnosis of SSc and 2013 American College of Rheumatology (ACR)/ European League Against Rheumatism (EULAR) classification criteria (9,10).

Nail fold videocapillaroscopic (NVC) examinations provide information for early diagnosis, prognosis and treatment efficacy in SSc (11), but its effects on functional status, hand function, fatigue, and pain are currently not known. This study aimed to 1) determine the frequency of scleroderma-type capillaroscopic patterns and 2) identify the associations of capillaroscopic scleroderma patterns with clinical parameters, functional status, fatigue, and pain in SSc.

MATERIAL AND METHOD

The study was carried out with the permission of Marmara University Faculty of Medicine Clinical Researches Ethics Committee (Date: 06.01.2017, Decision No: 09.2017.095). All procedures were performed in accordance with the 1964 Helsinki declaration. Informed consent was obtained from all participants.

Study Design and Cohort

This cross-sectional study included 32 patients diagnosed as SSc, according to the ACR/EULAR criteria for SSc (10). The SSc patients aged ≥18 years who presented to the outpatient clinic of Rheumatology were consecutively included in the study between January 2017 and January 2019. The exclusion criteria for the study were being younger than 18 years of age, having hand disorders related to Diabetes mellitus, neurological disorders, erosive osteoarthritis, and hand surgery or trauma.

Demographic and Clinical Variables

Data on age, sex, disease duration, age at diagnosis, smoking status, and body mass index (BMI) were recorded, and physical examinations were performed. Skin involvement was assessed using the modified Rodnan skin score (mRSS), and patients were grouped into diffuse cutaneous SSc (dcSSc) or limited cutaneous SSc (lcSSc) according to the extent of skin involvement (12,13). The presence of the Raynaud phenomenon and digital ulcer were evaluated separately, and the severity of these parameters was assessed on Visual Analogue Scales (VAS). Interstitial lung disease, pulmonary hypertension, cardiac, gastrointestinal system (GIS), renal, joint and muscle involvement were recorded in each patient. Pain, fatigue, and patient global assessment (PGA) were evaluated on VAS. The Health Assessment Questionnaire (HAQ) was used to evaluate physical disability (14). Hand function was assessed with Duruoz Hand Index (DHI), which is a valid scale for SSc (15,16).

NVC examinations of the patients were performed, and they were classified into four groups according to the findings of capillaroscopy: normal/non-specific, early, active, and late scleroderma patterns (8). The second-fifth fingernails were examined bilaterally in each patient. Before the examination, the patients rested for at least 15 minutes at room temperature (22–25 °C). Fingers affected by recent local trauma were excluded from analysis (17).

Statistical analysis

The statistical analysis was done using SPSS Statistics (IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY: IBM Corp). A two-tailed P-value of <0.05 was considered significant. Categorical variables were summarized as frequency and percentages, and continuous variables were summarized as mean, standard deviation, median, minimum, maximum, 25th and 75th percentiles. Continuous variables were compared using the Mann-Whitney U and Kruskal-Wallis tests (with a post hoc test) because the variables had a non-normal distribution, according to the normality tests. Chi-square and Fisher's exact tests were used to assess the differences between categorical variables.

RESULTS

The mean age of 32 patients with SSc (31 female, one male) was 48.93±12.77. The median disease duration was 24 months (min-max: 3-216), and the mean age at diagnosis was 45.21±12.84 (**Table 1**).

Normal capillaroscopy findings were found in 6.3% of the patients. Capillaroscopic examination findings of the patients are given in **Table 2**. Capillary loss was detected in 53% of the patients, and digital ulcer (p=0.006), dcSSc (p=0.003), telangiectasia (p=0.031), pulmonary hypertension (p=0.006), and GIS involvement (p<0.001) were more common in those with capillary loss.

The comparison of scleroderma pattern groups revealed no difference in age (p=0.224) and age at diagnosis (p=0.677), but disease duration was shorter in the early pattern group (p=0.005). Although groups were not different regarding the presence of the Raynaud phenomenon (p=0.819), the duration and severity of the Raynaud phenomenon were lower in the early pattern group (p=0.004, and p=0.009, respectively). The digital ulcer was more common in the late pattern group (p=0.011). Mean mRSS was lower in the early pattern group (p=0.001). The dcSSc (p=0.016) was more common in the late pattern. The percentage of interstitial lung disease (p=0.274), renal

disease (p=0.470), and six-minute walking test (p=0.335) were similar between the capillaroscopy patterns. The percentage of pulmonary hypertension (p=0.011) and GIS involvement (p<0.001) were higher in the late pattern group. The percentage of arthralgia was higher in the late pattern group (p=0.027), but arthritis was not (p=0.254). Fatigue (p=0.575), pain (p=0.536), PGA (p=0.861), HAQ (p=0.164) and DHI (p=0.064) scores were not different between the capillaroscopy pattern groups (**Table 3**).

Table 1. Demographic and clinical characteristics of the patients

Variable	
Female (n, %)	31 (96.9)
Age (mean±SD)	48.93±12.77
Body Mass Index, kg/m2, (mean±SD)	29.40±5.79
Smoker (n, %)	7 (21.9)
Duration of disease, months, (median, min-max)	24 (3-216)
Age at diagnosis, year, (mean±SD)	45.21±12.84
Raynaud phenomenon (n, %)	30 (93.8)
Duration, months, (median, min-max)	24 (3-192)
Severity, VAS, (mean±SD)	6±2.21
Digital ulcers (n, %)	6 (18.8)
Severity, VAS, (mean±SD)	4.3±2.16
History of digital ulcers (n, %)	11 (34.4)
mRSS (median, min-max)	6.5 (0-29)
Diffuse cutaneous SSc (n, %)	8 (25.0)
Limited cutaneous SSc (n, %)	17 (53.1)
Telangiectasia (n, %)	5 (15.6)
Calcinosis (n, %)	3 (9.4)
Interstitial lung disease (n, %)	11 (34.4)
Pulmonary hypertension (n, %)	6 (18.8)
Cardiac disease (n, %)	0
Renal disease (n, %)	1 (3.1)
Gastrointestinal involvement (n, %)	8 (25.0)
Arthralgia (n, %)	21 (65.6)
Arthritis (n, %)	2 (6.3)
Myositis (n, %)	0
Tendon friction rub (n, %)	0
Fatigue, VAS, (mean±SD)	4.29±3.37
Pain, VAS, (median, min-max)	2 (0-9)
PGA, VAS, (mean±SD)	5.09±1.93
HAQ (median, min-max)	0.42 (0-1.35)
DHI (median, min-max)	3 (0-66)

SSc: Systemic Sclerosis, VAS: Visual Analogue Scale, mRSS: modified Rodnan skin scores, PGA: Patient global assessment, HAQ: Health Assessment Questionnaire, DHI: Duruoz Hand Index.

Table 2. Capillaroscopic examination findings of the patients

Variable	
Hemorrhages (n, %)	22 (68.8)
Enlarged/dilated capillaries (n, %)	19 (59.4)
Giant capillary (n, %)	24 (75.0)
Avascular area (n, %)	17 (53.1)
Disorganization of capillary architecture (n, %)	17 (53.1)
Neoangiogenesis (n, %)	3 (9.4)
Patterns	
Normal/non-specific pattern (n, %)	2 (6.3)
Early scleroderma pattern (n, %)	12 (37.5)
Active scleroderma pattern (n, %)	13 (40.6)
Late scleroderma pattern (n, %)	5 (15.6)

Table 3. Comparisons of patients between capillaroscopic pattern groups

	Normal/ Early Pattern N=14	Active Pattern N=13	Late Pattern N=5	P value
Age	45 (30.5-58)	52 (45.5-60.5)	54 (48.5-60)	0.224
Female	13 (92.9)	13 (100)	5 (100)	0.515
Body Mass Index	27.5 (20.7-32.5)	31.2 (26.6-34.5)	32.4 (23.9-35.4)	0.385
Smoking	3 (23.1)	4 (30.8)	0 (0)	0.458
Disease duration	12 (7-19.5)	48 (24-72)	60 (30-174)	0.005
Age at diagnosis	44 (27.7-56.5)	47 (37-57)	46 (43-50)	0.677
Raynaud phenomenon	13 (92.9)	12 (92.3)	5 (100)	0.819
Duration	12 (5-24)	48 (24-99)	132 (36-186)	0.004
Severity, VAS	4 (4-5.5)	7 (3.5-8)	8 (7.5-8.5)	0.009
Digital ulcers	0	3 (23.1)	3 (60)	0.011
Severity, VAS	0	4 (3-7)	3 (2-7)	0.500
History of digital ulcers	0	6 (46.2)	5 (100)	<0.001
mRSS	2 (0-5.5)	7 (4-11)	17 (9.5-26.5)	0.001
Diffuse cutaneous SSc	0	5 (38.5)	3 (60)	0.016
Limited cutaneous SSc	8 (57.1)	7 (53.8)	2 (40)	
Telangiectasia	0	3 (23.1)	2 (40)	0.067
Calcinosis	0	2 (15.4)	1 (20)	0.264
Interstitial lung disease	3 (21.4)	5 (38.5)	3 (60)	0.274
Pulmonary hypertension	0	3 (23.1)	3 (60)	0.011
Cardiac disease	0	0	0	
Renal disease	0	1	0	0.470
Gastrointestinal involvement	0	3 (23.1)	5 (100)	<0.001
Arthralgia	12 (85.7)	5 (38.5)	4 (80)	0.027
Arthritis	2 (14.3)	0	0	0.254
Myositis	0	0	0	
Fatigue, VAS	3.5 (0-7.5)	5 (1.2-7.7)	4 (3-7)	0.575
Pain, VAS	3.5 (0-7.5)	0.5 (0-6.5)	1 (0-5)	0.536
PGA, VAS	5 (3.7-6.2)	5.5 (4.2-6)	5 (3.5-7)	0.861
HAQ	0.25 (0-0.5)	0.5 (0.2-0.7)	0.55 (0.2-0.8)	0.164
DHI	1.5 (0-6)	4 (1-16.5)	6 (4-12.5)	0.064

SSc: Systemic Sclerosis, VAS: Visual Analogue Scale, mRSS: modified Rodnan skin scores, PGA: Patient global assessment, HAQ: Health Assessment Questionnaire, DHI: Duruoz Hand Index.

DISCUSSION

The present study identified the frequency of scleroderma-type capillaroscopic patterns and the associations of capillaroscopic patterns with clinical features and functional status in SSc patients.

Anormal capillaroscopic examination findings were detected in 93.7% of the patients, and the most common capillaroscopic pattern was the active pattern in our study. Similarly, scleroderma-type patterns have been reported to be present in more than 90% of SSc patients with clinically significant disease (2).

In the present study, disease duration, duration and severity of Raynaud phenomenon, and mRSS were lower in the early pattern group. However, digital ulcers, dcSSc, and arthralgia were more common in the late pattern group. Although there were no differences in interstitial lung disease and the six-minute walking test between the groups, pulmonary hypertension and GIS involvement were more common in patients with late capillaroscopy pattern. Our study results are in line with data on the associations of NVC patterns with organ involvement in SSc. Previous reports have found capillaroscopy to be useful in detecting patients with more severe disease who will have new involvement in the future, and there is an increased risk for moderate to severe organ involvement in SSc patients with late pattern (3,11,18-20).

In our study, the capillary loss was detected in 53% of the patients, and digital ulcer, pulmonary hypertension, and GIS involvement were more common in those with capillary loss. Similarly, a previous study found capillary loss as a risk factor for new digital ulcers (21). Nailfold capillary density was also associated with pulmonary arterial hypertension in another study (22).

Additionally, we found no significant differences in fatigue, pain, PGA, HAQ and DHI scores between the SSc patients regarding NVC patterns. Impaired hand function was identified as the major contributor to overall disability in SSc patients (23,24). A previous study found that patients with early dcSSc had a high burden of disability, fatigue, and pain (23). Other studies reported that patients with dcSSc had poorer hand functions (25), and in early dcSSc, the degree of hand impairment correlated with skin thickening (23). Digital ulcers have been identified as one of the disease manifestations affecting hand function (26).

On the other hand, to the best of our knowledge, there is no data revealing the relationship between NVC patterns and function, pain, and fatigue in SSc patients. The fact that there was no difference between the scleroderma type capillaroscopic patterns in terms of function, fatigue, and pain in our study may be due to the small sample size and needs to be confirmed in larger epidemiologic studies.

CONCLUSION

NVC provides valuable data on the diagnosis, activity, and prognosis of SSc. Digital ulcers, pulmonary hypertension, and GIS involvement are more common in SSc patients with late scleroderma-type capillaroscopic pattern and capillary loss. Since hand function is an important determinant of physical function in patients with SSc, it should be determined by further studies whether this can be predicted with capillaroscopy findings.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Marmara University Faculty of Medicine Clinical Researches Ethics Committee (Date: 06.01.2017, Decision No: 09.2017.095).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

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

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Original Article

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The mortality predictors in non-vaccinated COVID-19 patients

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ABSTRACT

Aim: The novel coronavirus (SARS-CoV-2) causes COVID-19 disease. From December 31, 2019, to the present (July 2022), 545 million new cases have been detected, while the number of deaths due to the disease has reached 6.3 million. This study aims to reveal mortality-related risk factors, including comorbid conditions, clinical course, imaging, and laboratory parameters in COVID-19 patients hospitalized in a tertiary hospital.

Material and Method: An observational, retrospective study was conducted among hospitalized COVID-19 patients at the tertiary health center in Turkey between November 2020 and April 2021. A total of 601 patients were treated in this period and vaccinated 85 patients were excluded. The remaining 516 patients' demographical data, clinical severity, laboratory parameters, thorax computed tomography (CT) involvement, and mortalities were recorded.

Results: In evaluating the factors affecting COVID-19 mortality, it was observed that male gender and advanced age were significantly associated with mortality, and the mortality rate was higher in patients with involvement in thorax CT and patients with a clinically severe course. In the evaluation of the patients in terms of comorbidities, DM, HT, and CAD were significantly higher in the patients who died. It was determined that patients who died during hospitalization needed respiratory support at a higher rate.

Conclusion: Identifying predicting factors is essential for the early recognition the vulnerable patients for hospitalization decisions and early aggressive treatment. In this study, male gender, advanced age, comorbidities (DM, HT, CAD), pulmonary involvement, and severe clinical presentation were identified as significantly related factors associated with mortality.

Keywords: COVID-19, mortality, risk factors

INTRODUCTION

The novel coronavirus (SARS-CoV-2) causes COVID-19 disease. From December 31, 2019, to the present (July 2022), 545 million new cases have been detected, while the number of deaths due to the disease has reached 6.3 million. While 15 million new patients have been seen in Turkey since the beginning of the pandemic, the number of deaths due to the disease has reached ninety-nine thousand (1). Many studies have been conducted on the risk factors that cause mortality in COVID-19 patients. The most important risk factors in these studies were hypertension (HT), diabetes mellitus (DM), obesity, cardiovascular diseases (CAD), chronic obstructive pulmonary disease (COPD), and malignancies. Male gender and advanced age are other risk factors found in studies (2-5). In a multicenter study in Turkey, risk factors affecting mortality were advanced age, male gender, concomitant malignancy, and interstitial lung disease. In the same study, when laboratory values were examined, high blood urea nitrogen (BUN), lactate dehydrogenase (LDH), c-reactive protein (CRP), d-dimer, procalcitonin, neutrophil count, and low albumin and lymphocyte levels were associated with mortality (6). Romero-Gameros et al. (7) found a significant relationship between mortality and higher d-dimer, ferritin, LDH, and CRP levels.

This study aims to reveal mortality-related risk factors, including comorbid conditions, clinical course, imaging, and laboratory parameters in COVID-19 patients hospitalized in a tertiary hospital.

MATERIAL AND METHOD

An observational, retrospective study was conducted among hospitalized COVID-19 patients at the tertiary health center in Turkey between November 2020 and April 2021 after Ondokuz Mayıs University Clinical Researchs Ethics Committee approval (Date: 25.06.2021, Decision No: 2021/336). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration

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of Helsinki. Inclusion criteria for patients: 18 and older with a positive real-time polymerase chain reaction (RT-PCR) test; complete laboratory results are needed for the study protocol. A total of 601 patients were treated in this period and vaccinated 85 patients were excluded. The remaining 516 patients' demographical data, clinical severity [non-severe, severe (sPO2<90%, respiratory rate >30/min, signs of severe distress), and critical (requires life-sustaining treatment, acute respiratory distress syndrome, sepsis, septic shock)] (8) laboratory parameters (leukocyte, neutrophil, monocyte, platelet counts, hemoglobin levels, C reactive protein (CRP), D-dimer, ferritin, aspartate aminotransferase (AST), alanine transaminase (ALT), gamma-glutamyl transferase (GGT), lactate dehydrogenase (LDH), and procalcitonin levels), thorax computed tomography (CT) involvement, and mortalities were recorded. The patients were divided into two groups survivors and non-survivor. We compared the acquired data between the groups.

RESULTS

In the evaluation of the factors affecting COVID-19 mortality, it was observed that male gender and advanced age were significantly associated with mortality, and the mortality rate was higher in patients with involvement in thorax CT and patients with a clinically severe course. In the evaluation of the patients in terms of comorbidities, DM, HT, and CAD were significantly higher in the patients who died. It was determined that patients who died during hospitalization needed respiratory support at a higher rate (**Table 1**).

Table 1. The evaluation of demographics, comorbid conditions, and ventilation support on mortality

	Survivor (n=315)	Non-survivor (n=201)	p
Gender (Male), n (%)	167 (53.0)	127 (63.1)	0.023
Age (years)	58.69 ± 14.96	69.03 ± 12.64	0.000
Thorax CT (involved), n (%)	292 (92.6)	198 (98.5)	0.003
Clinical condition (Severe), n (%)	123 (39.0)	175 (87.0)	<0.001
Comorbidities			
DM, n (%)	81 (25.7)	72 (35.8)	0.014
HT, n (%)	128 (40.6)	120 (59.7)	<0.001
CAD, n (%)	54 (17.1)	65 (32.3)	<0.001
COPD, n (%)	22 (6.9)	22 (10.9)	0.116
Asthma, n (%)	22 (6.9)	15 (7.4)	0.837
Malignancy, n (%)	32 (10.1)	28 (13.9)	0.192
Smoking, n (%)	217 (68.8)	134 (66.6)	0.598
Ventilation Support			
No MV, n (%)	238 (75.5)	58 (28.8)	<0.001
HFNO, n (%)	66 (20.9)	92 (45.7)	<0.001
NIMV, n (%)	7 (2.2)	15 (7.4)	0.004
Intubation, n (%)	4 (1.2)	36 (17.9)	<0.001

CT: computerized tomography, DM: diabetes mellitus, HT: hypertension, CAD: coronary artery disease, COPD: chronic obstructive pulmonary disease, MV: mechanical ventilation, HFNO: high-frequency nasal oxygenation, NIMV: non-invasive mechanical ventilation

In the evaluation of the effect of laboratory findings on mortality, it was observed that leukocyte and neutrophil levels were higher in patients who died. In contrast, lymphocyte count and hemoglobin level were significantly lower. It was noted that inflammation markers such as ferritin, d-dimer, CRP and procalcitonin, and AST and LDH levels were significantly higher in the mortal group (**Table 2**).

Table 2. The evaluation of laboratory values on mortality

	Survivor (n=315)	Non-survivor (n=201)	p
WBC (/μL)	7,380.21±3,927.33	9,457.11±5,949.47	0.001
Neutrophil (/μL)	5,637.71±3,646.41	7,829.2±5,586.82	0.000
Lymphocyte (/μL)	1,166.86±682.74	984.93±1,017.11	0.000
Hemoglobin (g/dL)	12.69±2.14	12.06±2.23	0.005
Monocyte (/μL)	505.4±775.82	565.32±1,162.17	0.826
Platelet (10^3/μL)	211.54±97.42	200.42±90.57	0.100
AST (U/L)	41.88±39.45	53.64±64.43	0.015
ALT (U/L)	35.64±43.24	35.57±39.37	0.734
GGT (U/L)	56.45±83.34	65.68±115.64	0.140
LDH (U/L)	377.63±192.11	577.8±404.92	0.000
Ferritin (ng/mL)	585.91±691.36	1,277.19±1,888.57	0.000
CRP (mg/L)	81.68±77.56	137.85±106.61	0.000
D-dimer (ng/mL)	1,467.16±2,100.42	3,391.62±3,454.3	0.000
Procalcitonin (ng/mL)	0.49±4.23	3.85±12.16	0.000

WBC: white blood cells, CRP: c-reactive protein, AST: aspartate transaminase, ALT: alanine transaminase, GGT: gamma-glutamyl transferase, LDH: lactate dehydrogenase

In the study, logistic regression analysis was performed to determine the risk factors affecting the mortality of COVID-19 patients. Patient's age, gender, comorbid diseases (DM, HT, CAD, COPD, Asthma, Malignancy), presence of involvement in thorax CT, clinical severity of the disease, need for mechanical ventilation, WBC, neutrophil, lymphocyte, monocyte, hemoglobin, thrombocyte, CRP, d-dimer, ferritin, AST, ALT, GGT, LDH, and procalcitonin levels were primarily analyzed by univariate logistic regression analysis. Age, gender, comorbid diseases (DM, HT, CAD), presence of involvement in thorax CT, clinical severity of the disease, need for mechanical ventilation, WBC, neutrophil, lymphocyte, hemoglobin, CRP, d -dimer, ferritin, AST, LDH and procalcitonin levels were included in the multivariate logistic regression analysis. In the multivariate logistic regression analysis, the variable selection was performed using the forward addition method. In the final step, the variables found to be significant in the model were age, clinical severity, NIMV, intubation, hemoglobin, ferritin, and LDH levels (**Table 3**). In the final step, it was determined that each unit's increase in age, ferritin, and LDH levels in the model increased mortality by 1.074, 1.001, and 1.002 times, respectively, and each unit's increase in hemoglobin level decreased mortality by 11%. Clinical severity, NIMV, and intubation increased the mortality risk by 7.37, 4.09, and 6.47 times, respectively.

Table 3. Logistic regression analysis

	Wald	p	OR	95% CI for EXP(B)	
				Lower	Upper
Age	51.600	0.000	1.074	1.054	1.096
Clinical Severity	47.780	0.000	7.367	4.182	12.978
NIMV	6.219	0.013	4.091	1.352	12.380
Intubation	8.728	0.003	6.473	1.875	22.344
Hemoglobin	4.171	0.041	0.887	0.791	0.995
Ferritin	12.615	0.000	1.001	1.000	1.001
LDH	10.076	0.002	1.002	1.001	1.003

NIMV: non-invasive mechanical ventilation, LDH: lactate dehydrogenase
Model χ^2= 256.432; p<0.001, Hosmer and Lemeshow Test: p=0.699

DISCUSSION

Our study revealed that male gender and advanced age were significantly effective in mortality. The mortality rate was higher in patients with pulmonary involvement and severe clinical course. Also, DM, HT, and CAD were effective comorbidities in mortality. Additionally, while leukocyte and neutrophil counts were higher, lymphocyte count and hemoglobin levels were significantly lower in dead patients. High inflammation markers such as ferritin, d-dimer, CRP, procalcitonin, and AST, LDH levels were associated with mortality.

Male sex and advanced age were frequently reported predictors of mortality. Zhou et al. (9) reported that advanced age, severe disease, and high levels of D-dimer were associated with the risk of in-hospital death. The UK OpenSAFELY study (3) also reported that increasing age, male gender, and comorbidities such as diabetes, severe asthma, liver disease, and kidney disease were associated with high mortality risk. A nationwide retrospective large cohort in Turkey also reported that older age, male sex, and severe disease were independent predictors of mortality (6). Jin et al. (10) also found that male gender and increased age were related to severe disease and mortality. The present study also demonstrated that male sex and advanced age were significantly associated with mortality in hospitalized COVID-19 patients. Age-related conditions such as comorbidities and frailty can affect disease progression. Additionally, aging affects the proper functioning of the adaptive and innate immune system, which can lead to vulnerability to several infections.

Yuan et al. (11) reported that patients with pulmonary involvement have a higher mortality rate. A meta-analysis including 7,106 COVID-19 patients also showed that thorax CT involvement in these patients could predict mortality (12). In accordance with the literature, our results showed a higher mortality rate with pulmonary involvement.

A meta-analysis, including 61 cohort studies with 31,089 patients about the negative impact of comorbidities on COVID-19, reported chronic kidney disease, cardiovascular disease, cerebrovascular disease, COPD, HT, malignancy, DM, and immunodeficiency were associated with increased risk of mortality (5). A large data set with 331,928 positive COVID-19 patients from Mexico analyzed that DM, obesity, HT, COPD, CKD, and immunocompromised patients were at greater risk for mortality (13). Similarly, in the current study, DM, HT, and CAD were significantly associated with mortality.

In many investigations; decreased white blood cell, platelet count, and increased d-dimer, AST, urea, creatinine, and LDH were associated with mortality (14-17). Romero-Gomeros et al. (7) reported that high levels of d-dimer, LDH, and CRP levels were related to mortality as in our study in which we observed CRP, d-dimer, LDH, and procalcitonin levels were significantly higher in the mortal group.

The exclusion of vaccinated patients, an essential factor influencing mortality, may represent the strength of our study. In contrast, retrospective design and the relatively small sample size in a single center may represent the limitations of the current study.

CONCLUSION

Identifying predicting factors is essential for the early recognition of vulnerable patients for hospitalization decisions and early aggressive treatment. In this study, male gender, advanced age, comorbidities (DM, HT, CAD), pulmonary involvement, and severe clinical presentation were identified as significantly related factors associated with mortality.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Ondokuz Mayıs University Clinical Researches Ethics Committee (Decision No: 2021/336).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and they have approved the final version.

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Original Article

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Comparison of the effects of hydroxyethyl starch and succinylated gelatin infusion on the perfusion index in elective caesarean sections under spinal anaesthesia

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ABSTRACT

Aim: This study is to compare the alterations of three different replacement fluids on Perfusion Index, Pleth Variability Index (PI, PVI) and hemodynamic data in cases planned to experience caesarean surgery under spinal anaesthesia.

Material and Method: 94 ASAII class patients aged 18–40 that were planned to experience caesarean surgery were included in the study. The patients were divided into three groups according to the fluid replacement to be applied. Patients in Group H received 10 ml/kg of hydroxyethyl starch (HES) up to a maximum of 500 ml over 20 minutes. Patients in Group G got 10 ml/kg of modified liquid gelatin(GEL) up to a maximum of 500 ml over 20 minutes. Patients in Group I got 20 ml/kg of isotonic sodium chloride (0.9% NaCl) over 20 minutes. Routine monitoring and perfusion index, pleth variability index were recorded baseline and at the first, third and tenth min after spinal anaesthesia for all participants

Results: A significant increase in the PI value over time was observed in Groups G and I (p=0.001*). According to the PVI results, the amount of decrease in Group G was statistically less than in the other two groups (p=0.015*).

Conclusion: In conclusion, 0.9% NaCl and gelatine were more effective on PI in caesarean section under spinal anesthesia. Isotonic has a positive effect on both PI and PVI. We detected that PI increased compared to baseline values, and believe that this increase may a positive effect on tissue circulation in the patient.

Keywords: Hydroxyethyl starch, succinylated gelatin, perfusion index

INTRODUCTION

Spinal anaesthesia is the opted anaesthesia method in caesarean surgeries because it eliminates the potential risks associated with airway management in pregnant women (1). Hypotension result of spinal anaesthesia during caesarean section occurs due to reduced vascular resistance from sympathetic blockade. Reduced cardiac output causes blood pooling formation in plugged regions of the body (2). The incidence and severity of hemodynamic instability can be reduced by tilting the patient in a left lateral position and applying various techniques, including vasopressor drug administration, manual uterine displacement maneuvers, and preloading with crystalloid or colloid (3,4). Crystalloid and colloid solution loadings from these applications can effectively normalise blood volume and arterial blood pressure (5). The PI is calculated as the ratio of pulsatile blood flow to nonpulsatile flow in the peripheral extremity and is utilized as a rapid indicator of microcirculatory changes. It provides continuous information about tissue perfusion in a non-invasive manner. Because of its ease of use, it has become the preferred hemodynamic monitoring method for patient follow-up (6). PI can be described as the rate of nonpulsatile current (AC) to pulsatile flux (DC) in the capillary area. PI (%)=(AC: DC) x 100 using the maximum and minimum PI, PVI can be calculated as follows. PVI (%)= [(PI max - PI min) / PI max] x 100 provides dynamic automatic assessments in the course of the respiratory period. It is used via a non-invasive, finger/ear connected oximetry probe (7). In addition, PVI has been demonstrated as a firm indicator of hypotension in anaesthesia induction (8) and can be used as a guide for maintaining fluid response in mechanically ventilated cases (9). However, in spontaneously breathing cases, the determination of fluid response transforms difficult as tidal volume and respiratory frequency differ (10)Primary the present research is aimed to compare the alterations of three different replacement fluids on PI, PVI and hemodynamic

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data in cases planned to experience caesarean surgery under spinal anaesthesia. Secondarily, the replacement fluids we gave were to provide hemodynamic stability and to determine the amount of increase in blood PI.

MATERIAL AND METHOD

The study was carried out with the permission of Malatya Turgut Özal University Clinical Researches Ethics Committee (Decision No: 2021/65). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. Clinical study was planned prospectively.

A total of 94 ASA II class patients aged 18–40 that were planned to experience caesarean surgery were included in the study. The number of patients was planned based on the number of patients in similar studies (11-13). The emergency cases, aged smaller than 18 or over 40 years, gestational aged smaller than 36 weeks or over 41 weeks, the value of body mass index (BMI) ≥ 40, refusal to participate, presence of placenta previa, preeclampsia, cardiovascular disease, Raynaud's disease, fetal complications, or contraindications to spinal anesthesia were created as exclusion criteria.

Patients who developed insufficient block and perioperative hypotension were excluded from the study.. All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki. Written informed consent was obtained from all participants who participated in this study. The patients were divided into three groups according to the fluid replacement to be applied. All participants fasted for at least 8 hour In the operation room venous access was maintained and standard monitoring electrocardiography (ECG), partial oxygen saturation (SpO_2), non-invasive blood pressure (NIBP) was applied. Basal systolic arterial pressure (SAP), mean arterial pressure (MAP), as well as diastolic arterial pressure (DAP), heart rate (HR), and SpO_2 were recorded. A Masimo Radical 7 pulse oximeter probe (Masimo Corp., Irvine, CA, USA) was connected to the forefinger of the right hand and protected from light. Then, PI, PVI, peripheral blood oxygen content (SPOC) (mL/dL) and peripheral blood haemoglobin (SpHb) (g/L) were recorded. Patients in Group H received 10 ml/kg of hydroxyethyl starch (Corn-based 130/0.4 hydroxyethyl starch, sodium chloride (HES)) up to a maximum of 500 ml over 20 minutes. Patients in Group G got 10 ml/kg of modified liquid gelatin (500 ml solution 20.0 g succinylated gelatin, 3.505 g sodium chloride (GEL)), up to a maximum of 500 ml over 20 minutes. Patients in Group I got 20 ml/kg of isotonic sodium chloride (0.9% NaCl) over 20 minutes.

Fluids were planned as infusions over 20 min and ended at the 10th minute following spinal anaesthesia. The patients were set in a sitting position, and 12–15 mg of hyperbaric bupivacaine was managed by reaching the subarachnoid area with a 25 G Quincke spinal needle from the L4–L5 space. Following the application, the patients were placed on their backs. The sensory block levels were evaluated. The patients SAB, MAP, DAP, SpO_2, HR, PI, PVI, SPOC and SpHb were recorded at the first, third and tenth min after spinal anaesthesia. A reduction of more than 30% from the basal rate or a reduction below 90 mm Hg was considered hypotension.Data analysis was carried out using the IBM SPSS version 26.0 statistical programme (Chicago, IL, USA). Skewness and kurtosis values were utilized to evaluate the normality distribution. Descriptive data were presented as mean and standard deviation values for the quantitative variables. The **demographic data** of the groups were compared with a one-way analysis of variance (ANOVA). A mixed-design ANOVA was utilized to test for significant differences among the groups with repeated measurements. Mauchly's test was used to test the sphericity assumption, and MANOVA was also used. The Duncan test was chosen as a between-group post hoc test, and the Bonferroni correction was used for confidence interval adjustment. A p value of <0.05 was accepted as significant.

RESULTS

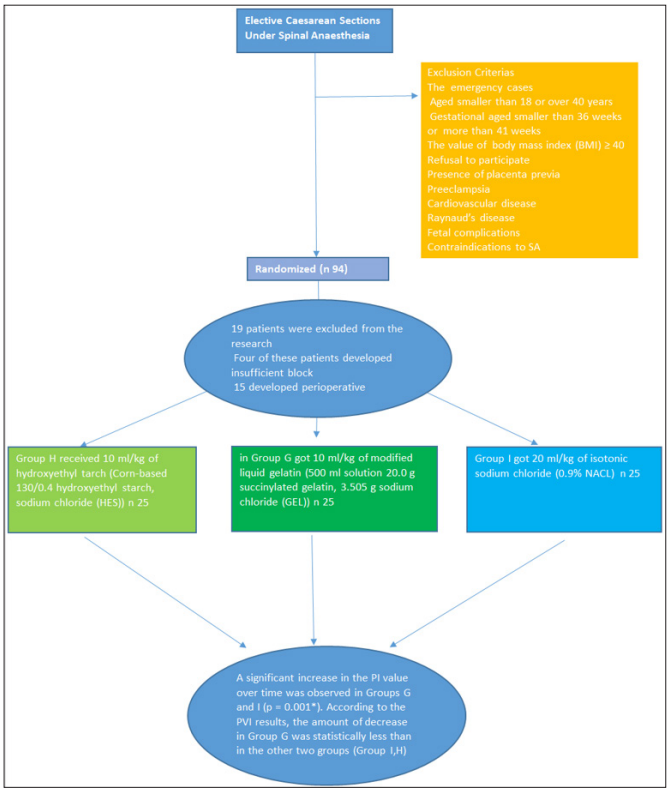
Seventy-five ASA II class cases were included. In total, 19 patients were excluded from the research. Four patients developed insufficient block, and 15 developed perioperative hypotension.

The age was determined as mean±standard deviation (30.32±4.779). Patient BMI was determined as mean ± standard deviation (29.4278±6.36174). Demographics were similar in three groups. A significant increase in the PI value over time was observed in Groups G and I (p=0.001*). A statistically significance was observed

Demographics Datas					
	Group G Mean±Std.deviation (n=25)	Group I Mean±Std.deviation (n=25)	Group H Mean±Std.deviation (n=25)	TOTAL Mean±Std.deviation (n=75)	P value
Age	29.12±4.333	31.68±5.266	30.16±4.525	30.32±4.779	0.164
Height (cm)	163.80±6.357	160.60±10.966	157.64±5.634	162.01±8.013	0.359
Weight (kg)	76.12±8.136	79.60±12.007	73.84±10.032	76.52±10.322	0.139
BMI	28.4494±3.41831	31.6228±9.79918	28.2111±3.08570	29.4278±6.36174	0.105
BMI. Body Mass Index					

between the groups for the third and fourth measurements (p=0.029*). Group H values were more stable and lower than those of the other two groups. The PI values showed more variation and were increased in Groups G and I compared to Group H (**Table 1**) The between-group evaluation of PI showed significant differences between Groups I and H for the third measurement (p=0.028*) and between Groups G and H for the fourth measurement (p=0.007*) (**Figure 1**). When PI values were evaluated according to time, a significance was obtained between the second and third measurements in Group G (p=0.001) and between the second and third evaluations in Group I (p=0.005*). In Group H, no significance was obtained in terms of time.When the change in PVI was evaluated according to time, a significant decrease was observed (p=0.001*). According to the PVI results, the amount of decrease in Group G was statistically less than in the other two groups (p=0.015*) (**Figure 2**). When PVI was evaluated among the groups, there was a statistical significance was observed between Group G and Group I in the second measurement (p=0.026*). When evaluated in terms of time, there was no difference in Group G, whereas in Group I, there was a difference in the second and third measurements (p=0.014*) and the third and fourth measurements (p=0.012*). A significance was found among the first and second measurements in Group H (p=0.027*) (**Table 2**).

Examination of SAP, DAP, MAP, HR, SPOC and HGB



Flow Chart

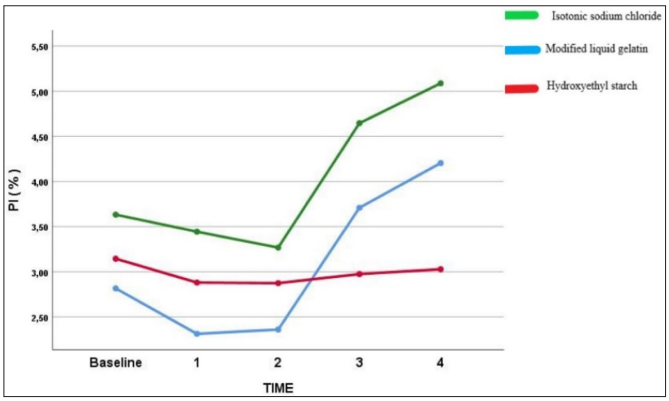


Figure 1. Perfusion Index Between Group

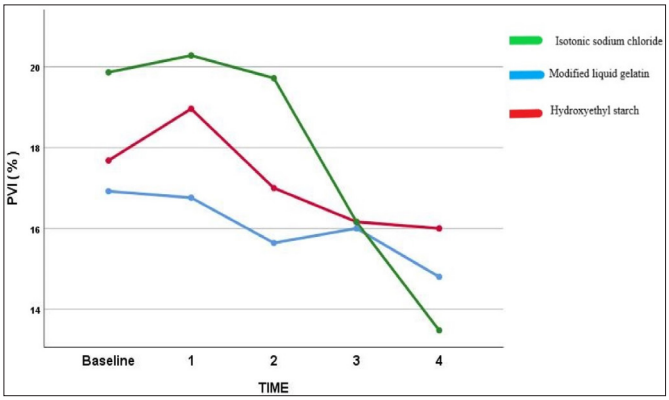


Figure 2. Pleth Variability Index Between Groups

Table 1. Perfusion index value

Perfusion Index	Group G	Group I	Group H	Total	Main effect		Interraction effect
	Mean±Std.deviation (n=25)	Mean±Std.deviation (n=25)	Mean±Std.deviation (n=25)	Mean±Std.deviation (n=75)	Time	Group	
Baseline	2.816±1.25422	3.632±2.04037	3.144±2.02322	3.197±1.81711			
1st measurement	2.3124±1.36763	3.4440±1.75145	2.8800±1.86257	2.8788±1.71550			
2nd measurement	2.3596±1.13138	3.2680±2.07580	2.8736±2.04917	2.8337±1.82063	p=0.001*	p =0.029*	p=0.025*
3rd measurement	3.7088±2.20316	4.6460±2.24174	2.9740±2.02398	3.7763±2.23768			
4th measurement	4.204±2.29265	5.088±2.57331	3.028±1.72736	4.107±2.35408			

Source of Difference for Interaction (Group x Time) for Group		Source of Difference for Interaction (Group x Time) for Time	
Pairwise Comparison (Group)		**Pairwise Comparison (Time)**	
Baseline	-	Group G	(2-3) p=0.001*
1st measurement	(P=0,064)	Group I	(2-3) p=0.005*
2nd measurement	(P=0,211)	Group H	No significant difference
3rd measurement	P=0.028* (Group I - Group H) (Group G - Group H)	* There is a significant difference	
4th measurement	P=0.007* (Group G -C) (Group G - Group H)		

Table 2: Pleth variability index value

Pleth Variability Index	Group G	Group I	Group H	TOTAL	Main Effect		Interraction Effect
	Mean±Std. deviation (n=25)	Mean±Std.deviation (n=25)	Mean±Std. deviation (n=25)	Mean±Std. deviation (n=75)	Time	Group	
Baseline	16.92±6.670	19.864±6.667	17.680±5.422	18.155±6.321			
1st measurement	16.76±5.861	20.28±5.990	18.96±5.184	18.67±5.799			
2nd measurement	15.64±5.574	19.72±6.655	17.00±3.227	17.45±5.544	p =0.001*	p=0.015*	p=0.037*
3rd measurement	16.00±3.841	16.16±6.169	16.16±5.031	16.11±5.034			
4th measurement	14.80±5.354	13.476±4.551	16.000±4.282	14.759±4.800			

Source of Difference for Interaction (Group×Time) for Group		Source of Difference for Interaction (Group x Time) for Time	
Pairwise Comparison (Group)		**Pairwise Comparison (Time)**	
Baseline	-	Group G	No significant difference (p>0,05)
1st measurement	(p=0.094)	Group I	(2-3)=0.014 * . (3-4)=0.012*
2nd measurement	(Group G - Group I) p= 0.026*	Group H	(1-2)=0.027*
3rd measurement	(p=0.992)	* There is a significant difference	
4th measurement	(p=0.178)		

values revealed no significant difference among the groups. Results showed that changes according to time and hemoglobin values displayed no significance among the groups for all three replacement fluids (**Table 3**).

Table 3. Hemodynamic Value

M (measurement) B (Baseline)	Group G	Group I	Group H	TOTAL	Main Effect		Interraction Effect
	Mean±Std. deviation (n=25)	Mean±Std. deviation (n=25)	Mean±Std. deviation (n=25)	Mean±Std. deviation (n=75)	Time P value	Group	
SAP						p =0.664	p=0.387
B	120.84±12.392	123.40±9.857	121.80±17.923	122.01±13.661	-		
1st M	117.04±13.466	118.48±11.594	118.92±15.354	118.15±13.45	0.386		
2nd M	104.24±13.386	112.60±15.147	111.76±14.914	109.53±14.798	0.014		
3rd M	113.64±16.124	115.52±13.522	116.04±13.545	115.07±14.290	0.249		
4th M	113.24±12.417	114.84±14.141	107.88±14.855	111.99±13.695	0.995		
DAP						p=0.324	p =0.651
B	73.76±12.869	76.12±9.833	76.88±12.755	75.59±11.816	-		
1st M	72.64±12.767	72.48±11.917	72.72±11.141	72.61±11.798	0.637		
2nd M	61.28±14.965	68.32±12.750	65.88±14.406	65.16±14.188	0.163		
3rd M	64.88±11.245	68.76±9.658	70.60±10.809	68.08±10.720	0.628		
4th M	62.76±14.624	62.96±11.929	65.64±12.540	63.79±12.971	0.593		
MAP						p =0.523	p =0.472
B	87.36±13.181	90.80±8.134	90.68±13.050	89.61±11.646	-		
1st M	88.36±12.714	87.40±10.618	88.04±12.061	87.93±11.676	0.368		
2nd M	74.32±13.582	81.28±11.585	79.72±13.437	78.44±13.074	0.089		
3rd M	78.48±11.369	80.80±12.069	82.88±10.256	80.72±11.250	0.493		
4th M	79.40±13.054	77.16±13.015	107.88±13.478	88.15±13.053	0.452		
HR						p=0.186	p=0.870
B	92.72±12.908	96.60±12.332	98.72±15.085	96.01±13.543	-		
1st M	99.96±20.535	102.96±17.932	103.36±19.213	102.09±19.056	0.875		
2nd M	96.36±21.614	101.72±12.749	103.08±24.449	100.39±20.165	0.811		
3rd M	94.20±17.630	96.56±16.917	105.36±14.660	98.71±16.932	0.406		
4th M	132.72±11.096	97.08±13.823	101.12±17.624	110.31±14.376	0.356		
SPOC						p =0.764	p =0.501
B	15.60±1.683	15.60±1.803	15.36±1.729	15.52±1.719	-		
1st M	15.60±1.683	14.96±1.399	15.80±2.041	15.45±1.742	0.089		
2nd M	15.52±1.418	15.32±1.345	15.88±2.128	15.57±1.662	0.374		
3rd M	14.76±1.665	14.80±1.528	14.96±2.208	14.84±1.801	0.666		
4th M	14.796±1.528	14.680±2.0207	14.800±1.8868	14.759±2.0842	0.898		
SpHb						p=0.523	p=0.328
B	11.808±1.1751	11.640±.9354	12.008±1.4212	11.819±1.1873	-		
1st M	16.400±1.2747	11.516±1.0526	12.044±1.4933	13.320±1.2879	0.801		
2nd M	11.884±1.0562	11.424±1.1727	12.004±1.4490	11.771±1.2458	0.335		
3rd M	14.992±1.2339	14.756±1.2164	15.416±1.7781	15.055±1.4222	0.529		
4th M	11.496±1.2512	11.264±1.3610	11.220±1.4972	11.327±1.3603	0.100		

SAP Systolic Arterial Pressure, DAP Diastolic Arterial Pressure, MAP Mean Arterial Pressure, HR Heart Rate, SPOC Peripheral BloodOxygen Content, SpHb Peripheral Blood Haemoglobin.

DISCUSSION

Spinal anaesthesia is a proven method for caesarean section and has prevented common complications of general anaesthesia, including difficult intubation, rised risk of gastric acid aspiration and fetal hypoxia during pregnancy (14,15).A regular pregnancy is characterised by a reduction in systemic vascular resistance and an increase in total blood volume as well as cardiac output. In particular, after the thirtieth week of pregnancy, extra blood volume is retained in the extremities as an outcome of reduction in vascular tone caused by pregnancy (16). Despite its advantages (e.g., direct inception of activation and sufficient quality of both sensory and motor blockage), hemodynamic impairment following spinal anaesthesia for caesarean section remains a prevalent and severe complication (3).Hypotension induced by spinal anaesthesia due to reduced systemic vascular resistance and reduced cardiac output, mainly as a result of the preganglionic sympathetic fibres block (17).Crystalloid preloading before spinal anaesthesia procedure is suggested to decrease the ratio of hypotension, but its usefulness is controversial (18). Despite the controversy, prehydration with crystalloid or colloid is widely used prior to spinal anaesthesia. Marx and Wollman noted the significance of fluid infusion in counteracting the relative hypovolemia caused by spinal anaesthesia. Several fluids, such as crystalloids and colloids, have been utilized for preloading prior to spinal anaesthesia for caesarean procedure (19). Park et al. (20) examined the alterations of modified volumes of crystalloid application and their alterations on maternal hemodynamic and colloid osmotic pressure. They pointed out that the groups administered 20 and 30 ml/kg crystalloid indicated a greater decrease in maternal colloid osmotic pressure than the one administered 10 ml/kg crystalloid .

Mathru et al. (21) pointed out that the infusion of 5% albumin in 5% dextrose (15 ml/kg) in lactated ringers with left uterine displacement was an efficient way of preventing hypotension in the course of caesarean section under spinal anaesthesia. Crystalloid solutions classically have short half-lives and weak plasma volume-expanding effects. For these reasons, crystalloid preload may be insufficient to eliminate hypotension due to spinal anaesthesia. Crystalloid preloading at high volumes may additionally reduce oxygen-carrying ability and rise the risk of peripheral and pulmonary edema in pregnant women (22). Colloids that can remain in the circulation for a long time seem to be more suitable options for preventing hypovolemia due to spinal anaesthesia (23). However, any solution including artificial macromolecules such as dextran, HES or gelatin may carry an increased viscosity compared with plasma, based on the size and organization of these molecules and their exact concentration in the blood may induce increased plasma viscosity, especially following extended or recurrent utilization of dextran 40,000-60,000-70,000 and HES (22). Although these alterations are quite small and well below the values that occur physiologically in the late period, some authors have hypothesised that organ blood flow and tissue oxygenation may be compromised (24).In our study, we observed that PI values increased in all groups. Administration of 500 cc of colloid fluids did not impair the PI, and improvement was observed in the peripheral circulation as a result of volume replacementIn a study conducted in healthy volunteers, 1 L infusion of 4% succinylated gelatin in 0.7% saline and 6% HES in 0.9% saline, (over 1 h) had a significant effect on the blood volume expansion capacity, and no difference was observed (25). A novel human research showed that in the early stage following cardiac surgery, the influence of one dose of HES solution on cardiac index was superior to that of the gelatin-administered group (26). Rittoo and colleagues demonstrated that microvascular perfusion and oxygenation were sufficiently maintained with HES infusion than with Gelofusin (27). In another study, circulatory disorders were more common in patients who took gelatin than in those who took albumin (28). Peripheral PI alterations a respond to local blood volume pulsations and changes in intravascular pulse pressure; both of them are influenced by the elasticity of the vascular wall or vascular tone. Low PI peripheral vasoconstriction with or without severe hypovolemia, and high PI generally indicates dilation of peripheral blood vessels (29).The PVI maintains monitoring of continuous, noninvasive dynamic follow-up of circulating blood volume and has been pointed out to assess fluid resuscitation (30). PVI is a better predictor of the dynamic alterations in the PI that may occur in the course of respiratory cycles (31). The PVI is a beneficial technique due to its advantages, such as being noninvasive characteristic , utilizing a feasible to attach an sensor, and its providing of continuous bedside evaluation method (32). Coeckelenbergh et al. (33) pointed out that PVI was a better predictor for appropriate fluid therapy similarly to pulse pressure alterations due to the length of hospital stay and incidence of postoperative complications in cases, particularly following low-to-moderate-risk abdominal surgery.

Kumar et al. (34) defined changes in the PI value as an outcome of local vasoconstriction (low PI) or vasodilation (increased PI) in the skin. Anxiety that occurs before spinal anaesthesia causes alterations in blood pressure, PVI and PI due to an increase in sympathetic tone. To minimise this, we allowed patients to rest and supported them to remain calm prior to the measurement of hemodynamic variables. Sympathetic block levels are higher than sensory block levels during spinal anaesthesia.

There are some limitations to our study. First, the presence of spontaneous breathing and the patient's stress, anxiety and movement may have affected the PI values. In addition, increased sympathetic activation after spinal block and vasoconstriction in unblocked areas and changes with PI.

CONCLUSION

In conclusion, 0.9% NaCl and gelatine were more effective on PI in caesarean section under spinal anesthesia. Isotonic has a positive effect on both PI and PVI. We detected that PI increased compared to baseline values, and believe that this increase may a positive effect on tissue circulation in the patient.

ETHICAL DECLARATIONS

Ethics committee approval: The study was carried out with the permission of Malatya Turgut Ozal University Clinical Researches Ethics Committee (Decision No: 2021/65).

Informed consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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Concordance of histopathological and radiological grading in soft tissue sarcomas

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ABSTRACT

Aim: The grade of the tumor is essential for planning the treatment strategy in soft-tissue sarcomas (STS). The goal of this study is to determine magnetic resonance imaging features related to histopathological grade and aggressiveness of STS.

Material and Method: This retrospective single-center study involved preoperative contrast-enhanced MRI examinations of 64 patients with STS. MRI findings evaluated were; heterogeneity, necrosis, hemorrhage, and relationship with surrounding tissue in T1-weighted (T1W), T2-weighted (T2W), and T1W post-contrast sequences of the lesion. Histological grade was determined with the Fédération Nationale des Centres de Lutte Contre Le Cancer (FNCLCC) grading system, and the aggressiveness of the lesion was measured with the Ki-67 index.

Results: Sixty-four patients (mean age 45.5±21.6, M/F ratio 34/30) with STS were included. 33 (51.6%) patients graded as FNCLCC grade 3. On MRI examinations, the absence of necrosis was significantly associated with FNCLCC grade 1 and a low Ki-67 index ($p<0.001$). The presence of hemorrhage signal distinguished as a hyperintense signal on T1W, tail sign, and post-contrast peritumoral enhancement was significantly higher in FNCLCC grade 3 soft tissue sarcomas (p:0.008, p:0.001, p:0.004, respectively). The presence of peritumoral edema on T2W imaging in all high-grade patients also showed a strong relationship between these two (p:0.001).

Conclusion: Our study found that the presence of hemorrhage signal, tail sign, peritumoral enhancement, clear borders of 50% and less obtained from conventional MRI features of soft tissue sarcomas are associated with high grade tumors. The absence of necrosis signal, clear borders of 90% and above in MRI were significantly associated with FNCLCC grade 1.

Keywords: Magnetic resonance imaging, FNCLCC grading, soft-tissue sarcoma

INTRODUCTION

Soft tissue sarcomas (STS) are rare malignant tumors that are aggressive, with over 100 different histologic subtypes. They are of mesodermal origin with an incidence of 5/100000 per year. They can arise from any part of the body, but most occur in extremities, and the patients usually present with a painless enlarging mass. The most common ones include liposarcoma, leiomyosarcoma, and undifferentiated pleomorphic sarcoma (1). Tumors are grouped as adipocytic, fibroblastic, skeletal muscle, vascular, smooth muscle, pericyte, and uncertain differentiation category according to the WHO classification of soft tissue tumors (2).

Tumor grade is vital as high-grade tumors may benefit from neoadjuvant chemotherapy and radiotherapy for achieving local control after upcoming resection surgery. The histological grade of the tumor is shown to be strongly linked to the patient's risk of metastasis and overall survival (3-5). Also, proper diagnosis of the grade of the tumor is essential for planning the treatment strategy. A pre-treatment percutaneous needle biopsy is mandatory to diagnose a STS and determine its grade. However, biopsy results may be non-diagnostic as tissue specimens may not represent the whole; this mainly occurs where the tumor contains a mixture of low and high-grade areas (6-8).

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The histological grade of the tumor is determined according to the grading system of Fédération Nationale des Centres de Lutte Contre Le Cancer (FNCLCC), which considers tumor differentiation and mitotic activity and necrosis (9). However, the mitotic count, cell size, and tumor cellularity may be affected by the interval between the surgical resection and fixation of the specimen. The Ki-67 protein, which is encoded by the MKI67 gene, is a cellular marker for proliferation. The high Ki-67 count of STS suggests its high potential for reproducibility and aggressiveness (10,11).

Combining pre-treatment conventional magnetic resonance imaging (MRI) features of the lesion with histopathologic findings is necessary for the decision to repeat the biopsy (12-14). This may be required to find out the correct diagnosis and grade of the tumor, especially in regions where limited resources are available.

Precise determination of the grade of the STS has a critical role in planning an appropriate treatment strategy. MRI is vital for imaging the characteristics and anatomical details of the soft tissue. Although there are some studies in the English-speaking literature, the correlation between MRI features and histopathological findings of STS has still been assessed to a limited degree. This study aimed to determine the MRI features related to the FNCLCC histological degree and Ki-67 grading of STS in a tertiary reference oncology center in a developing country.

MATERIAL AND METHOD

The study was carried out with the permission of Istanbul Medeniyet University Göztepe Prof. Dr. Süleyman Yalçın City Hospital Noninvasive Clinical Researches Ethics Committee (Date: 30.06.2021, Decision No: 2021/0347). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

We retrospectively conducted this single-center study in our clinic. Inclusion criteria were as follows; patients diagnosed with STS between 2016 and 2021 had a baseline intravenous contrast-enhanced MRI study prior to any treatment, histopathologic grade assessment on core needle biopsy before any history of neoadjuvant therapy. Exclusion criteria were patients whose tumor grade was unclear due to insufficient pathological findings and patients without preoperative MRI. Sixty-four patients suited the criteria and baseline characteristics, and histopathological & MRI features were considered (**Figure 1**). Therapeutic and prognostic values have not been in concern of this study.

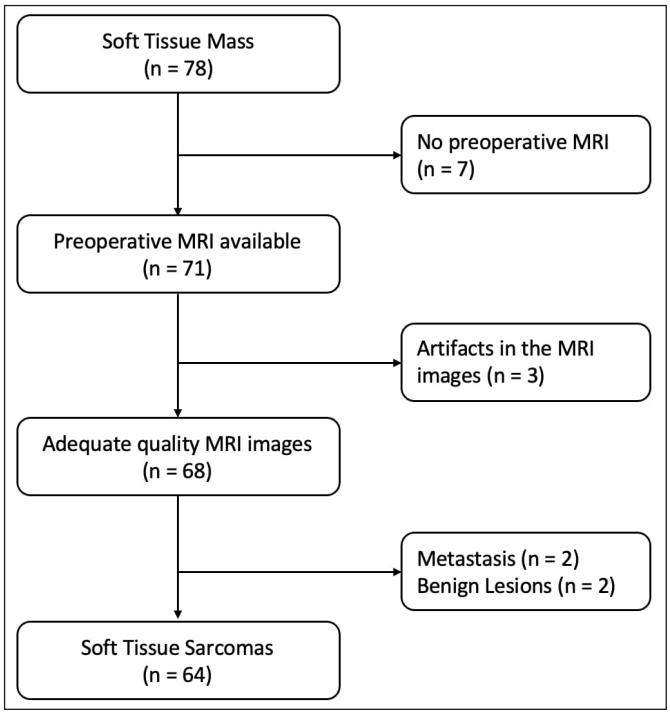

Figure 1. Flowchart of study (n = number of patients).

Baseline characteristics were noted as; age, sex, tumor location (extremity or trunk), and histological type of STS. For histopathological assessment, FNCLCC grade on core needle biopsy, Ki-67 index, necrosis ratio, and mitosis ratio on the specimen. MRI features are as follows; size, depth (deep, superficial, or both), heterogenous signal ratio (homogenous, <%50 or ≥%50 heterogenous), necrosis ratio (none, <%50 or ≥%50), presence of a hemorrhagic signal, border and sharpness (<50%, between 50% and 90%, >%90), tail sign (defined as thickening and enhancement of the aponeurosis with contrast), peritumoral edema, peritumoral enhancement, bone invasion, extension to vessel or nerve on T1, T2, and contrast-enhanced T1W images.

Single senior orthopaedic oncologist routinely performed the core needle biopsies from our institution (K.O., with 16 years of experience in percutaneous biopsy and surgery of STS) with a 14-16 gauge fully automatic core-needle biopsy device (Geotek Maxicore-M® Reusable Biopsy Gun, Ankara, Turkey). The MRI images were analyzed by a senior radiologist (BB, with 20 years of experience in soft-tissue imaging) and a radiology resident (MBD, with 3.5 years of experience in MRI, including 6-months of musculoskeletal rotation). These radiologists first recorded all data blind from the pathology results. The conflicting data was then finalized with a second consensus meeting. Histopathologic evaluation performed by two pathology experts in musculoskeletal oncology (TZ and ANT, over 10 years of experience in pathology).

MRI Protocol

This study was performed with two different MRI devices consisting of 1.5 Tesla GE Optima MR450w and MR360 (General Electric, Chicago, IL, USA). The MR imaging protocol basically consists of T1-weighted (T1W) sequence prior to contrast injection, T2-weighted (T2W) sequence with or without fat-suppression, DWI sequences and T1W sequence after contrast injection. Since the examined tumors were located in different localizations, the parameters were arranged for the relevant area and therefore varied. Repetition time and echo time intervals for T1W imaging were 450-720 ms and 7-12 ms, respectively. For T2W imaging, the same values were 2600-6900 ms and 82-120 ms. Section thicknesses vary between 3mm and 6mm. DWI images were acquired using b values of 0 and 800 s/mm².

Statistical Analysis

Number Cruncher Statistical System (NCSS 2007, Kaysville, Utah, USA) program was used for statistical analysis. While evaluating the study data, besides the descriptive statistical methods (Mean, Standard Deviation, Median, Frequency, Ratio, Minimum, Maximum), the distribution of the data was evaluated with the Shapiro-Wilk Test. Kruskal-Wallis test used in the comparison of quantitative data of three or more groups that do not show normal distribution; Mann-Whitney U Test was used to compare two groups that did not show normal distribution. Chi-square analysis was used to determine the relationship between qualitative data. Significance was evaluated at $p<0.01$ and $p<0.05$ levels.

RESULTS

The mean diameter of STS was measured as 90.1 mm in MRI evaluation. The mean age of sixty-four patients (M/F=34/30) was 45.5 (1.5-85). The majority (59/64) of these tumors were in the extremity. When examined for depth, only three were seen as both deep and superficial, all of which were high-grade tumors. Approximately half of the patients in the study were FNCLCC grade 3. The mean Ki-67 proliferation index value was 32.7 (**Table 1**).

The relationship between MRI findings and histopathological findings was examined in detail and summarized in **Table 2**. The ratio of heterogeneous signal area to tumor volume on T1W or T2W imaging was not associated with the aggressive behavior of the tumor. While the absence of necrosis signal in MRI findings was significantly associated with FNCLCC grade 1 (**Figure 2**) and low Ki-67 index (p:0.001), it was also shown that a ratio of more than 50% necrosis observed in contrast-enhanced T1 and T2W imaging to tumor volume was seen significantly in high-grade sarcomas (p:0.001).

Table 1. Baseline distribution of data of the population study

Characteristic	All Patients (n=64)	FNCLCC grade 1 (n=13)	FNCLCC grade 2 (n=18)	FNCLCC grade 3 (n=33)
Age (y)	45.5 ± 21.6 (1.5–85)			
Sex				
Men	34 (53%)			
Women	30 (47%)			
Size (mm)	90.1 ± 102.1 (10.4-835)	83.2 ± 43.7 (21.8-176.7)	70.1 ± 47.8 (14-196.7)	102.3 ± 135.9 (10.4-835)
Location				
Extremity	59 (92.2%)	11 (18.6%)	18 (30.5%)	30 (50.8%)
Trunk	5 (7.8%)	1 (25%)	0	3 (75%)
Depth				
Superficial	29 (45.3%)	7 (24.1%)	11 (37.9%)	11 (37.9%)
Deep	32 (50%)	6 (18.8%)	7 (21.9%)	19 (59.4%)
Both	3 (4.7%)	0	0	3 (100%)
FNCLCC grade				
Grade 1	13 (20.3%)			
Grade 2	18 (28.1%)			
Grade 3	33 (51.6%)			
Ki-67 index (%)	32.7 ± 27.6 (1-90)			

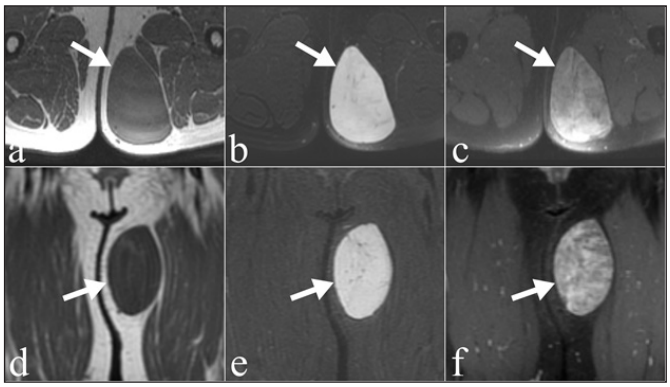

Figure 2. Images show MRI features in a 20-year-old woman with low grade (FNCLCC grade 1) liposarcoma. MRI protocol included, a, axial T1-weighted imaging, b, axial T2-weighted imaging with fat-suppression, c, axial post-contrast T1-weighted imaging, d, e and f, respectively, coronal plans of the same sequences. There is no hemorrhage, peritumoral enhancement, or peritumoral edema in this low-grade tumor.

The presence of hemorrhage signal distinguished as a hyperintense signal on T1W (**Figure 3**), tail sign (**Figure 4**), and post-contrast peritumoral enhancement (**Figure 5**) was significantly higher in FNCLCC grade 3 (**Figure 6** and **7**) STS (p:0.008, p:0.001, p:0.004, respectively). Absence of these features were correlated with low Ki-67 index as well (p:0.015, p:0.006, p:0.010, respectively). The presence of peritumoral edema on T2W imaging in all high-grade patients also showed a strong relationship between these two (p:0.001). Also, according to border and sharpness observed in T2W images in MRI, clear borders of 90% and above were associated with a grade 1 tumor (p:0.009). The presence of bone invasion, vessel, and nerve extension did not significantly correlate with tumor grade or the Ki-67 index.

Table 2. Association of MRI features with Ki-67 proliferation index and FNCLCC grade of specimen

Characteristic	All Patients (n=64)	Ki-67 Index % (mean)	p Value	FNCLCC grade 1	FNCLCC grade 2	FNCLCC grade 3	p Value
Heterogenity (T1W)			0.090				0.563
Homogenous	12 (18.8%)	18.9		4 (33.3%)	4 (33.3%)	4 (33.3%)	
<50%	24 (37.5%)	31.7		5 (20.8%)	7 (29.2%)	12 (50%)	
≥50%	28 (43.7%)	39.5		4 (14.3%)	7 (25%)	17 (60.7%)	
Heterogenity (T2W)			0.156				0.113
Homogenous	2 (3.1%)	25		0	2 (100%)	0	
<50%	28 (43.7%)	25.9		7 (25%)	9 (32.1%)	12 (42.9%)	
≥50%	34 (53.1%)	38.9		6 (17.6%)	7 (20.6%)	21 (61.8%)	
Necrosis			0.001*				0.001*
None	15 (23.4%)	11.07		10 (66.7%)	4 (26.7%)	1 (6.7%)	
<50%	20 (31.3%)	36.4		1 (5%)	6 (30%)	13 (65%)	
≥50%	29 (45.3%)	41.5		2 (6.9%)	8 (27.6%)	19 (65.5%)	
Hemorrhagic signal			0.015*				0.008*
No	26 (40.6%)	24.2		10 (38.5%)	7 (26.9%)	9 (34.6%)	
Yes	38 (59.4%)	38.6		3 (7.9%)	11 (28.9%)	24 (63.2%)	
Border and sharpness			0.402				0.009*
≥90%	17 (26.6%)	27		8 (47.1%)	4 (23.5%)	5 (29.4%)	
Btw 50% -90%	32 (50%)	32.8		3 (9.4%)	12 (37.5%)	17 (53.1%)	
<50%	15 (23.4%)	39.1		2 (13.3%)	2 (13.3%)	11 (73.3%)	
Tail sign			0.006*				0.001*
No	21 (32.8%)	21.4		10 (47.6%)	6 (28.6%)	5 (23.8%)	
Yes	43 (67.2%)	38.3		3 (7%)	12 (27.9%)	28 (65.1%)	
Peritumoral edema			0.001*				0.001*
No	10 (15.6%)	6.5		6 (60%)	4 (40%)	0	
Yes	54 (84.4%)	37.6		7 (13%)	14 (25.9%)	33 (61.1%)	
Peritumoral enhancement			0.010*				0.004*
No	30 (46.8%)	25		10 (33.3%)	11 (36.7%)	9 (30%)	
Yes	34 (53.1%)	39.6		3 (8.8%)	7 (20.6%)	24 (70.6%)	
Bone invasion			0.782				0.360
No	56 (87.5%)	33.1		12 (21.4%)	17 (30.4%)	27 (48.2%)	
Yes	8 (12.5%)	30.7		1 (12.5%)	1 (12.5%)	6 (75%)	
Extension to vessel and nerves			0.224				0.696
No	40 (62.5%)	30.4		9 (22.5%)	12 (30%)	19 (47.5%)	
Yes	24 (37.5%)	36.6		4 (16.7%)	6 (25%)	14 (58.3%)	

DISCUSSION

Histological grading is one of the essential criteria in the treatment planning of STS. Predicting the grade of the tumor from the first diagnosis provides benefits at every step, from the biopsy stage to systemic and local treatments. The heterogeneous internal structure of the tumor may lead to errors in the grading performed by biopsy. Jones C. et al. (15) and several other authors challenged the adequacy of core needle biopsy for determining the treatment (16-18). At this point, MRI stands out because it offers a global examination opportunity before total excision. Zhao et al. (12) previously showed independent MRI feature predictors of high-grade STS. Followed by Crombe A. et al. (13) with supporting imaging features correlating with the grade of the STS. Our study found that the following MRI features were essential predictors for high-grade tumors: Presence of hemorrhage signal distinguished as a hyperintense signal on T1W, tail sign, and post-contrast peritumoral enhancement.

After detecting a soft tissue tumor, MR imaging is performed to distinguish the tumor as benign or malignant and determine its extent and size. Then, if malignancy is suspected, a biopsy is performed for histopathological examination. In addition to the sarcoma diagnosis, the tumor grade is determined by biopsy. It has been reported that the grade of tumor determined by biopsy is lower than the final grade obtained after excision, with a rate of 13.5 to 55% in previous studies (19-21). This shows that biopsy adequacy and repetition should be reviewed in patients whose biopsy results were reported as low grade, while there are MRI findings pointing to a high-grade tumor. In the future projection, it will be aimed to predict tumor grade with MRI, independent of biopsy.

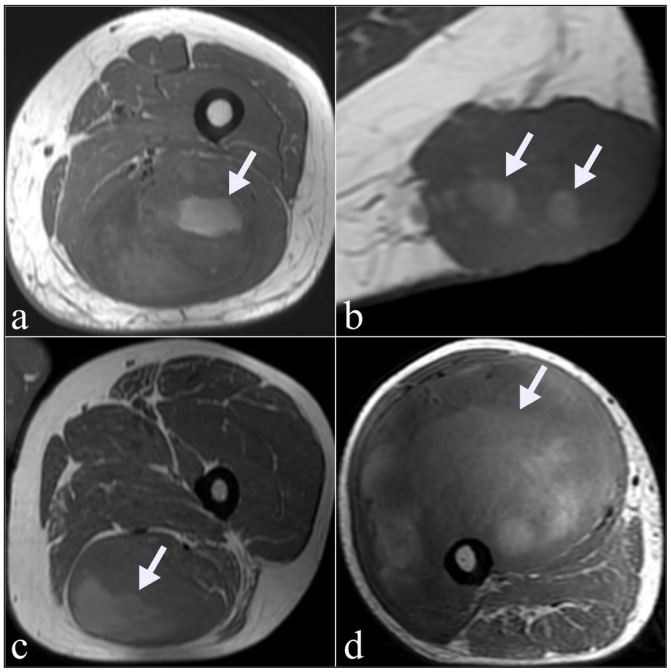

Figure 3. The presence of hemorrhage signal distinguished as a hyperintense signal on T1W is an important indicator for high grade. a. In the thigh MRI of a 41-year-old male patient, pleomorphic sarcoma and the hemorrhage area within the tumor were shown with an arrow. b. In the pelvic MRI of a 28-year-old female patient, angiosarcoma and the hemorrhage area within the tumor were shown with arrows. c. In the thigh MRI of a 40-year-old male patient, undifferentiated pleomorphic sarcoma and the hemorrhage area within the tumor were shown with an arrow. d. Right thigh MRI of a 50-year-old male patient showed myxofibrosarcoma and hemorrhage areas within the tumor with arrows.

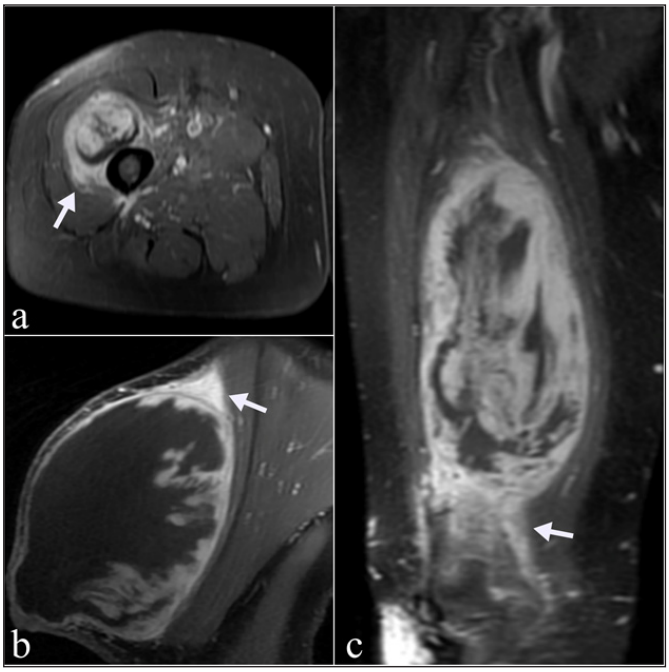

Figure 5. Peritumoral enhancement is more common in high-grade tumors. a. In the thigh MRI of a 62-year-old female patient, pleomorphic sarcoma and peritumoral enhancement were shown with an arrow. b. In the shoulder MRI of a 33-year-old male patient, fibrosarcoma and peritumoral enhancement were shown with an arrow. c. In the thigh MRI of a 67-year-old female patient, undifferentiated pleomorphic sarcoma and peritumoral enhancement were shown with an arrow.

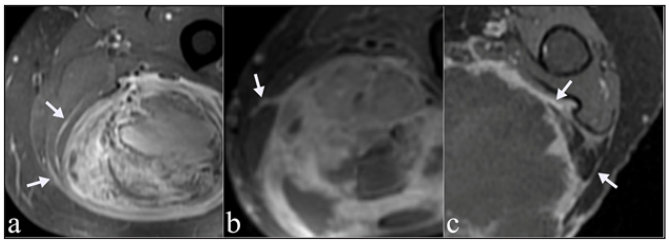

Figure 4. The tail sign is a signal extending curvilinearly along a certain plane with the same enhancement as the main mass in contrast-enhanced series. a. In the thigh MRI of a 41-year-old male patient, pleomorphic sarcoma and tail-sign were shown with an arrow. b. In the thigh MRI of a 62-year-old male patient, myxofibrosarcoma and tail-sign were shown with an arrow. c. Right arm MRI of a 85-year-old female patient, leimyosarcoma and tail-sign were shown with an arrow.

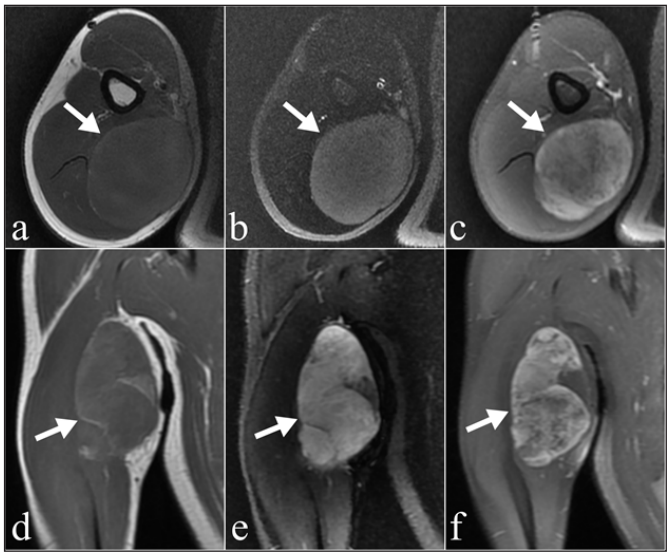

Figure 6. Images show MRI features in a 34-year-old male with FNCLCC grade 2 myxoid liposarcoma. MRI protocol included, a, axial T1-weighted imaging, b, axial T2-weighted imaging with fat-suppression, c, axial post-contrast T1-weighted imaging, d, e and f, coronal plans of the same sequences, respectively. There is a high T1 signal of hemorrhage in the tumor and mild peritumoral edema is present in the inferior.

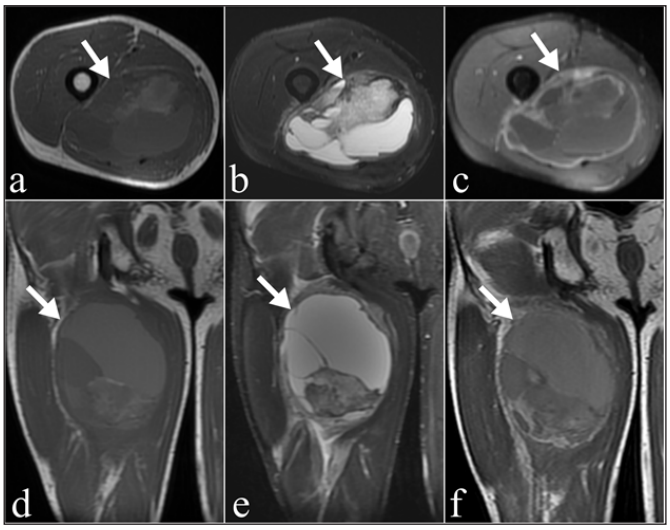

Figure 7. Images show MRI features in a 40-year-old male with FNCLCC grade 3 undifferentiated pleomorphic sarcoma. MRI protocol included, a, axial T1-weighted imaging, b, axial T2-weighted imaging with fat-suppression, c, axial post-contrast T1-weighted imaging, d, e and f, coronal plans of the same sequences, respectively. The tumor is highly heterogeneous in all sequences and has a high T1 signal of hemorrhage. Peritumoral edema and enhancement are present.

In our study, the rate of patients with grade III tumors was 51.6% (12,22,23), similar to the literature (46.1-59.7%). Crombé et al. (13) identified peritumoral enhancement as an independent predictor for high-grade tumors. However, they did not make a recommendation for a hemorrhage signal distinguished as a hyperintense signal on T1W and tail sign. They highlighted the findings of a necrotic component of the tumor, heterogeneous SI greater than or equal to 50% at T2W imaging. The absence of necrosis and a homogeneous signal were also found to be features of low-grade tumors in our study. Combining low-grade tumors into grades 1 and 2 may explain the differing results between studies.

In the analysis by Zhao et al. (12), tumor margin regularity was associated with a high degree of peritumoral edema and contrast enhancement. Our results showed a high degree of correlation between tail sign, peritumoral edema and contrast enhancement, and border irregularity. Presumably, since these indicators suggest invasion into surrounding tissues, it can be expected to be related to the tumor's aggressiveness.

Contrary to previous publications, the association of heterogeneity with high-grade tumors in T1 and T2W imaging showed no significant relevance. Revealing a precise heterogeneity ratio rather than an observation will increase the usability of this feature. Radiomics parameters are gaining in popularity today (24-26). Also, artificial intelligence-assisted pattern-based classification methods show promising results for future use in the quantitative analysis of tumor heterogeneity (27-29).

CONCLUSION

Our study found that the presence of hemorrhage signal, tail sign, peritumoral enhancement, clear borders of 50% and less obtained from conventional MRI features of STS are associated with high-grade tumors. The absence of necrosis signal, clear borders of 90% and above in MRI were significantly associated with FNCLCC grade 1. In case of discordance of these features and the biopsy result, it should be kept in mind that the tumor may be high grade considering that MRI can perform global tumor examination. MRI findings of the tumor will continue to be of increasing importance as a guide in clinical decision-making.

Abbreviations: STS: soft-tissue sarcoma; FNCLCC: Fédération Nationale des Centres de Lutte Contre Le Cancer

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Istanbul Medeniyet University Göztepe Prof. Dr. Süleyman Yalçın City Hospital Noninvasive Clinical Researches Ethics Committee (Date: 30.06.2021, Decision No: 2021/0347).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version

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Original Article

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Effect of different surface pretreatment methods on repair bond strength of resin composite subjected to pH-cycling

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ABSTRACT

Objectives: The aim of this study was to evaluate the effect of two different repair methods (Er:YAG laser and bur) with or without silane application on the microtensile bond strength of a nanohybrid resin composite aged with two different aging methods (pH cycling and thermocycling).

Material and Method: Resin composite blocks (Clearfil Majesty Esthetic, Kuraray, Japan) were randomly assigned into two groups for aging process: (a) pH cycling (b) thermocycling (5,000 cycles). After aging, the blocks were assigned to one of the following repair procedures: (1) Er:YAG laser (LightWalker STE-E, Fotona Medical Lasers, Ljubljana, Slovenia) (2) Er:YAG laser+silane (3) bur (4) bur+silane and (5) no-pretreatment group and (6) Cohesive control (cohesive strength of the resin). Resin composite (Clearfil Majesty Esthetic) was bonded to the conditioned substrates incrementally and light polymerized. Repaired samples were thermocycled (5.000 cycles). The microtensile bonding test was performed. The data were analyzed using Scheirer-Ray-Hare, Kruskal-Wallis Mann-Whitney U tests, Chi-square and Z tests with Bonferroni correction (p=0.05).

Results: No statistically significant difference was found between the aging methods applied to filling material (p=0.821) and the interaction of applied surface treatments and aging (p=0.289). All repair procedures achieved bond strength values higher than the no-pretreatment group but they did not reach the resin composite's cohesive bond strength. Failure modes distribution was found statistically different according to repair procedure and also aging methods (p<0.05).

Conclusion: The bond strengths of the resin composites were similar to those of applied thermal cycling and the pH cycling model with no difference between the different repair methods.

Keywords: Aging, dental restoration repair, lasers, pH, silanes

INTRODUCTION

In recent years, resin composites have shown significant developments regarding their technical and aesthetic properties. The mechanical/physical properties and diversity of these restorative materials vary depending on the size, morphology, amount, distribution and chemical composition of the filler (1). After the introduction of nanotechnology into the field of dentistry, nano-filled resin composites that can be better polished, have higher fracture and wear resistance, lower polymerization shrinkage, and can be used in both anterior and posterior restorations with more aesthetic properties have been introduced to the market (2).

Restorative dentistry does not only show its conservative approach in the treatment of caries with conservative restorations, but also maintains this attitude with the repair of the existing restoration instead of replacing the defective restoration completely. Restoration repair is more practical and economical approach, with less compromise on tooth hard tissues (3). In addition, it is timesaving, requires less extensive cavity preparation thus reduce the risk of pulp exposure, less traumatic to the tooth. It has been reported that the repair of resin composite restorations yields promising results in terms of longevity and quality of the restoration (4).

Bonding between the two composite layers is achieved by the presence of an oxygen inhibition layer over the unpolymerized resin. The oxygen inhibition layer is a layer of unreacted monomers that increase inter-material adhesion. The amount of unreacted monomers present in the restoration decrease with aging (5). Therefore, the bond formed between the old and new resin composite during the repair procedure could be unreliable. When

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restorations are exposed to the oral environment, absorb water and the activity of free radicals inside the material terminates (6). In order to increase the repair bond strength between the old and new composite materials, many techniques such as bur (7-14), silane (6-8,11,15-21), bonding agent (6-9,11,13-15,17,19,22,23) and Erbium Yttrium Aluminum Garnet (Er:YAG) laser (7,10,12,24,25) pretreatment modalities are used.

Artificial aging is preferred to mimic the intraoral environmental changes and helps to evaluate the effect of various aging factors on the resin composite. For simulating intraoral temperature changes, thermal cycling (12,15,18,19,24,26,27) is frequently used in vivo. It is one of the most commonly employed methods, which is found to be much more effective than other aging methods for mimicking aging by creating stress on the bonding interface (18). Generally, samples are subjected to extreme temperatures of 5 -55℃. High temperatures are known to weaken the physicochemical properties of resin composites. Additionally, temperature changes can also reduce the number of unreacted double bonds within the composite or on the composite surface, thereby can negatively affect the composite-to-composite repair bond strength (18).

In the oral cavity, resin composite restorations are under the impact of many dynamics such as water, saliva, thermal stress, chemical attacks, and chewing forces. Intraoral pH varies depending on the organic acid in the plaque, bacterial metabolism, saliva and eating habits (28). These factors, individually or collectively, may degrade the resin composite or lead to inter-material debonding. Although pH cycling models have been used widely to create artificial demineralization and remineralization (29,30), little is known about their effects on dental materials (31,32). Different in vitro artificial aging methods such as thermal cycling and pH cycling may have different effects on the degradation of resin composites.

The aim of this study is to evaluate the effect of two different repair methods (Er:YAG laser and bur) applied with and without silane on the microtensile bond strength of nanohybrid resin composite (Clearfil Majesty Esthetic, Kuraray, Japan) aged using two different methods (pH cycle and thermal cycle). The null hypotheses tested were that (1) the success of the repair bond strength of resin composites is not dependent on the surface treatments evaluated, and (2) the aging conditions will not have the same effect on the repair bond strength of resin composites.

MATERIAL AND METHOD

Ethics committee approval is not required for in vitro material studies that do not use human and animal subjects. All procedures were performed adhered to the ethical rules and principles of the Helsinki Declaration.

The materials used in this study are shown in **Table 1**. The experimental design is outlined in **Figure 1**.

Table 1. Materials and contents used in the study.

Brand name	Manufacturer	Batch no.	Composition Filler loading
Clearfil Majesty Esthetic (A1, A3,5 shade)	Kuraray, Tokyo, Japan	00033A B50001 00022C 00022C	Silanated barium glass (mean particle size 0.7 µm), pre-polymerized organic filler including nanofiller, Bis-GMA, hydrophobic aromatic dimethacrylate, hydrophobic aliphatic methacrylate 78% by weight 66% by volume
Clearfil Ceramic Primer	Kuraray, Tokyo, Japan	2S0001	MPS, MDP, ethanol
Clearfil SE Bond	Kuraray, Tokyo, Japan	000093	Primer: MDP, HEMA, hydrophilic dimethacrylate, water, photoinitiator Bond: MDP, HEMA, Bis-GMA, hydrophobic dimethacrylate, silanized colloidal silica, photoinitiators

**Bis-GMA: Bisphenol A-glycidyl methacrylate, HEMA: 2-hydroxyethyl methacrylate, MDP: 10-methacryloyloxydecyl dihydrogenphosphate, MPS: 3-Methacryloxypropyltrimethoxysilane

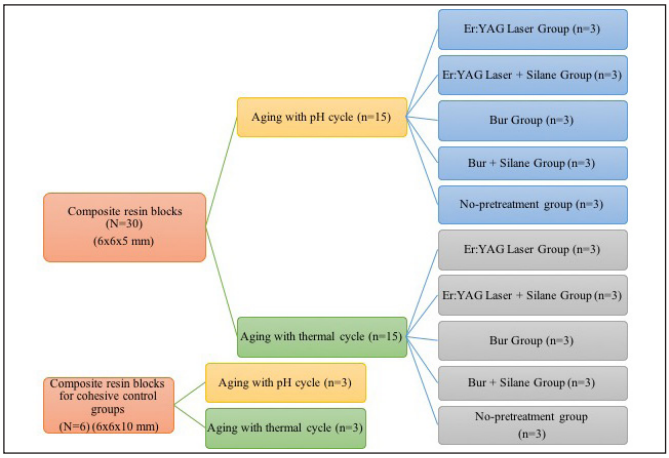

Fig. 1. Study design.

Preparation of Aged Resin Composites

Thirty resin composite blocks with dimensions of 6x6 mm and 5 mm in height were built up incrementally with a three increments of nanohybrid resin composite (Clearfil Majesty Esthetic, Shade A1, Kuraray, Tokyo, Japan) placed inside a silicone matrix (Elite HD Putty soft setting, Zhermack, Italy) according to the manufacturer's instructions. Each 2-mm increment was light-cured for 20 seconds with a LED lightcuring unit (Elipar Freelight 2, 3M ESPE, Germany). After the samples were removed from the mold, curing light was applied to the bottom and sides of the composite that were previously in contact with the silicone mold for a total of 100 seconds to ensure their full polymerization. All surfaces of the specimens to be treated were polished with 320-grit silicone carbide papers under cooling, and then the specimens were ultrasonically cleaned for 3 min using distilled water.

As the cohesive control group and to determine the inherent cohesive strength of the resin composite, six resin composite blocks (6×6×10 mm) were prepared and cured in a similar manner as previously described using the same composite (Clearfil Majesty Esthetic (Shade A1)).

Aging of the Resin Composites

The resin composite blocks were then randomly and equally divided into two groups, according to the aging method applied.

In pH cycling model for the cariogenic challenge specimens were immersed in demineralizing solution [2.0 mmol/L $Ca(NO_3)_2.4H_2O$, 2.0 mmol/L $Na_2HPO_4.2H_2O$, 75 mmol/L acetate buffer, 0.04 ppm F, pH 4.7] for 6 h (30 mL per specimen) and in remineralizing solution [1.5 mmol/L $Ca(NO_3)_2.4H_2O$, 0.9 mmol/L $Na_2HPO_4.2H_2O$, 150 mmol/L KCl, 0.02 mol/L Tris buffer, 0.05 ppm F, pH 7.0] for 18 h (30 mL per specimen) at 37°C. This sequence was repeated for 5 days, In the last two days, the specimens were kept in the remineralizing solution only. Both solutions were replaced daily (30).

Other samples were thermally aged in distilled water between 5°C and 55°C for 5000 cycles with a dwell time of 30 seconds and a transfer time of 5 seconds.

Conditioning of the Aged Resin Composites

After the aging protocols resin composite blocks were randomly and equally divided into five subgroups (n=3) and different surface pretreatment methods were employed to one surface of the samples as follows:

Er:YAG laser: An Er:YAG laser (LightWalker STE-E, Fotona Medical Lasers, Ljubljana, Slovenia) with a wavelength of 2940 nm coupled with a handpiece (R02-C) having a spot size of 0.9 mm in diameter in a noncontact mode under continuous water spray (40–60 mL/min) at a focal distance of 7 mm from the target point was used. Laser was applied with the following parameters; 5 W power, 250 mJ energy, 20 Hz pulse repetition rate and 100 μs pulse duration (10).

Er:YAG laser+Silane: Er:YAG laser application was performed as described in the Er:YAG laser only group, followed by application of Clearfil Ceramic Primer (Kuraray Medical, Okayama, Japan) for 60 seconds and dried by blowing mild oil-free air for 10 seconds to evaporate the solvent.

Bur Group: Composite surface was roughened with a diamond cylinder bur attached to a highspeed air turbine (KaVo Dental, Bismarckring, Germany) under air and water cooling. Five consecutive strokes with minimal pressure were applied on the sample surface to remove a similar composite thickness from each sample surface. A new bur was used for each sample.

Bur+Silane: Roughening with bur was performed as described in the Bur-only group followed by, Clearfil Ceramic Primer application as described in Er:YAG laser + Silane group.

No-pretreatment group: No surface treatment was performed on the surface of resin composite blocks (5 mm × 6 mm × 6 mm) to be repaired.

Cohesive Control: Longer resin composite blocks (10 mm × 6 mm × 6 mm) were used to measure the cohesive strength of the resin composite. No surface pretreatment was performed, and no repair composite was bonded. Specimens were directly subjected to tensile forces.

Bonding Procedure and Specimen Storage

Following pretreatment procedures, specimens were rinsed with water and dried with air. A two-step self-etch adhesive system (Clearfil SE Bond, Kuraray, Okayama, Japan) was then applied to all the pretreated surfaces except cohesive control group. Self-etching primer was applied to the surface and left undisturbed for 20 seconds, followed by drying with mild air flow for 5 seconds. Bond was applied and gently air-thinned with to provide a uniform resin film and cured for 10 seconds with the LED lightcuring unit.

The samples were then placed inside the bottom portion of the 10 mm-deep silicone mold, with the adhesive applied surface on top. To distinguish between the new repair and the aged composite, a different shade (A3.5) nanohybrid resin composite of the same brand (Clearfil Majesty Esthetic) was placed with 2 mm-thick increments. Each increment was light-cured for 20 seconds with the LED lightcuring unit. Then, the mold was removed, and samples were post-cured for a total of 80 seconds from all four lateral surfaces.

All specimens, including the cohesive control groups, were subjected to 5000 thermal cycles in distilled water bath between 5°C-55°C before the microtensile bond strength test.

Microtensile Bond Strength Test

The samples were glued to the L-shaped acrylic blocks with cyanoacrylate adhesive for micro-sectioning, then were placed in a precision cutting device (Microcut Precision Cutter 201, Metkon, Turkiye) for sectioning. Serial sections were taken at a cross-sectional area of 1mm x 1mm (approximately $1mm^2$) under water cooling with a low-speed diamond saw. At least 13 test sticks were obtained from each sample, resulting in at least 40 test sticks for each group.

For the microtensile bond strength test, test sticks were glued to the microtensile test device (Microtensile tester, Bisco, Schamburg, USA) from both ends with cyanoacrylate adhesive (Polibond, Polidol, Istanbul, Turkiye) parallel to the long axis of the device. The loading speed of the test

device was determined as 0.5 mm/min until failure. The results were recorded as megapascals (MPa).

After the microtensile bond strength test, the failure modes of the samples were determined by examining the failed bonding interface under a stereo microscope (Leica MZ12; Wetzlar, Germany) at x40 magnification. Failure modes were classified as cohesive failure (within the filling material or repair material) and adhesive failure (between the filling and repair material).

Statistical Analysis

Normality of data distribution and homogeneity of group variances were evaluated with Levene's test. After observing non-homegeneous variance the non-parametric counterpart of the two-way ANOVA, Scheirer-Ray-Hare analysis was used. The interaction between the surface treatment and aging methods was not statistically significant, therefore Kruskal-Wallis test was used to compare the microtensile bond strength values according to the surface pretreatment methods used. Mann-Whitney U test with Bonferroni correction was used for the multiple comparisons. Chi-square tests were used to compare failure patterns in the aging and surface pretreatment groups. Bonferroni corrected z-test was used for pairwise comparisons (α=0.05)

IBM SPSS Statistics 21.0 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.) software was used for statistical analyses and calculations. For the Scheirer-Ray-Hare analysis Real Statistics Resource Pack software (Release 4.3. Copyright (2013 – 2015) Charles Zaiontz.) was used.

RESULTS

No statistically significant interaction was found between the aging methods and surface pretreatments (p=0.289). While aging methods had no significant effect on repair bond strength (p=0.821), surface pretreatment had (p<0.001) (**Table 2**). Comparison of μTBS values according to the surface pretreatment methods are given in **Table 3**. Cohesive strength of the resin composite was significantly higher than all repaired composite groups (p<0.05). No-pretreatment group had significantly lower μTBS values than that of the cohesive control group, Er:YAG laser+silane and bur+silane groups. (p<0.05). Er:YAG laser-only, Er:YAG laser+silane, bur-only and bur+silane groups had similar μTBS values (p>0.05).

Comparison of μTBS values according to the aging methods are given in **Table 4**. Cohesive strength of the resin composite was significantly higher than all repaired composite groups and no-pretreatment group had significantly lower μTBS values than that of the cohesive control group, Er:YAG laser+silane and bur+silane groups in both pH cycle and thermal cycle aging methods (p<0.05).

Table 2. Comparison of aging methods and surface treatments applied to the filling materials.

Effects	H statistic	p
Aging methods	1.125	0.821
Surface treatments	71.660	<0.001
Aging methods*Surface treatments	-2.197	0.289

Table 3. Comparison of the overall microtensile bond strength values according to the surface pretreatments applied to the filling materials

	Mean±SE Median (Min-Max)	χ^2	p
Surface pretreatments		68.270	<0.001
Er:YAG Laser Group	28.68±6.37 28.76 (14.24 - 42.95)[1]		
Er:YAG Laser + Silane Group	30.02±5.85 30.14 (17.69 - 45.12)[2,3]		
Bur Group	29.67±8.49 29.42 (15.20 - 49.20)[4]		
Bur+Silane Group	30.96±6.29 30.92 (17.33 - 49.00)[5,6]		
No-pretreatment Group	26.83±4.91 26.52 (17.44 - 37.43)[2,5,7]		
Cohesive Control Group	35.93±7.11 35.36 (20.79 - 49.79)[1,3,4,6,7]		

** Data are given as mean ± standard error (Mean±SE) and median (Minimum-Maximum) (Min-Max). There is a statistically significant difference between groups with the same number ([1,2,3,4,5,6,7] p<0,05).

Table 4. Comparison of microtensile bond strength values according to aging methods

	Aging methods	
	pH cycling	Thermal cycling
Surface pretreatments	Mean ± SE Median (Min-Max)	Mean ± SE Median (Min-Max)
Er:YAG Laser Group	28.52±6.89 28.09 (14.24 - 41.40)[1]	28.85±5.84 29.99 (18.04 - 42.95)[1]
Er:YAG Laser + Silane Group	29.97±6.52 29.45 (21.44 - 45.12)[2,3]	30.08±5.16 30.28 (17.69 - 39.37)[2,3]
Bur Group	29.23±6.76 29.42 (17.79 - 41.80)[4]	30.15±10.09 29.16 (15.20 - 49.20)[4]
Bur+Silane Group	30.68±6.63 31.06 (17.33 - 49.00)[5,6]	31.25±6.01 30.79 (21.30 - 44.54)[5,6]
No-pretreatment Group	25.81±4.71 25.62 (17.44 - 37.43)[2,5,7]	27.83±4.95 27.6 (18.72 - 36.80)[2,5,7]
Cohesive Control Group	35.55±6.70 34.8 (20.79 - 48.52)[1,3,4,6,7]	36.30±7.54 35.46 (24.00 - 49.79)[1,3,4,6,7]

** Data are given as mean ± standard error (Mean ± S) and median (Minimum-Maximum) (Min-Max). There is a statistically significant difference between groups with the same number in each column. ([1,2,3,4,5,6,7] p<0,05).

The distribution of failure modes is shown in **Figure 2**. There is a statistically significant difference in terms of failure modes according to aging methods (p=0.007). Adhesive failures were significantly fewer and cohesive failures (in repair material) were significantly more frequent in pH cycle compared to thermal cycle (p<0.05). Adhesive failure was statistically significantly more frequent in Er:YAG laser and no-pretreatment group compared to other groups (p<0.05). When the surface

treatments were compared in terms of failure modes among the aging methods, adhesive failure in both pH cycle and thermal cycle applied samples was statistically significantly more frequent in the Er:YAG laser group compared to Er:YAG laser+Silane, Bur and Bur+Silane groups ($p<0.05$).

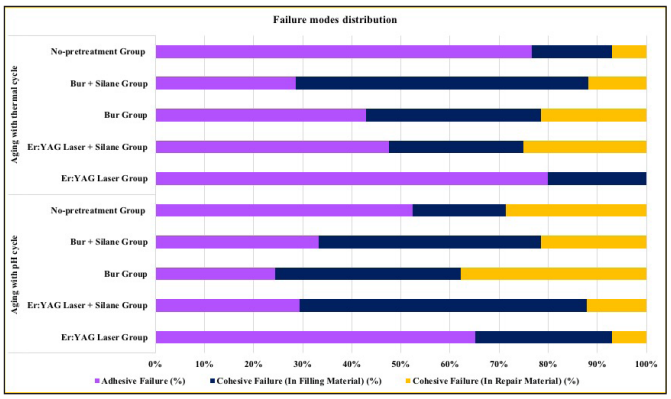

Fig. 2. Failure modes distribution.

DISCUSSION

Dental resin composites can degrade over time due to mechanical factors such as wear, abrasion and fatigue; or chemical factors such as enzymatic hydrolytic and acidic action or due to temperature changes. This degradation may result in discoloration, microleakage, marginal misadaptation or minor fractures of the restoration and may require repair or replacement of the restoration (17). Restoration repair is less time consuming and less costly than replacement (3,4).

Özcan et al. (18) found that the 5000 thermal cycles they applied were a more effective aging method than other aging methods in reducing the repair bond strength of the resin composite. When the repair bond strength studies are examined, it is seen that 5000 thermal cycles are generally performed (19,26,27,33), therefore resin composites before and after the repair was thermally aged for 5000 cycles in our study. On the other hand, there are limited studies examining the effects of pH cycling on dental materials (31,32), and these pH cycling models used to evaluate artificial caries formation or remineralization (29,30) differ in terms of solution pH and application periods. Since the enamel surface is the first surface exposed to the oral environment and the histological and chemical changes of caries are observed on this tissue first, with the pH cycle model applied to the enamel tissue for cariogenic challenge, the pH of the remineralization solution in which we kept our samples for 18 hours was determined as 7 and the pH of the demineralization solution, which we kept for 6 hours, was determined as 4.7 (30).

In composite repair, bonding between the old and new resin composite is achieved through three mechanisms such as chemical bonding to organic matrix, chemical bonding to exposed filler particles, and micromechanical adhesion (8). Microretentive interlocking is the most important factor that provides bonding between the new and old resin composite and increases the bonding between the resin matrix and the exposed filler particles (8,17). Roughening the surface increases the availability of free carbon atoms on the surface (34). Due to the fact that the most frequently used material in daily dental applications is diamond burs and researchers have focused on the effectiveness of Er:YAG lasers for composite repair in recent years, but there are limited studies on this subject (7,10,12,24,25,35), the burs and Er:YAG laser were used as different roughening methods in our study. The repair bond strengths of nanohybrid resin composites have been evaluated in a limited number of studies using the Er:YAG laser applied at 1.5W, 25 Hz, 75 μs (7), 50 mJ and 200 mJ, 10 Hz and 10 seconds (35). Since there are not enough studies examining the effect of Er:YAG lasers on the repair bond strength of nanohybrid resin composites, the Er:YAG laser we used in our study was applied with parameters 250 mJ, 20 Hz, 5 W, wavelength 2.94 μm and pulse duration 100 μs (very short pulse) to determine whether the parameters we chose for these resin composites are suitable or not and to shed light on other studies.

In addition to micromechanical adhesion, it has been reported that chemical bonding to the exposed filler particles and organic matrix is effective in the repair process between the old and new resin composite (8). For this purpose, silanes were are frequently evaluated in many studies (6-8,11,13,15-20). Resin composite restorations may lose the silane layers around the fillers due to both aging in the oral environment after polishing and finishing, and mechanical surface applications such as pretreatment with bur, airabrasion, lasers etc. It is reported that with the use of silane, covalent bonds are re-established between the inorganic fillers of the resin composite and the monomers in the adhesive system, and at the same time, the wettability of the adhesive increases and it infiltrates the surface irregularities more easily (8,16,18,20). In our study, silane was used to evaluate its effect of roughening methods on bond strengths.

Temperature changes and water absorbed by the resin composites as a result of thermal cycling can cause the resin composite organic matrix to swell, lead to microcracks, resin destruction, disintegration of the silane layer on the surface of the filler particles, separation of the filler particles, and a decrease in the number of unreacted double bonds on the resin composite surface or inside the composite, which may affect the composite-composite

repair bond strength (15,36). Depending on the chemical composition of the resin composite, chemical degradation can cause changes in the physico-mechanical properties of the resin composite, such as a decrease in tensile bond strength, fracture toughness and hardness, or increase in wear (36). The degradation of resin composites in an acidic environment has not been widely studied, but it is known that strong acids can dissolve filler particles from the composite surface (37). In a study investigating the effects of solutions used at different pHs and times on the solubility and sorption properties of resin composites, it was shown that the solubility and sorption properties of resin composites are related to the hydrophilicity of the matrix and the chemical composition of the fillers used (32). Resin composites with large filler particle sizes are more prone to degradation by acids (38). Filler particles are released with aging in microhybrid nanohybrid and nanofilled resin composites aged with 5000 thermal cycles, storage in water for 6 months or immersion in citric acid for 1 week (27). In a study, it was observed that thermal cycling yielded the lowest μTBS values of microfilled resin composites (18). In another study, it was observed that immersion in water for 2 months yielded the lowest shear bond strength values of resin composites and immersion in citric acid caused loss of filler along with deterioration of the organic matrix of the resin composite in SEM images (15). Our findings differ from the studies (15,18) found that thermal cycling and immersion in water for 2 months yielded the lowest bond strength values of resin composites. The difference in the chemical structure of the composite used in our study and the use of the pH cycle model instead of citric acid in aging may have led to different results from these studies (15,18). According to our findings, no difference between the two aging methods shows that the pH cycle model we used in our study on resin composites has a similar aging degree with the thermal cycle. For this reason null hypothese tested that the aging conditions will not have the same effect on the repair bond strength of resin composites was rejected.

In a study comparing the roughness of the diamond bur and the Er:YAG laser applied at different power on microhybrid resin composite using scanning electron microscopy, it has been shown that the diamond bur forms smear layer and grooves on the surface of the resin composites and produces much lower roughness surfaces compared to the surfaces treated with the Er:YAG laser and the Er:YAG laser applied up to 5W exhibits more irregular and microporous surfaces, while the Er:YAG laser applied at 6W causes degradation in the resin composite (10). In studies (25,35), where higher roughness values were obtained with Er:YAG laser application compared to burs, it was stated that surface pretreatment methods performed with burs on resin composites did not provide a significant increase in bond strength. This outcome was confirmed in our study.

Consistent with our study, in a study in which different energy parameters of the Er:YAG laser were evaluated in order to determine the best surface treatment for the repair bond strength of the microhybrid resin composite, it was shown that there was no statistically significant difference between the experimental groups including the control group with no treatment (25). Er:YAG laser applied with 150 mJ 10 Hz, 1.5 W 0.119 W/mm² and a pulse duration of 700-ms was reported to provide repair bond strength similar to that of the bur treatment in microfilled hybrid resin composite thermally aged (39). This result is in accordance with our study. Contrary to our study, another study evaluating the repair bond strength of microhybrid resin composite aged with 6000 thermal cycles using 75, 100, 200 and 300 mJ Er:YAG laser energies, found that the highest shear bond strength values (25.98 MPa) in the laser groups were in the group using 75 mJ power and the lowest bond strength values were in the groups using 200 mJ and 300 mJ, and these bond strength values were statistically different compare to the control group with no treatment (24). It was found that Er:YAG laser with 1.5W, 25 Hz, 75 μs increased the repair shear bond strength of nanofilled resin composite aged with 500 thermal cycles compared to the control group with no treatment, unlike our study (7). When the repair bond strengths of nanofilled resin composites applied with 50 mJ and 200 mJ 10Hz and 10 seconds Er:YAG laser were compared, it was seen that the Er:YAG laser groups were not statistically different from the diamond bur group in accordance with our study, but unlike the no-pretreatment group, contrary to our study (35). Differences in the bond strength test method used in our study, the materials, the aging methods used before and after the repair process, and the application parameters of the Er:YAG laser may have led us to obtain different results compared to other studies (7,24,35).

In this study, statistically higher repair bond strength of silane applied bur and Er:YAG laser compared to the no-pretreatment group and the repair bond strength of the bur and Er:YAG laser applied with and without silane is higher than the ones without silane, with no statistical difference between them and the repair bond strengths of the silane-free bur and the Er:YAG laser exhibit statistically similar bond strengths with the control group with no treatment shows the specific effect of silane on repair bond strength. For this reason, null hypothese tested that the success of the repair bond strength of resin composites is not dependent on the surface treatments evaluated was rejected. Our finding that the use of silane increases the repair bond strength is also supported by other studies (15,23,33).

Failure modes provide important information that allows estimation of possible clinical performance limits of the tested material. In this study, failure patterns were mostly cohesive (repair material/filling material), while adhesive failures were more frequent in Er:YAG laser and no-pretreatment group of samples aged with both pH and thermal cycling. It shows that the cohesive failures represent the weak point in the resin composites because of its own composition or the presence of voids or contamination between composite layers, whereas the bond between the filling material and the repair resin composite is reliable (26). In our study, we think that aging methods affect the mechanical and physical properties of resin composites. In the Er:YAG laser group, we believe that the adhesive failures, which were seen intensely similar to the no-pretreatment group, were that the laser parameters we used in the study could not provide sufficient morphological changes on the resin composites, and therefore, the surface change created by the laser was not much different from that of the no-pretreatment group.

CONCLUSION

Within the limitations of this in vitro study, it was observed that the bond strengths of resin composites were similar to those applied with the pH cycle model and thermal cycle. Although there was no difference between the other repair methods such as Er:YAG laser-only or bur-only, surface pretreatment methods with silane such as Er:YAG laser+silane and bur+silane groups had significantly higher μTBS values than that of no-pretreatment group.

ETHICAL DECLARATIONS

Ethics Committee Approval: No interventional procedure was performed with the method and study protocol infrastructure of the study. Due to the absence of clinical studies, ethics committee approval is not required for in vitro material studies that do not use human and animal subjects.

Informed Consent: Because the study was designed on materials, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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Papillary thyroid carcinoma prevalence and its predictors in patients with primary hyperparathyroidism

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ABSTRACT

Aim: Papillary thyroid carcinoma (PTC) and primary hyperparathyroidism (PHPT) are among the most common endocrine diseases. Although it has been shown that hyperparathyroidism may be associated with various cancers, the question of whether there is an association between hyperparathyroidism and PTC remains controversial. To evaluate the incidence of concomitant PTC among patients with PHPT and to identify possible risk factors for the development of PTC in these patients.

Material and Method: The data of 543 patients who had been operated on due to PHPT in our institution were reviewed retrospectively. Patients who underwent thyroid surgery in conjunction with parathyroidectomy and patients whose diagnosis of PTC was confirmed histopathologically were compared in terms of their clinical, biochemical, and histopathological features. The prevalence of PTC found in patients with PHPT was compared with national rates to estimate standardized incidence ratios (SIRs).

Results: Of the 456 PHPT patients enrolled in the study, 281 (61.6%) had concomitant thyroid nodules on thyroid ultrasonography, and PTC was detected in 53 (11.6%) patients during their thyroid surgeries. Compared to the general population, the incidence of papillary thyroid cancer was increased in both women and men with PHPT (SIR: 272.2, 95% CI: 201.6-360.0, p<0.001 and SIR: 736.5, 95% CI: 322.1-1457.0, p<0.001, respectively). Patients who were found to have PTC were older than non-PTC patients and had higher serum calcium levels (p=0.026 and p= 0.012, respectively). In multivariate analysis, a high serum calcium level and advanced age were independent predictors of PTC in patients with PHPT (OR: 1.402, 95% CI: 1.046-1.878, p=0.024 and OR: 1.024, 95% CI: 1.001-1.047, p=0.043, respectively).

Conclusion: Our study showed a significant increase in the prevalence of PTC in patients with PHPT compared to the general population in association with both older age and higher levels of serum calcium. Due to their higher levels of risk, such patients should particularly be comprehensively screened for PTC preoperatively, and the indications for thyroid surgery entailing parathyroidectomy should be updated in the current guidelines.

Keywords: Primary hyperparathyroidism, papillary thyroid carcinoma, prevalence, hypercalcemia, advanced age

INTRODUCTION

Among the most commonly seen mineral metabolism disorders, primary hyperparathyroidism (PHPT) occurs as a result of parathyroid hormone (PTH) being abnormally secreted without complete regulation from one or more of the parathyroid glands. Upon determining which glands are hyperactively secreting PTH, the standard treatment is parathyroidectomy (PTX) (1). Although the primary target organs for potential complications of hyperparathyroidism are the skeleton and kidneys, some studies have shown that hyperparathyroidism may also have cancer-promoting potential and that it may be associated with thyroid or non-thyroid cancers (2-7). However, it remains to be determined whether these complications arise primarily as the result of a genetic predisposition to the development of cancer or whether physiological associative effects, be they environmental or intrinsic, are at play. Similarly, it has not yet been established whether this risk is generally related to PHPT or is specifically related to only severe cases and whether the complications are associated more generally with malignancies or with specific types of cancer (4). Because PHPT is one of the most common endocrine disorders

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and predominantly affects postmenopausal women, it is important to address these questions and learn more about the disorder's relationship with cancer.

Among the various types of thyroid cancer, papillary thyroid carcinoma (PTC) is most commonly seen, with researchers reporting that PTC accounts for roughly 84% to 90% of all cases of malignancies of the thyroid (8,9). Although its general prognosis is very good, PTC spreads to the cervical lymph nodes in 20% to 50% of all cases and distant metastasis is observed in <5% of cases. Thus, early diagnosis is important (10). In the literature, the incidence rate for PTC associated with PHPT has been reported to range between 2% and 17% (6,7,11-14); however, these are mostly reports on small case series, and the identified frequency of PTC has rarely been compared with the general population. Although some studies have sought answers to whether this association is coincidental or related to the more frequent performance of preoperative neck US evaluations for patients with PHPT, the findings remain controversial (3,4).

The objectives of the present study are to determine the prevalence of concomitant PTC in patients with PHPT in our patient population, representing one of the largest case series reported to date; to evaluate whether the frequency of PTC increases in patients with PHPT compared to the general population; and to determine whether there are possible predictors of the development of PTC in patients with PHPT.

MATERIAL AND METHOD

The study was approved by the Ondokuz Mayıs University Clinical Researches Ethics Committee (Date: 27.12.2019, Decision No: 2019/882). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Patient Characteristics and Study Protocol

We analyzed the data of 543 patients who underwent PTX due to PHPT in our tertiary care hospital between January 2010 and August 2019. A total of 87 patients were excluded because regular follow-up data, operation notes, pathology reports, or preoperative neck US results were unavailable; because they were <18 years of age; because they had previously undergone parathyroid or thyroid surgery; or because they were found to have medullary thyroid carcinoma/multiple endocrine neoplasia. The distinction between multiglandular parathyroid disease and uniglandular parathyroid disease was made according to current guidelines (15). In the preoperative period, it was confirmed that the patients met the biochemical diagnostic criteria for PHPT in the presence of at least one of the indications for the operation (1). Neck ultrasonography (US) was performed for all patients included in the study in order to evaluate whether there was synchronous thyroid disease. For thyroid nodules that yielded suspicious US results based on the guidelines of the American Thyroid Association, thyroid fine-needle aspiration biopsies (FNABs) were conducted, and the results of those biopsies were reported within the framework of the standards of the Bethesda System for Reporting Thyroid Cytopathology (16). In addition, lymph node biopsies were performed for patients who were found to have pathological lymphadenopathy of the neck. In cases where an operation was planned for patients with PHPT due to concomitant synchronous thyroid disease, when there was an indication for thyroid surgery, the patients were evaluated in multidisciplinary meetings attended by an endocrinologist, thyroid/parathyroid surgery specialist, and pathologist, and the type of thyroid surgery to be performed in conjunction with PTX was determined (total thyroidectomy, lobectomy, thyroidectomy with central/lateral lymph node dissection, etc.) (9, 16). All operative procedures were conducted by a surgeon who had over 20 years of experience performing thyroid and parathyroid surgeries. Patients presenting with thyroid nodules underwent intensive exploration of the thyroid during the course of their operations, as well. The final histological diagnosis was based on the histopathologic examination of permanent sections of lesions by the same pathologist.

For all patients enrolled in the study, age and gender were recorded as demographic data. All relevant laboratory findings were also recorded, including the values of intact PTH (iPTH), 25-OH vitamin D3 ($25(OH)D_3$), serum calcium (Ca) and phosphate (P), 24-h urine calcium, glomerular filtration rate (GFR) according to the equation proposed in the Modification of Diet in Renal Disease (MDRD) Study, and serum alkaline phosphate (ALP). The serum values of creatinine, ALP, albumin, Ca and P, and 24-h urine Ca were evaluated spectrophotometrically using a Cobas 8000 Modular Analyzer (C701 Module, Roche Diagnostics). Measurements of iPTH were performed by electrochemiluminescence assay (Cobas 8000 Modular Analyzer, e602 Module, Roche Diagnostics). Levels of $25(OH)D_3$ in plasma were measured using an appropriate instrument for high-performance liquid chromatography (Ultimate 3000, Thermo Scientific). The neck US results of the patients were also evaluated, as were the available thyroid FNAB results, preoperative parathyroid imaging results, type of operations performed (PTX alone or PTX with thyroid surgery), thyroid surgery indications, operation notes, and postoperative histopathology results. Patients were divided into two groups according to whether they were found to have or not have PTC in the presence of PHPT, and these groups were compared in terms of clinical,

biochemical, and histopathological features. For patients who were found to have PTC, histopathological features of PTC such as bilaterality, multifocality, subtype, extrathyroidal extension, lymphatic invasion, vascular invasion, and cervical lymph node metastasis were also recorded and tall cell, columnar cell, and hobnail subtypes were considered as aggressive subtypes based on WHO classification (17).

Statistical analysis

Standardized incidence ratios (SIRs) were calculated by dividing observed numbers of cases by expected numbers of cases. The expected number of cancer cases was calculated by multiplying the number of cases per year by the relevant gender, age, and region-specific cancer incidence rate for each age group and year of observation. Cancer incidences in the general population were calculated based on the Public Health Cancer Statistics published by the Ministry of Health of the Republic of Turkey, last updated in 2017 (18). While performing descriptive statistical studies, continuous data were given as mean ± standard deviation and categorical data as frequency (percentage). Independent sample t-tests were used in comparing the continuous data of the groups, while chi-square or Fisher exact tests were used to compare categorical data between the groups. As a result of univariate analysis, a binary logistic regression model was created using parameters with significance of $p<0.05$. This model was used to determine independent factors that could predict PTC. In this study, we applied two-way analyses and used $p<0.05$ to indicate statistical significance. IBM SPSS Statistics 25 (IBM Corp.) was used for statistical analysis.

RESULTS

Among the 456 patients with PHPT included in this study, 377 (82.7%) were female and 79 (17.3%) were male. The age at diagnosis was 48±13.3 years for female patients and 49±12.8 years for male patients (p=0.680). The mean duration of follow-up was 24±23.9 months. Twenty-seven of the patients had multiglandular parathyroid disease and 429 had uniglandular parathyroid disease. Of the patients who underwent PTX, 281 (61.6%) had synchronized thyroid nodularity. Among them, 211 (75.1%) patients had multiple thyroid nodules and 70 (24.9%) had solitary nodules. While 353 patients underwent isolated PTX, 103 patients underwent PTX with thyroid surgery. PTC was detected in 53 of the patients who underwent thyroid surgery (F/M=46/7). We assessed SIRs for PTC in female and male patients separately. Among female patients, the incidence of PTC was increased compared to the general population (SIR: 272.2, 95% CI: 201.6-360.0, p <0.001). Among male patients, the incidence of PTC was similarly increased compared to the general population (SIR: 736.5, 95% CI: 322.1-1457.0, p<0.001). **Table 1** presents the SIRs for PTC in PHPT patients compared to the general population and cancer incidence data by gender.

Table 1. Papillary thyroid carcinoma incidence data by gender

	Observed	Expected	SIR (95% CI)	p value
Female (n=377)	46	0.169	272.2 (201.6-360.0)	<0.001
Male (n=79)	7	0.009	736.5 (322.1-1457.0)	<0.001

CI: Confidence interval, SIR: Standardized incidence ratio

The mean age of patients at diagnosis was 52±11.1 years in the PTC group and 48±13.4 years in the non-PTC group, thus being higher in the PTC group (p=0.026). While the mean serum Ca value was 11.7±1.21 mg/dL in the PTC group, it was 11.4±0.83 mg/dL in the non-PTC group, being significantly higher in the PTC group (p=0.012). The groups were similar in terms of gender, serum iPTH, serum P, 25(OH)D_3, serum ALP, GFR, 24-h urine calcium, osteoporosis, kidney stones on imaging, and histopathologic characteristics (**Table 2**). To determine the preoperative predictors of concomitant PTC in the presence of PHPT, a binary logistic regression model was created with variables identified as having significance of $p<0.05$ between the groups in univariate analysis (age at diagnosis, serum Ca; **Table 3**). In multivariate analysis, age at diagnosis and serum Ca were found to be independent factors predicting the presence of PTC in patients with PHPT (OR: 1.024, 95% CI: 1.001-1.047, p=0.043 and OR: 1.402, 95% CI: 1.046-1.878, p=0.024, respectively).

Table 2. Clinical features of PHPT patients with/without papillary thyroid carcinoma

	Non-PTC (n= 403)	PTC (n=53)	p value
Age at diagnosis (years) (mean ± SD)	48±13.4	52±11.1	0.026
Gender (Female n%)	331 (82.1)	46 (86.8)	0.400
Serum iPTH (pg/mL)	219±159.9	208±146.5	0.622
Serum Ca (mg/dL)	11.4±0.83	11.7±1.21	0.012
Serum P (mg/dL)	2.5±0.5	2.7±0.5	0.056
25-OH vitamin D (µg/L)	16.4±11.5	15.7±11.5	0.688
Serum ALP (U/L)	120±90.3	108±44.4	0.344
24hr urine Ca (mg/day)	402±387.9	387±219.6	0.815
GFR (MDRD)	97±28.0	95±23.6	0.709
Osteoporosis*	117 (47.3)	17 (48.6)	0.893
Kidney stones **	98 (31.7)	16 (37.2)	0.471
Thyroid nodularity	229 (56.8)	52 (98.1)	NA
Histopathologic examination			
Uniglandular parathyroid disease	378 (93.8)	51 (96.2)	NA
Multiglandular parathyroid disease	25 (6.2)	2 (3.8)	
Follow-up period (months)	23±22.9	36±27.9	<0.001

25-OH vitamin D: 25-hydroxy vitamin D, ALP: Alkaline phosphatase, Ca: Calcium, iPTH: Intact parathyroid hormone, GFR: Glomerular filtration rate, MDRD: Modification of Diet in Renal Disease, P: Phosphate, PHPT: Primary hyperparathyroidism *Bone densitometry was performed in 282 patients **Kidney imaging was performed on 352 patients

Table 3. Predictive factors of papillary thyroid carcinoma by multivariable analysis

	OR (95% CI)	p value
Age at diagnosis (years)	1.024 (1.001-1.047)	0.043
Serum calcium	1.402 (1.046-1.878)	0.024

CI: Confidence interval, OR: Odds ratio

Of the 103 patients who underwent thyroid surgery with PTX, 65 (63.1%) underwent PTX with total thyroidectomy and 38 (36.9%) underwent PTX with thyroid lobectomy. Indications for thyroid surgery performed in conjunction with PTX are shown in **Table 4**. PTC was found in 39 (60%) of the patients who underwent total thyroidectomy, in 14 (36.8%) of the patients who underwent lobectomy, and in 53 (51.5%) of the patients who underwent PTX with thyroid surgery. PTC was detected incidentally in 37 (69.8%) of 53 patients who were found to have PTC, and 36 (67.9%) of these cases involved papillary microcarcinoma. Five (9.4%) of the patients with PTCs had aggressive subtypes, 4 (7.5%) had lymphatic invasion and/or vascular invasion, and 3 (5.7%) had cervical lymph node metastases. Other histopathological features of PTCs are shown in **Table 5**.

Table 4. Indications for simultaneous parathyroidectomy and thyroid surgery for patients with PHPT

Indications for thyroid surgery (n=103)	n (%)
Nontoxic guatr with compression symptoms	57 (55.3%)
Thyroid FNAB results	30 (29.1%)
Bethesda I (recurrent)	2 (1.9%)
Bethesda III (recurrent)	12 (11.7%)
Bethesda IV	7 (6.8%)
Bethesda V	4 (3.9%)
Bethesda VI	5 (4.9%)
Graves disease	2 (1.9%)
Toxic multinodular goiter	6 (5.8%)
Toxic adenoma	3 (2.9%)
Cosmetic reasons due to goiter	2 (1.9%)
Suspicion of thyroid cancer on intraoperative inspection	2 (1.9%)
Concomitant parathyroid carcinoma	1 (1%)

Bethesta: System for Reporting Thyroid Cytopathology, Bethesda I: nondiagnostic or unsatisfactory; Bethesda II: benign; Bethesda III: atypia of undetermined significance or follicular lesion of undetermined significance; Bethesda IV: follicular neoplasm or suspicious for a follicular neoplasm; Bethesda V: suspicious for malignancy; Bethesda VI: malignant, PHPT: Primary hyperparathyroidism,

Table 5. Histopathological characteristics of PTC in patients with PHPT

PTC (n=53)	n (%)
Incidental	37 (69.8)
Tumor size (mm) (mean ± SD)	11.3±12.9
≤ 10	36 (67.9)
> 10	17 (32.1)
Bilaterality	13 (24.5)
Multifocality	22 (41.5)
Subtypes	
Follicular	26 (49.1)
Classic	15 (28.3)
Oncocytic	7 (13.2)
Tall cell	5 (9.4)
Extrathyroidal extension	1 (1.9)
Lymphatic invasion	2 (3.8)
Vascular invasion	2 (3.8)
Cervical LNM	3 (5.7)

LNM: Lymph node metastasis, PHPT: Primary hyperparathyroidism, PTC: Papillary thyroid carcinoma

DISCUSSION

This study showed that the prevalence of PTC is significantly higher in patients with PHPT compared to the general population and that PHPT is a risk factor for the development of PTC. This study also showed that advanced age and high Ca levels may be predictors for the development of PTC in patients with PHPT. Our results suggest that the current guidelines should include additional recommendations on the treatment of patients with PHPT and synchronized thyroid disease; the indications for thyroid surgery with PTX for patients with PHPT should not be the same as those applied for other thyroid patients. Our results also emphasize the importance of conducting routine and comprehensive preoperative thyroid assessments for all patients with PHPT.

Previous studies have reported the association of PHPT with multiple types of cancer, including breast cancer, hematopoietic cancer, thyroid cancer, urinary tract carcinomas, colon cancer, and squamous cell skin cancer, with this association recently being considered as a risk factor for the occurrence of PTC (2-7). For example, one previous study found thyroid malignancy to be the most prevalent type of cancer (SIR: 21.19, 95% CI: 4.3-61.9) among patients presenting with PHPT as the primary disorder (5). Some studies have suggested that this relationship between PHPT and cancer reflects the existence of a genetic predisposition to cancer with disturbed vitamin D receptor alleles triggering poor regulation of the parathyroid glands together with defective processes of apoptosis and higher rates of preneoplastic lesions (19, 20). Other findings of previous research suggest that increased levels of PTH, decreased levels of vitamin D, and the presence of hypercalcemia are involved in thyroid carcinogenesis because of their ability to promote the release of vascular endothelial growth factor and fibroblast growth factor, with these growth factors possibly impacting the thyroid follicular cells via mitogenic and differential effects while impacting endothelial cells via angiogenic effects (3,21-23). Although the underlying mechanism has yet to be clarified, previous studies generally support the hypothesis that the association between these two diseases is not accidental and that there is a strong relationship between them.

According to many researchers, concomitant thyroid nodules are observed in 15% to 75% of all cases of PHPT, while PTC presenting in association with PHPT has been found to have incidence rates ranging from 2% to 17% (6,7,11-14). In our large case series, we found that the frequency of thyroid nodularity was 61.6% while the frequency of PTC was 11.6% in patients with PHPT, and these results are consistent with the literature. Among

patients with PHPT, thyroid nodules were found to have an overall malignancy rate of 18.5%, which was higher than the previously reported malignancy rate of thyroid nodules in the general patient population (5-15%) (24). These results suggest that PHPT may be a risk factor for the development of malignancies in thyroid nodules. The wide ranges indicated in reports on the PHPT-PTC association are likely due to the fact that reports are generally created based on small case series or the higher rates of confirmed PTC among patients with PHPT as these patients undergo neck US more frequently for the preoperative determination of synchronized thyroid disease. However, the increase in the accessibility of thyroid US as a result of the development of health services over the years has increased the incidence of not only PTCs detected in the presence of PHPT but also that of latent PTCs in the general population. Therefore, comparing PHPT patients with the general population while evaluating the prevalence of PTC may give more accurate results. In our study, in comparison to the general population, both male and female patients with PHPT had significantly higher risks of PTC. We think that all patients with PHPT should be evaluated with comprehensive preoperative neck US to ensure the early diagnosis of PTC and prevent the complications that may develop in the event of a second surgery.

In the literature, there are very few studies arguing for a relationship between PHPT and PTC and identifying the risk factors for that relationship, and their results are contradictory (6,25,26). In a study conducted with 318 patients, moderate elevations of serum PTH independently predicted thyroid cancer among patients with PHPT (25). Conversely, in another study conducted with a similar population, decreased serum PTH measurements were obtained for patients with both PHPT and PTC in comparison to patients diagnosed with benign thyroid lesions (26). In another study conducted with 155 patients, a significant inverse association was identified between nonmedullary thyroid carcinoma and levels of preoperative serum Ca in cases of PHPT, similarly to the research reported by Liu et al. (6,26). Contrary to those findings, our study, conducted with a much larger patient population compared to previous studies, showed that high serum Ca levels and advanced age were independent risk factors for PTC. In the literature, the carcinogenic effects of significantly extended exposure to higher levels of Ca have been reported as a result of the goitrogenic effects of calcium with inhibited thyroxine synthesis occurring due to higher levels of kidney iodine clearance, and this supports the results of our study (3). The current guidelines set forth the same indications for thyroid surgery combined with PTX for both patients with concomitant thyroid disease and PHPT and patients presenting with isolated thyroid disease (27). Our finding that PHPT patients with high serum Ca levels and older patients with PHPT have significantly increased risks of PTC will hopefully contribute to these indications in the guidelines, and we think that the criteria for thyroid surgery in the guidelines should be customized, particularly for these patients. Our opinion is supported by the finding that 69.8% of the detected cases of PTC were incidental in our study despite the fact that we complied with the current guideline recommendations for the indications of thyroid surgery performed in conjunction with PTX.

In the literature, it has been reported that approximately 10% to 15% of all thyroid cancers are aggressive subtypes (28). In our study, we found this rate to be 9.4% in cases of PTCs accompanying PHPT, which is similar to the rates for isolated PTCs reported in the literature. Some studies have suggested that PTC detected in patients with PHPT may be more aggressive than cases of isolated PTC due to features such as high rates of tumor capsule invasion and multicentricity (29) or high lymph node ratio (number of metastatic lymph nodes divided by number of lymph nodes removed) (30). However, it remains necessary to determine whether PHPT is seen in association with cases of more invasive PTC according to morbidity rates, tumor pathology, and prognosis.

The limitations of our study arose from its retrospective nature. In addition, the rate of co-occurrence of PHPT and PHPT may reflect detection bias. On the other hand, our study is one of the largest case series in the literature, and it is also one of the rare studies comparing the prevalence of PTC in PHPT patients with the prevalence of PTC in the general population. We have also shown that the risk of PTC in patients with PHPT is associated with only advanced age and high serum Ca levels among the many other variables considered in this study. We hope that these findings will contribute to the current guidelines and that the upcoming guideline updates will ensure that these patients are not evaluated in the same way as patients who need other thyroid surgeries.

CONCLUSION

Since patients with PHPT, and particularly the elderly and patients with high Ca levels, have significantly higher risks of PTC, the indications for performing thyroid surgery in conjunction with PTX should be revised considering the benefits of early clinical diagnosis of PTC for patients and the complications that a second surgery may cause. We believe that PTC may have an important place in the early diagnosis and treatment of these patients and our results may change the surgical procedures to be performed in clinical practice, particularly for high-risk patients.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was approved by the Ondokuz Mayıs University Clinical Researches Ethics Committee (Date: 27.12.2019, Decision No: 2019/882).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper and that they have approved the final version.

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Prognostic importance of thrombospondin-1, VEGF, PDGFR-β in diffuse large B-cell lymphoma

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ABSTRACT

Aim: In this study, we aimed to investigate the relationship between the staining rates of thrombospondin-1, VEGF, and PDGFR-in tissue preparations in patients diagnosed with DLBCL and their clinical features at the time of diagnosis, and response to treatment and prognosis.

Material and Method: A total of 44 patients with a diagnosis of DLBCL and 13 patients diagnosed with control reactive lymphadenopathy were included in this study. After immunohistochemical staining of the pathology preparations of the patient and control groups with VEGF, PDGFR-β and thrombospondin-1 stains, the clinical characteristics of the patients and the relationship between survival analysis and staining rates were statistically analyzed.

Results: When the patients were compared with the control group in terms of VEGF, PDGFR-β, and thrombospondin-1 staining rates, we found that staining with PDGFR-β was lower in patients (p=0.009). Although it was not statistically significant for PDGFR-β, it was observed that 5-year OS and PFS values were low in patients with high levels of expression, on the contrary, 5-year OS was low in patients with high thrombospondin staining rate. A negative correlation was observed between thrombospondin-1 and PDGFR-β (p=0.003, r=-0.440).

Conclusion: As a result, although no relationship was found between VEGF and survival in our study, it was observed that PDGFR-β and thrombospondin-1 were effective in prognosis. A negative correlation was observed between thrombospondin-1 and PDGFR-β.

Keywords: Diffuse large B cell lymphoma, thrombospondin-1, VEGF, PDGFR-β, prognosis

INTRODUCTION

Diffuse large B cell Lymphoma (DLBCL) is the most common subtype of Non-Hodgkin Lymphoma (NHL)(1). Angiogenesis is regulated by the balance between angiogenic and anti-angiogenic factors. Microvascular density and tumor angiogenesis were found to be poor prognostic factors in patients with DLBCL receiving anthracycline-based chemotherapy (2). VEGF (Vascular endothelial growth factor), and PDGF (Platelet derivated growth factor) are the leading angiogenic factors, and thrombospondin-1 is the leading antiangiogenic factor. In studies performed with serum VEGF levels of patients with a diagnosis of DLBCL, those with high serum VEGF levels were found to be associated with poor prognosis (3). In mice models with DLBCL, apoptosis in pericytes, one of the important elements of angiogenesis, and a decrease in tumor volume were observed with imatinib treatment targeting PDGFR-β (4). It was observed that a high level of thrombospondin expression in many tumor cell lines inhibits tumor cell angiogenesis and progression (5,6). The aim of this study is to immunohistochemically investigate the relationship between the staining rates of mainly antiangiogenic factor thrombospondin-1, VEGF, and PDGFR-in tissue preparations in patients diagnosed with DLBCL as a result of lymphadenopathy biopsy and their clinical features at the time of diagnosis, response to treatment and prognosis.

MATERIAL AND METHOD

The study was carried out with the permission of Kocaeli University Noninvasive Clinical Ethics Committee (Date: 13.01.2015, Decision No: 1-23). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

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Study Design and Data Collection

Between June 2007 and September 2014, 44 patients with DLBCL diagnosed by lymph node biopsy in the haematology department were included in the study. Patients whose treatment and polyclinic follow-ups were accepted in our department were included in the study. Pathology preparations of 13 patients for whom reactive hyperplasia was found in the lymph node biopsy and no other malignancies were taken as the control group.

The cut-off values of the laboratory parameters used in the study were chosen as the upper limit of their normal values. The term partial remission was defined as a total reduction of more than 50% of the product of the perpendicular dimensions of the measurable lesions, whereas the term complete remission is defined for patients who show complete recovery with no signs of disease in laboratory values and imaging after treatment (7).

Method of Staining

Immunohistochemical VEGF, PDGFR-β, and Thrombospondin stains were performed with the streptovidin-avidin-biotin method. The preparations were evaluated at 40 magnification under light microscopy with a hematologist and a pathologist were scored as 0, 1, 2, and 3 as per the staining ratio of the cells. The sections were deparaffinized by holding them in a 56oC incubator overnight. Deparaffinization was completed by keeping the sections taken in xylene for 15+15 (30) minutes after they were removed from the oven. Then the absolute alcohol was poured first, kept for 15 minutes in the second, and then the same procedure was performed with 96% ethyl alcohol and hydrated. Washed with distilled water for 5 minutes. The slides were placed in a microwave-resistant plastic bowl. 10% citrate buffer solution was prepared and placed on them (10 cc citrate buffer is prepared with 90 cc distilled water). The microwave oven was operated at maximum power (100%) for 10 minutes. At the end of the time, the power of the oven was reduced by 50% and operated for 5+5 minutes. After the preparations were taken out of the microwave oven, they were kept at room temperature for 20 minutes. They were washed with distilled water. The sections were kept in a mixture of 3% HO (Hydrogen peroxide) for 20 minutes to perform peroxidase blockage. Then the sections were washed with distilled water. After sections were kept in Phosphate buffer saline (PBS) for 15 minutes, they were placed in an immunostaining container and protein blockade was performed for 15 minutes. After washing, VEGF, PDGFR, and Thrombospondin stains were dropped and left for 2 hours incubation. After the preparations were taken into PBS and shaken, they were kept in PBS for 15 minutes for the second time. Goat-Anti-Polyvalent was dropped on the sections taken into the staining container and left for 20 minutes. Then, after the preparations were taken into PBS and shaken again, they were kept in PBS for 15 minutes for the second time. Streptovidin peroxidase was dropped on the sections taken into the staining container and left for 20 minutes. Tissues that were passed through PBS again were incubated with AEC chromogen for 20 minutes. The colored preparations were washed in distilled water and then kept in Mayer hematoxylin for 2 minutes. After washing with distilled water, soaked through ammonia water. After the sections were washed with distilled water again and dried, they were sealed with a suitable covering medium. The preparations evaluated at 40 magnification under light microscopy with a hematologist and a pathologist were scored as 0, 1, 2, and 3 as per the staining ratio of the cells.

Statistical Analyses

The evaluation of the results was made using the SPSS Version.22.0 program. Variables in the study were evaluated in terms of normal distribution using the One-Sample Kolmogorov-Smirnov test. Data that are in compliance with the normal distribution were given with arithmetic mean and standard deviations, and data that are not in compliance with the normal distribution were given with median values (25% -75% percent). Chi-square and Fisher tests were used for comparison of categorical data. Non-parametric Mann-Whitney test was used to evaluate ordinal data. Kaplan-Meier method and Log-rank test were used to calculate progression-free survival (PFS) and overall survival times (OS). Progression-free survival time was considered as the time from diagnosis to progression, and total survival time from the date of diagnosis to the date of patient death or the termination of follow-up period. $P<0.05$ values were considered statistically significant.

RESULTS

A total of 44 cases with a diagnosis of DLBCL, 24 male, 20 female, diagnosed as a result of lymph node biopsy in Kocaeli University Medical Faculty Hospital, Hematology Department between June 2007 and September 2014 and 6 male and 7 female, totally 13 lymphadenopathy pathology preparations compatible with reactive hyperplasia were taken as the control group.

The mean age was found to be 60.55±11.23 years (median 60 years) in the patient group and 42.53±14.52 years (median 44 years) in the control group. **Table 1** presents clinical and laboratory characteristics of the patient and control groups. When the patients were evaluated as per the IPI score, it was observed that 31.8% were low risk, 27.3% low-intermediate risk, 27.3% high-intermediate risk, and 13.6% high risk. When we examine the treatment protocols given

to the patients in primary care, 35 patients (79.5%) R-CHOP (Rituximab, Cyclophosphamide, Adriamycin, Vincristine, Prednisolone), 2 patients (4.5%) RCVP (Rituximab, cyclophosphamide, vincristine, methylprednisolone), 2 patients (4.5%) CHOP (Cyclophosphamide, adriamycin, oncovin, prednisone), 1 patient (2.3%) CVP (cyclophosphamide, vincristine, methylprednisolone), 1 patient (2.3%) received cyclophosphamide and methylprednisolone. VEGF, PDGFR- and thrombospondin staining rates were depicted in **Table 2.** PDGFR-β staining was statistically significantly lower in the patient group compared to the control group (p=0.009). In terms of staining with thrombospondin-1, a low rate of staining was found in 41 (93.2%) of the patients and a high rate of staining in 3 patients (6.8%). In the control group, low staining was detected in 12 patients (92.3%) and high staining in 1 patient (7.7%). There was no statistically significant difference was found between the two groups in terms of thrombospondin-1 staining rates.

Table 2. Comparison of patient and control groups according to thrombospondin-1, VEGF, PDGFR-β staining rates

	Total n (%)	Control n (%)	Patient n (%)	p
VEGF				0.742
0-1	38 (66.7)	8 (61.5)	30 (68.2)	
2-3	19 (33.3)	5 (38.5)	14 (31.8)	
PDGFR-β				0.009
0-1	36 (63.2)	4 (30.8)	32 (72.7)	
2-3	21 (36.8)	9 (69.2)	12 (27.3)	
Trombospondin-1				1.00
0-1	53 (93)	12 (92.3)	41 (93.2)	
2-3	4 (7)	1 (7.7)	3 (6.8)	

When the statistical relationship between VEGF, PDGFR-β, and Thrombospondin was investigated, a significant relationship was found between PDGFR-β and Thrombospondin (p=0.003, r=-0.440). **Table 3** presented a comparison of the general characteristics of the patients and the staining rates of VEGF, PDGFR-B, and Thrombospondin-1. It was observed that as the PDGFR-β staining rate increased, it was observed the thrombospondin-1 staining rate decreased. VEGF was found to be stained at a statistically significant higher rate in men than in women (p=0.029). When the VEGF, PDGFR-β, thrombospondin-1 staining ratios of the patients and IPI score, whether they have B symptoms, disease stage, bone marrow involvement, hepatomegaly, splenomegaly, bulky disease, extranodal involvement, refractory to primary care and relapse were compared, no statistically significant difference was found. It was observed that PDGFR-β was less stained in patients with high IPI scores and stages. Although those with a high PDGFR-β staining rate were mostly female, the rate of males was higher in low staining rates. Less bone marrow involvement, hepatomegaly, splenomegaly, bulky disease, extranodal involvement, and relapse were found in patients with high PDGFR-β staining. However, no statistically significant relationship was observed. Although most of the thrombospondin-1 stained ones were male, all of those highly stained were male. All patients with high thrombospondin-1 staining had B symptoms, and none of them had bone marrow involvement, hepatomegaly, bulky disease, extranodal involvement, refractoriness to treatment, or relapse.

Table 1. Clinical characteristics of the patients and the control group

	Patients	Control group	p
Number (%)	44 (77.1%)	13 (22.9)	
Age (Median (min-max))	60.5 (35-88)	42.53 (16-67)	<0.001
Gender (Male) (%)	24/20 (54.5)	6/7 (46.2)	0.594
LDH elevation (%) (LDH>220 U/L)	35 (79.5)	2 (15.4)	
B symptom positivity (%)	19 (43.2)		
ECOG ½ (%)	38/6 (86.4/ 13.6)		
Stage (n)	8/13/14/9		
I/II/III/IV (%)	(13.8/ 29.5/ 31.8/ 20.5)		
IPI (n)	4/10/12/12/6		
(0/1/2/3/4) (%)	(9.1/ 22.7/ 27.3/ 27.3/ 13.6)		
Hepatomegaly (%)	6 (13.6)		
Splenomegaly (%)	11 (25)		
Bone marrow infiltration (%)	4 (9.1)		
Extra-nodal involvement (%)	8 (18.2)		
Bulky disaese (%)	5 (11.4)		
Partial remission (%)	4 (9.1)		
Complete remission (%)	30 (68.2)		
Relapse (%)	5 (11.4)		
Mortality (%)	11 (25)		
Treatment steps taken (n)	38/4/2		
(I/II/III) (%)	(86.4/ 9.1 /4.5)		

Table 3. Comparison of the general characteristics of the patients and the staining rates of VEGF, PDGFR-B and Thrombospondin-1

	VEGF (0-1) n (%)	VEGF (2-3) n (%)	p	PDGFR (0-1) n (%)	PDGFR (2-3) n (%)	p	Thrombospondin (0-1) n (%)	Thrombospondin (2-3) n (%)	p
Gender			0.029			0.477			0.377
Female	17 (56.7)	3 (21.4)		13 (40.6)	7 (58.3)		20 (48.8)	0 (0)	
Male	13 (43.3)	11 (78.6)		19 (59.4)	5 (41.7)		21 (51.2)	3 (100)	
IPI			0.419			0.332			0,558
<3	16 (53.3)	10 (71.4)		17 (53.1)	9 (75)		25 (61)	1(33,3)	
≥3	14 (46.7)	4 (28.6)		15 (46.9)	3 (25)		16 (39)	2(66,7)	
B symptoms in diagnosis	17 (56.7)	8 (57.1)	1.00	19 (59.4)	6 (50)	0.828	22 (53,7)	3 (100)	0,247
Stage			0.596						0.599
≤2	13 (43,3)	8 (57.1)		14 (43.7)	7 (58.3)	0,601	19 (46.3)	2 (66.7)	
>2	17 (56,7)	6 (42.9)		18 (56.3)	5 (41.7)		22 (53.7)	1 (33.3)	
Bone marrow involvement in diagnosis	3 (10)	1 (7.1)	1.00	3 (9.4)	1 (8.3)	1.000	4 (9.8)	0 (0)	1.000
Hepatomegaly in diagnosis	6 (20)	0 (0)	0,155	5 (15.6)	1 (8.3)	1.000	6 (14.6)	0 (0)	1.000
Splenomegaly in diagnosis	8 (26.7)	3 (21.4)	1.000	7 (21.9)	4 (33.3)	0.457	10 (24.4)	1 (33.3)	1.000
Bulky disease in diagnosis	2 (6.7)	3 (21.4)	0,307	4 (12.5)	1 (8.3)	1.000	5 (12.2)	0 (0)	1.000
Extranodal involvement in diagnosis	7 (23.3)	1 (7.1)	0.402	7(21.9)	1 (8.3)	0.403	8 (19.5)	0 (0)	1.000
Refractory disease	3 (10)	1 (8.3)	1.000	4 (12.9)	0 (0)	0.563	4 (10)	0 (0)	1.000
Relapse on follow-up	3 (10)	2 (15.4)	0,630	3 (9.7)	2 (16.7)	0.608	5 (12,5)	0 (0)	1.000

Considering in terms of VEGF staining rates, the estimated 5-year OS in low-stained and high-stained patients was 71% in both groups. While the estimated 5-year PFS value was found to be 63% in low-stained patients, it was 73% in high-stained patients. Although it was not statistically significant in those highly stained with PDGFR-β, a decrease in both OS and PFS values was observed in the 5-year estimate. In the PDGFR-β low staining group, the estimated 5-year OS was 72% PFS was 69%, while the OS was found as 70% and PFS was 45% in the high staining group. The 5-year OS was found to be 66% in the thrombospondin-1 high-staining group, compared to 72% in the low-stained patients (**Table 4**).

Table 4. Comparison of VEGF, PDGFR-β, Thrombospondin-1 staining rates and overall and progression-free survival of patients

	5-year OS (%)	p	5-year PFS (%)	p
VEGF		0.689		0.473
Low (<2)	71%		63%	
High (≥2)	71 %		71%	
PDGFR		0.773		0.276
Low (<2)	72 (%)		69 (%)	
High (≥2)	70 (%)		45 (%)	
Thrombospondin		0.500		0.679
Low (<2)	72		64	
High (≥2)	66		66	

DISCUSSION

DLCHL is the most common subtype of NHL in adults and constitutes approximately 40% of cases. It has been observed that the prognosis is worse and the long-term survival is less in patients who do not fully respond to the initial treatment. Therefore, studies have been conducted to investigate more effective and less toxic chemotherapy drugs and biological agents for this specific and large patient group. Among the treatment targets of these studies, angiogenesis, which is of increasing importance and the main subject of many cancer treatment studies, is included (8). Our aim in this study is to immunohistochemically study the relationship between the staining rates of mainly antiangiogenic factor thrombospondin-1, angiogenic VEGF, and PDGFR-in tissue preparations in patients diagnosed with DLBCL as a result of lymphadenopathy biopsy and their clinical features at the time of diagnosis, response to treatment and prognosis.

In the present study, it was observed that most of the patients were stained with VEGF, although PDGFR-β was lower in patient group. We found a statistically significant negative correlation between thrombospondin-1 and PDGFR-β staining. Over-staining of VEGF and under-staining of thrombospondin-1 shows us that the balance in lymphoma favors angiogenetic factors.. In addition, the fact that PDGFR β staining rate is prominent while thrombospondin-1 staining rate is low supports this. In another study by Paydas et al. (9), TSP-1 expression rate was found to be 14.8% in the pathological preparations of 88 patients with DLBCL, and no relation with prognostic factors and survival could be demonstrated.

When the literature is reviewed, there are studies showing the relationship between serum VEGF level and progression and prognosis of cancers. VEGF expression in aggressive lymphoma subtypes such as DLBCL, peripheral T-cell lymphoma, mantle cell lymphoma, and primary effusion lymphoma have been shown to

be highly and slightly increased in chronic lymphocytic leukemia/small lymphocytic leukemia (CLL/SLL) (10-12). Especially in acute lymphocytic leukemia and lymphomas, it was found that some tumor cells expressing VEGFR-1 and VEGFR-2 are involved in the survival, metastasis, and proliferation of tumor cells by autocrine mechanisms (13,14). The current study revealed that VEGF was found to be low in those with low IPI scores, and higher staining rates in patient group. Contrary to this extranodal involvement, hepatomegaly, bulky disease, splenomegaly, bone marrow involvement, refractory, and relapse rates were found to be high in patients with low VEGF staining. In the study conducted by Riihijarvi et al. (15). in 102 high-risk patients under 65 years of age, it was found that serum VEGF levels were statistically high in patients with high IPI scores, and performance score and no relationship was found between serum VEGF level and gender, B symptom, bulky disease. Again, in the study conducted by Salven et al. on 200 patients with NHL, no significant relationship was found between serum VEGF levels and stage, bulky disease, presence of B symptoms, extranodal involvement, and histological grade, but a significant relationship was found between performance score (ECOG) and IPI (16).

In another study, Hazar et al. demonstrated that VEGF negative NHL patients had better treatment responses than VEGF positive NHL patients (17). In the study of Salven et al., it was shown that serum VEGF level is an independent indicator of poor prognosis for NHL patients. In a study conducted on 200 NHL patients, high pre-treatment serum VEGF levels (462 pg ml 7-1) were found to be associated with high LDH levels, low-performance status, and low survival. The 5-year survival rate was found to be 31% in those with high serum VEGF levels, it was found 61% in those with low serum VEGF levels ($p<0.001$). The 5-year survival was found as 30% in patients with high VEGF serum levels and 53% in patients with low VEGF ($p <0.001$) in patients with DLBCL (n=78) (16). In our study, no significant relationship was found between VEGF expression at the tissue level and overall survival and progression-free survival.

Although the prognostic value of VEGF expression at the tissue level in patients with DLBCL is uncertain, it has been associated with poor prognosis in studies with serum VEGF levels (3,18). We thought that one of the reasons for its uncertain expression at the tissue level may be due to the fact that VEGF interacts with many inflammatory processes, and there are many factors affecting its expression at the tissue level.

When PDGFR-β staining rates were compared with demographic data, it was observed that patients with higher IPI scores and stages had less staining. When we looked at the survival analysis, we noticed that the group with a high PDGFR-β staining rate tended to have lower both OS and PFS values. Studies have also found conflicting results regarding angiogenic factors related to NHL depending on the heterogeneous population, tissue sample, or serum sample taken. Agreeably it was found that the rate of PDGFR-β staining was higher in the control group. It can be considered that this contradictory result may be influenced by the angiogenetic environment in reactive lymphadenopathy in the control group There are not many studies on the relationship between PDGFR-β expression in tissue preparations and prognosis in patients with DLBCL. As a result of its ligand binding to PDGFR-β, the PDGF receptor, the tyrosine kinase pathway is activated, which induces cell proliferation, differentiation, and migration. Tyrosine kinase inhibitors such as imatinib and sunitinib targeting PDGFR – β have been shown to be effective in some solid tumors by decreasing pericyte density around the vessel and weakening angiogenesis (19,20). In neonatal mouse models in which PDGFR was functional blockade, it was observed that a number of vascular smooth muscle cells were inhibited, apoptosis of vascular endothelial cells was induced and glomerular vascular network formation was negatively affected (21). In the study of Ruan et al. which they performed with imatinib targeting PDGFR -β in mouse lymphomas, they have demonstrated antiangiogenic effects in pericytes. It was observed that in mice with 3 types of DLBCL models, with 2-3 weeks of imatinib treatment, PDGFR -β + pericytes apoptosis and a significant decrease in tumor volume were observed. It was thought that the decrease in pericytes expressing PDGFR-β was due to increased apoptosis of CD 31+ vascular endothelial cells and decreased tumor vascularity (4).

TSP-1 is a multifunctional protein found in many biological processes such as angiogenesis, apoptosis, TGF-beta activation, and immune regulation. In some studies, thrombospondin has been shown to be a negative regulator of tumor progression and angiogenesis. In the current study, it was observed that the 5-year OS value in the group with a high level of thrombospondin-1 staining was lower than the patients with a low level of staining, although it was not statistically significant. It was observed that a high level of thrombospondin expression in tumor cell lines such as breast, skin, colorectal, glioblastome, and hemangioblastoma inhibits tumor cell angiogenesis and progression (5,6). On the other hand, thrombospondin is found as an adhesive protein in the extracellular matrix in many epithelial cancers and has been shown to be effective in cancer progression. Because thrombospondin-1 has been shown to activate the plasminogen/plasmin system in many adenocarcinoma models and increase tumor progression and metastasis (22). In the study of

Paydas et al. (23) on tissue preparations of 177 NHL patients, it was found that thrombospondin expression was associated with aggressive morphology. In addition, patients expressing both thrombospondin and survivin were shown to have both aggressive morphology and shorter OS. Furthermore, no relation was found between B symptoms, extranodal involvement, hepatomegaly, splenomegaly, and performance score used in daily practice.

This study has some limitations. The patients used as a control group in our study underwent lymph node excisional biopsy with suspicion of lymphoma and were evaluated as reactive lymph nodes on pathological examination. It should be kept in mind that factors such as viral infection that may cause reactive lymph nodes may impair the angiogenetic expression profile.

CONCLUSION

In this study, it was observed that the rate of VEGF staining was higher in patients with anemia, leukopenia, and lymphopenia with prognostic importance. Although it was not statistically significant 5-year OS and PFS values were low in patients with high levels of expression PDGFR-β and thrombospondin-1. However, another conclusion to be drawn from our study is that angiogenetic factor expression should not be used in the differentiation of reactive/tumoural lymph node enlargement. Special agents specific to the person and the type of DLBCL may be developed in the future with studies that target VEGF, PDGFR-β, and thrombospondin-1, with genetic studies and examining multiple immunohistochemical markers, in which there is a larger patient and control group for treatment in patients with DBBHL.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Kocaeli University Noninvasive Clinical Ethics Committee (Date: 13.01.2015, Decision No: 1-23).

Informed Consent: All patients signed the free and informed consent form.

Referee Evaluation Process: Externally peer-reviewed.

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

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Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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PUBLICATION RULES, PUBLICATION POLICY, GENERAL PRINCIPLES AND SUBMISSION RULES

AUTHOR GUIDELINES

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