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Research article

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Changes in dual energy X-ray absorptiometry parameters in postmenopausal women with osteoporosis who received at least 12 months of denosumab treatment

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All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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Abstract

Background/Aim: Denosumab is a human monoclonal antibody that binds to the receptor-activated nuclear factor kappa beta ligand (RANKL). Densosumab leads to a reduction in bone resorption by inhibiting RANKL and has been approved for treating postmenopausal osteoporosis (OP). The present study investigated real life data by evaluating the demographic data of postmenopausal patients with OP who received denosumab treatment and the changes in dual energy x-ray absorptiometry (DEXA) parameters before and after denosumab treatment.

Methods: This retrospective cohort study included 49 postmenopausal female patients followed in our OP outpatient clinic who were treated with 60 mg subcutaneous denosumab every six months for at least 12 months. The study retrospectively analyzed and recorded patient age, body mass index, age of menopause, fracture history, antiresorptive and/or anabolic drug treatment history, and pre- and post-denosumab T-scores in addition to L1–4, femoral neck, and total hip bone mineral densities (BMDs) on DEXA scans. The changes that occurred before and after the treatment in addition to those that occurred after the treatment based on whether previous anabolic or antiresorptive agents had been used were statistically compared.

Results: The L1–4 and total hip T-scores and L1–4 and total hip BMD values measured prior to denosumab treatment showed a statistically significant increase after denosumab treatment ($P < 0.001$, $P = 0.002$, $P = 0.028$, and $P = 0.002$, respectively). No statistically significant changes in the femoral neck T-score and BMD after denosumab treatment compared to that before denosumab use ($P = 0.056$ and $P = 0.138$, respectively) were found. Furthermore, no statistically significant difference between the pre- and post-denosumab DEXA parameters in the patients who used antiresorptive agents and those who did not ($P > 0.05$) was found. Additionally, pre- and post-denosumab parameters were not statistically significantly different between those who received and did not receive anabolic therapy before denosumab ($P > 0.05$).

Conclusion: Denosumab treatment for postmenopausal OP leads to a significant increase in lumbar and total hip T-scores and BMDs.

Keywords: Osteoporosis, Denosumab, Dual energy X-ray absorptiometry

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Introduction

According to the World Health Organization, osteoporosis (OP) is defined as bone mineral density (BMD) T-scores < −2.5 standard deviations (SD) on a dual energy x-ray absorptiometry (DEXA) scan [1]. OP is a systemic skeletal disease characterized by an increase in the risk of fracture due to defects in bone microarchitecture [2]. In the FRACTURK study, the prevalence of OP in women over 50 years in Turkey was reported to be 12.5%, and the risk of hip fracture was reported to be 14.5% [3]. Osteoporotic fractures adversely affect the quality of life as OP causes pain and impairs functional capacity as a result of its negative effects on the musculoskeletal system and body posture [2]. When OP is not detected and treated properly, the economic burden of OP-related fractures on the Turkish healthcare system increases as it is on the other countries in the world [4]. Antiresorptive agents (bisphosphonates and denosumab) that act by reducing bone resorption, and teriparatide, a recombinant human parathyroid hormone with an anabolic effect, are among the main agents used today in the pharmacological treatment of OP [5]. Postmenopausal estrogen deficiency causes an increase in the exposure of receptor-activated nuclear K (RANK) B receptors on the surface of osteoclasts to RANK ligand and consequently increases bone resorption and bone loss. Denosumab is a highly potent IgG2 human monoclonal antibody that binds to the RANK ligand via a mechanism resembling the action of osteoprotegerin that prevents the ligand from binding to the RANK receptor. Denosumab is administered at a dose of 60 mg via subcutaneous injections once every six months. Studies have reported that it causes an increase in bone density by causing a decrease in osteoclastic activity and bone resorption, thereby resulting in reduction of new vertebral and nonvertebral fractures [6–8]. Our study aimed to present the demographic characteristics of postmenopausal OP patients who received regular denosumab injection for at least 12 months in our clinic in addition to real-life data that we obtained by examining the changes in DEXA measurement parameters.

Materials and methods

The study included 49 postmenopausal female patients who were followed up in our OP outpatient clinic and administered 60 mg of denosumab subcutaneously every six months for at least 12 months. Power analysis was performed to determine the number of samples. A sample size of 34 was determined to be sufficient assuming that α was 0.05, effect size was 0.50, and power was (1 β) 0.80. G power (Version 3.1.9.6) was used for this calculation. Patients with diseases of bone metabolism, such as Paget's disease, osteomalacia, primary hyperparathyroidism, hyperthyroidism, malignancy, and malabsorption were excluded from the study. Those with T scores < −2.5 standard deviations (SDs) at three sites (total lumbar, total hip, or femoral neck) on DEXA scans were defined as having OP. This research was approved by University of Health Sciences Istanbul Fatih Sultan Mehmet Training and Research Hospital Ethics Committee (12.11.2020/ KAEK 2020/113) and the study was conducted in accordance with the Declaration of Helsinki. The files of the patients were retrospectively reviewed. Patients' ages, body mass indices (BMIs), ages at menopausal, fracture histories, and antiresorptive and/or anabolic drug treatment histories in addition to their L1–4, femoral neck, and total hip T-scores and BMD values on DEXA scans before and after denosumab injections were recorded.

The changes that occurred before and after the treatment and the changes that occurred after the treatment based on whether there had been an anabolic or antiresorptive agent used prior to the treatment were compared statistically.

Statistical analysis

The IBM SPSS Statistics 22 (IBM SPSS, Turkey) program was used for statistical analysis of the findings obtained in the study. While evaluating the study data, the conformity of the parameters to the normal distribution was evaluated using the Shapiro–Wilk test. Along with the descriptive statistics (mean, SD, and frequency) used in the data analysis, Student's t-test was also used for comparing two groups with normally distributed quantitative data. A paired samples t-test was used for the before/after comparisons of normally distributed quantitative data. Moreover, $P < 0.05$ was considered as statistically significant.

Results

The study was conducted with 49 postmenopausal women aged 48 to 92 years who were followed up in our OP outpatient clinic between 2014 and 2020. The mean age of the cases was 68.63 (8.27) years, and the mean BMI was 26.99 (4.76) kg/m^2. Menopausal ages ranged from 27 to 62 years with a mean of 47.22 (8.07) years. The months in which control DEXA was performed after denosumab ranged from the 12th to the 54th month with a mean of 23.8 (12.3) months. A total of 32.7% of the patients had at least one vertebral compression fracture, 4.1% had femur fractures, 24.5% had vertebral and non-femoral fractures, 93.9% had a history of antiresorptive drug use before denosumab, and 20.4% had a history of anabolic drug use before denosumab. Although 65.3% of these patients continued their treatment again with denosumab after the initial denosumab administration, 26.6% continued with antiresorptive treatment instead. The type of treatment that was continued after denosumab administration is unknown in 8.2% of the patient population (Table 1).

Table 1: Demographic data and clinical characteristics of the cases

		Min/Max	Mean /SD
Age		48/92	68.63 /8.27
BMI		16.8/36.5	26.99 /4.76
Menopausal age		27/62	47.22 /8.07
Months during which control DEXA was performed after denosumab		12/54	23.85 /12.3
		n	%
Vertebral compression	Yes	16	32.7
	No	33	67.3
Femur fracture	Yes	2	4.1
	No	47	95.9
Other fractures	Yes	12	24.5
	No	37	75.5
Antiresorptive use	Yes	46	93.9
before denosumab	No	3	6.1
History of anabolic treatment	Yes	10	20.4
before denosumab	No	39	79.6
Post-denosumab treatment	Denosumab continued	32	65.3
	Antiresorptive therapy	13	26.5
	Unknown	4	8.2

Min: minimum, Max: maximum, SD: standard deviation, BMI: body mass index, DEXA: dual energy X-ray absorptiometry

The increase in the T-score at L1–4 after denosumab administration versus the scores before denosumab administration was found to be statistically significant ($P < 0.001$ versus $P < 0.01$). Compared to the values prior to denosumab administration, the increase in the BMD values at L1–4 after denosumab was also statistically significant ($P = 0.028$). No statistically significant changes in T-scores and BMDs at the femoral neck were observed after denosumab administration compared with the values before administration ($P = 0.056$ and $P = 0.138$, respectively). The increase in total hip T-scores after starting denosumab treatment was found to be statistically significant compared to the scores before denosumab administration ($P = 0.002$). Moreover, the increase in the total hip BMD values after denosumab administration was found to be statistically significant compared to the values before denosumab administration ($P = 0.002$) as shown in Table 2.

Table 2: Comparison of bone mineral density (BMD) measurements of the cases before and after denosumab treatment

		Before denosumab		After denosumab		*P*-value
		Min/Max	Mean/SD	Min/Max	Mean/SD	
L1–4	T score	−3.8/−0.8	−2.6/0.66	−3.7/−0.1	–2.26/0.77	<0.001*
	BMD	0.68/1.05	0.82/0.08	0.6/2.08	0.89/0.21	0.028*
Femoral neck	T score	−3/−0.6	−1.99/0.63	−3.2/0	–1.87/0.62	0.056
	BMD	0.51/0.87	0.71/0.08	0.56/0.95	0.72/0.08	0.138
Total hip	T score	−3.1/−0.5	–1.79/0.68	−3.1/−0.5	–1.71/0.65	0.002*
	BMD	0.6/0.91	0.76/0.09	0.61/0.91	0.77/0.08	0.002*

BMD: bone mineral density, Min: minimum, Max: maximum, SD: standard deviation, Paired samples t test *$P < 0.05$

The pre- and post-denosumab L1–4 T scores, L1–4 BMDs, total hip T-scores and total hip BMDs were not statistically significantly different between patients who used antiresorptive agents and those who did not ($P = 0.427$, $P = 0.765$, $P = 0.110$, and $P = 0.11$, respectively). The femoral neck pre-denosumab T-scores and BMD values in those using antiresorptive agents before denosumab were found to be statistically significantly higher than those who did not use antiresorptive agents before denosumab treatment ($P = 0.013$ and $P = 0.020$ respectively); however, no significant differences in the same parameters after denosumab administration ($P = 0.081$ and $P = 0.093$ respectively) were noted (Table 3).

The pre- and post-denosumab L1–4 T scores ($P = 0.403$ and $P = 0.916$, respectively) and BMDs ($P = 0.251$ and $P = 0.473$, respectively), femoral neck T scores ($P = 0.504$ and $P = 0.600$, respectively), and BMDs ($P = 0.327$ and $P = 0.424$, respectively) did show pre- and post-treatment statistical differences. However, total hip T scores ($P = 0.668$ and $P = 0.684$, respectively) and corresponding BMDs ($P = 0.582$ and $P = 0.474$, respectively) did not demonstrate statistically significant differences between the patients who received anabolic agents before denosumab and those who did not (Table 3).

Table 3: Comparison of the BMD values after denosumab treatment based on the use of antiresorptive or anabolic treatment before denosumab

			Antiresorptive use before denosumab			Anabolic therapy use before denosumab		
			Yes Mean/SD	No Mean/SD	*P*-value	Yes Mean/SD	No Mean/SD	*P*-value
L1–4	T score	Before denosumab	−2.62/0.67	−2.3/0.35	0.427	−2.75/0.65	−2.55/0.66	0.403
		After denosumab	−2.27/0.8	−2.13/0.38	0.765	−2.29/0.95	−2.26/0.74	0.916
	BMD	Before denosumab	0.82/0.08	0.87/0.04	0.332	0.8/0.07	0.83/0.08	0.251
		After denosumab	0.89/0.22	0.89/0.05	0.988	0.97/0.43	0.86/0.1	0.473
Femoral neck	T score	Before denosumab	−1.92/0.6	−2.83/0.21	0.013*	−2.13/0.58	−1.95/0.65	0.504
		After denosumab	−1.82/0.6	−2.47/0.47	0.081	−1.99/0.39	−1.85/0.66	0.600
	BMD	Before denosumab	0.72/0.08	0.61/0.03	0.020*	0.68/0.09	0.72/0.08	0.327
		After denosumab	0.73/0.07	0.65/0.06	0.093	0.7/0.05	0.73/0.08	0.424
Total hip	T score	Before denosumab	−1.74/0.68	−2.4/0.44	0.110	−1.89/0.85	−1.77/0.64	0.668
		After denosumab	−1.66/0.64	−2.27/0.55	0.121	−1.8/0.71	−1.69/0.65	0.684
	BMD	Before denosumab	0.77/0.09	0.69/0.06	0.131	0.75/0.11	0.77/0.08	0.582
		After denosumab	0.78/0.08	0.71/0.07	0.122	0.75/0.08	0.78/0.08	0.474

BMD: bone mineral density, SD: standard deviation, Students t test *$P < 0.05$

Discussion

Initiating pharmacological treatment in OP patients who present an increase in risk of fractures is recommended. Although bisphosphonates have been the first-line treatment in the treatment algorithm for many years, denosumab treatment is among the first-line treatment options as an alternative to bisphosphonates [9]. Especially when compared to oral bisphosphonates, denosumab is a treatment with higher patient compliance [10, 11]. In their retrospective study, Cairoli et al. [12] compared findings in patients who received postmenopausal OP treatment with denosumab with those in patients who received postmenopausal OP treatment with oral bisphosphonates. At the end of 24 months, those who received denosumab treatment were found to have a higher reduction in alkaline phosphatase, higher increase in BMD, and lower incidence of new fractures and treatment unresponsiveness.

In our study, the mean age, BMI, and mean menopausal age of our cases were found to be consistent with those reported in literature [8]. According to the short- and long-term findings of the FREEDOM study, denosumab treatment leads to suppression of osteoclastic activity, slowing down of the bone remodeling process, and an increase in the total lumbar, total femur, and femoral neck BMDs in proportion to the duration of use, thus leading to a reduction in the risk of new vertebral fractures by 68%, nonvertebral fractures by 20%, and hip fractures by 40% [6, 8, 13]. The results from a transiliac biopsy performed in 41 patients who received denosumab treatment for five years showed that the bone quality of the patients was natural, and their bone turnover was low. This result, in line with literature, supports the effectiveness of denosumab treatment in producing an increase in BMD and reduction in the incidence of fractures [14]. Another study reported that changes in assessment and follow-up BMD values and T-scores on DEXA scans were strong indicators of fracture risk in cases undergoing denosumab treatment [15]. In our study, the average scan time after the treatment was approximately 23 months, and although the total lumbar and total hip BMDs and T-scores increased in our cases after denosumab treatment in line with results reported in the

literature, no increase was observed in femoral neck BMDs and T-scores, a result that is in contrast with that observed in literature. This finding could be explained by the fact that the treatment and follow-up periods of the cases in similar studies in literature were longer than those of our cases.

Several randomized controlled studies have evaluated the safety and the efficacy of denosumab and have found it to be generally well tolerated; it has also been reported that the frequency of possible side-effects, such as cancer, cardiovascular diseases, delayed fracture healing, hypocalcemia, development of opportunistic infections, neutralizing antibody formation, atypical femur fracture, and/or osteonecrosis of the jaw, did not increase compared to placebo. [6, 8, 13]. In a study evaluating the effects of denosumab treatment on fracture healing, denosumab was administered to patients with nonvertebral fractures within six weeks before and after the fracture, and no delay in fracture healing nor increased nonunion was observed compared to the placebo group [16]. However, it has been reported that the frequency of eczema increased compared to placebo [6].

Bisphosphonates accumulate in bone, whereas denosumab does not. Denosumab causes a rapid decrease in total lumbar, total hip, and femoral neck BMDs and a rapid increase in bone turnover markers in accordance with its mechanism of action, in case of treatment discontinuation [7, 17–19]. For this reason, re-examining patients receiving denosumab treatment for any fracture risk after five years and extending the treatment to 10 years in those with high fracture risk or switching to an alternative treatment, such as bisphosphonates, is recommended. In patients with low fracture risk, if cessation of denosumab treatment is desirable, discontinuing such treatment and developing an alternative treatment plan to manage the rapid BMD decrease and the potential vertebral fracture risk increase is recommended [19, 20]. Although the mean evaluation period of the cases in our study was two years, which is shorter than that of the existing studies, 65.3% of our cases continued with denosumab, 26.5% with antiresorptive agents, and 8.2% of the cases could not be followed.

Bisphosphonates are contraindicated in some cases, especially those in which the glomerular filtration rate is as low as <30 ml/min. On the other hand, although denosumab may be preferred in OP cases with chronic renal failure, exercising caution in terms of the risk of hypocalcemia is recommended [21, 22]. A retrospective study by Fraser et al. examined the changes in BMD after denosumab treatment administered to patients who had previously received bisphosphonate therapy and the effect of chronic renal failure on this change. According to the results reported in this study, denosumab treatment after bisphosphonates led to an increase in total lumbar, total hip, and femoral neck BMDs, whereas denosumab response was reported to be lower in terms of femoral neck BMD in proportion to the elevation in serum parathormone concentrations caused by chronic renal failure [23]. As the prevalence and duration of denosumab use in the treatment of OP increased, transitions between other treatments for OP and denosumab has gained further importance. In our study, the treatments that the cases received before denosumab were also evaluated, and it was seen that most cases, namely, 93.9% received antiresorptive treatment before starting denosumab. Consistent with the current study, a similar increase was observed in the total hip and total lumbar BMDs, except for the femoral neck BMD and T-scores in another study. In our study, pre- and post-denosumab BMD values and T-scores on DEXA scans were compared in patients with and without a history of antiresorptive treatment. Although only the femoral neck pre-denosumab BMD and T-scores were statistically higher in patients with a history of bisphosphonate treatment, no difference between the groups after treatment was observed. Again, no difference between those with and without a history of bisphosphonate use in terms of the total hip and total lumbar BMDs and T-scores before and after starting denosumab was noted. However, the results provide insufficient information as the number of patients who did not receive bisphosphonate therapy was very small. Furthermore, in our study, whether the cases received anabolic treatment history before the treatment or not was evaluated, but no significant difference was observed between the groups. According to the results of a randomized controlled trial that evaluated the outcome of the transition between denosumab and teriparatide treatments, BMD values continued to increase when switching from teriparatide to denosumab treatment in patients receiving postmenopausal OP treatment, whereas switching from denosumab to teriparatide treatment resulted in a progressive or temporary decrease in BMD [24].

Limitations

As our study was retrospectively conducted and did not have a control group, our primary limitations are not fully revealing the side-effects of the cases that received treatment, the short follow-up durations of the cases, and the small number of cases herein. However, it is notable that our study presents real-life data concerning denosumab use in postmenopausal OP treatment in Turkey. In Turkey, the need for randomized controlled clinical studies with longer follow-up periods and larger patient groups exists with the aim of demonstrating the efficacy of denosumab treatment and how the efficacy of treatment is affected in transitions between denosumab and other treatments.

Conclusion

Denosumab treatment may be an effective treatment option for postmenopausal OP as it leads to an increase in BMD values and T-scores on DEXA scans. As denosumab is one of the first-line treatments for OP, treatment transitions between denosumab and other antiresorptive or anabolic agents have also gained importance. This study provides real-life data addressing denosumab therapy, which has an important place in the treatment of osteoporosis.

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Research article

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Reclassification as non-invasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP): A retrospective review in a single institution and outcome study

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Ethics Committee Approval
The study was approved by Ethics Committee of Kayseri Training and Research Hospital, Turkey (Protocol No:652/2022).
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

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No conflict of interest was declared by the authors.

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Abstract

Background/Aim: Since non-invasive follicular thyroid neoplasm (NIFTP) was first defined in 2016, past overtreatment status, impact for the risk of malignancy, and incidence of NIFTP have been the subject of study. Retrospective cohort studies have been published and present widely varying results in different geographic regions. This study aimed to reclassify follicular variants of papillary thyroid carcinoma (FVPTC) cases diagnosed in a single center using the defined stringent NIFTP criteria and to determine incidence, clinicopathological features, and survival of NIFTP cases.

Methods: This retrospective cohort study was conducted in a single center and consisted of patients with diagnosed follicular variant papillary thyroid carcinoma in thyroidectomy/thyroid lobectomy specimens between 2014 and 2021. Reports of FVPTC cases between 2014 and 2018 were evaluated by two experienced pathologists to identify candidates for NIFTP. Archived glass slides of the potential NIFTP cases were retrieved and reviewed independently by two pathologists.

Results: Between 2014 and 2021, 84 patients who underwent surgery were diagnosed with FVPTC. Reports of 49 patients diagnosed before 2018 were re-evaluated by two pathologists, and 20 cases were identified as candidates for NIFTP. After blind evaluation of pathology slides, five cases (10%) were diagnosed as NIFTP according to the criteria established before 2016, and two cases between 2016 and August 2018 were still diagnosed as NIFTP. Fourteen patients were diagnosed with NIFTP between 2014 and 2021. The median follow-up of the NIFTP patients was 4.3 years, and no recurrence and/or metastasis was reported.

Conclusion: NIFTP represents 7.6% of the papillary thyroid carcinoma (PTC) cases in our cohort, which is higher than the incidence rate in our country. The follow-up results of our cases were uneventful considering the indolent nature of NIFTP, but we had high thyroidectomy rates. Due to the concomitant PTC, multifocality, and uncertainties in the follow-up routine, we think it would be appropriate for these patients to remain in active follow-up.

Keywords: Noninvasive follicular thyroid neoplasm with papillary-like nuclear features, Thyroid cancer, Follicular thyroid neoplasm, Incidence, Outcome

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Introduction

Despite the increase in the incidence of papillary thyroid cancer (PTC) over the last 30 years, mortality rates from PTC have remained stable [1]. Increased ultrasonographic scans and fine-needle aspiration biopsy (FNAB) rates result in overdiagnosis and unnecessary treatment. One of the reasons for this situation is the presence of low-grade/non-aggressive tumors within the PTC subgroup. FVPTC indicates a predominately follicular growth pattern with nuclear features of classic PTC [2, 3]. FVPTC is the least aggressive subtype of PTC and has shown the highest increase rate in recent years [4, 5]. FVPTC is classified as infiltrative/ non-encapsulated and encapsulated FVPTC (E-FVPTC) [6]. The infiltrative FVPTC may be associated with recurrence or metastasis and shows a molecular profile similar to classic PTC and E-FVPTC that exhibits indolent behavior and is often associated with a molecular profile seen in follicular neoplasms [7, 8]. The Endocrine Pathology Society Working Group examined E-FVPTC in 2016, and the terminology of non-invasive follicular thyroid neoplasm (NIFTP) with papillary-like nuclear features was defined along with new diagnostic criteria [9]. The diagnosis of NIFTP is based on the absence of invasion along with other histological criteria, including nuclear and architectural features. The indolent nature of NIFTP, based on this definition, allows for less radical treatment, and this terminology change is expected to reduce overtreatment and the psychological burden associated with a thyroid cancer diagnosis [9]. Patients treated for such tumors are expected to have an excellent prognosis. Rosario et al. [10] reported no NIFTP-related deaths, but a case series with one pulmonary metastasis and lymph node metastases has been reported. It has also been reported that the cases should be followed up as low-risk PTC or that current PTC follow-up routines are unnecessary [11–13]. Canberk et al. [14] reported that concomitant tumors in the contralateral lobe were not negligible (18%), and most were malignant.

The goal of this study was to retrospectively review and reclassify FVPTC cases diagnosed in a single center using the defined stringent NIFTP criteria. It also aims to determine the incidence of NIFTP and examine the clinicopathological features and survival of the cases that were diagnosed with NIFTP.

Materials and methods

The Institutional Review Board approved the study at Kayseri Training and Research Hospital (Protocol No: 652/2022). A retrospective review was performed of diagnosed papillary carcinoma in thyroidectomy/thyroid lobectomy specimens in Kayseri City Training and Research Hospital Pathology Clinic between 2014 and 2021. During this period, a search of the hospital medical record system was done using several keywords: (1) “thyroid”, (2) “follicular variant”, (3) “encapsulated”, and (4) “papillary thyroid carcinoma” for the index lesion.

Follicular thyroid cancers were excluded, and FVPTC or NIFTP pathology reports were retrieved to identify possible cases of NIFTP. After that step, reports of those cases diagnosed with FVPTC between 2014 and 2018 were evaluated by two experienced endocrine-specific pathology specialists to identify candidates for NIFTP. Archived glass slides of the potential NIFTP cases were retrieved and reviewed independently by two pathologists. The modified current criteria revised in 2018 by Nikiforov et al. [15] for NIFTP were used (Table 1). Since no BRAFV600E mutation information about the patients was available, they were not included in the evaluation. Locoregional recurrence or metastasis during the follow-up period was defined as an adverse event.

Table 1: Consensus diagnostic criteria for Noninvasive follicular thyroid neoplasm with papillary-like nuclear features (NIFTP), adapted from Nikiforov et al. [15]

Revised diagnostic criteria for NIFTP	Exclusion criteria
Encapsulation or clear demarcation	Any true papillae
Follicular growth pattern with:	Psammoma bodies
No well-formed papillae	Infiltrative border
No psammoma bodies	Tumor necrosis
<30% solid/trabecular/insular growth pattern	High mitotic activity
Nuclear score 2–3	Cell/morphologic characteristics of other variants of papillary thyroid cancer
No vascular or capsular invasion	
No tumor necrosis or high mitotic activity	

Statistical analysis

To summarize data obtained in the study, descriptive statistics were given as mean (standard deviation [SD]) and minimum–maximum (min–max) depending on the distribution of the continuous variables. Categorical variables were summarized as numbers and percentages. The Shapiro–Wilk test controlled the normality test of the numerical variables. Chi-squared and Fisher’s exact tests were used to calculate the categorical demographic characteristics of the patients. Analyses were performed with IBM SPSS Package Program version 24.0 (IBM Corporation, Armonk, NY, USA).

Results

Between 2014 and 2021, 84 out of 184 cases of papillary carcinoma with FVPTC were diagnosed. Pathology slides from 20 patients with possible NIFTP in 49 patients with FVPTC diagnosed in 2018 and before were reviewed by two experienced pathologists. Five cases were diagnosed as NIFTP according to the new criteria before 2016. Two cases between 2016 and 2018 were still diagnosed as NIFTP when re-evaluated according to the revised criteria in August 2018. The patient selection diagram and exclusion criteria of patients not accepted as NIFTP are shown in Figure 1. The clinical features of all cases are summarized in Table 2. Total thyroidectomy was performed on all patients (one with lobectomy had previously undergone contralateral lobe surgery). NIFPT represents 16.7% of FVPTC and 7.6% of PTC in our cohort of all PTCs from 2014 to 2021.

Table 2: Clinical features of cases reclassified as NIFTP

	Age	Sex	FNAC Bethesda classification	Surgery	Tumor size (mm)	Duration of follow-up (years)	RAI	Contralateral lesion
1	64	F	2	Total thyroidectomy	22	6	No	Nodular colloidal goiter
2	61	F	1	Total thyroidectomy	23	5	Yes	Papillary microcarcinoma**
3	50	F	0	Total thyroidectomy	13	6	Yes	No
4*	49	F	#	Lobectomy	10	7	No	No
5	59	M	#	Total thyroidectomy	12	8	No	No
6	44	F	2	Total thyroidectomy	15	4	Yes	Nodular colloidal goiter
7	44	M	4	Total thyroidectomy	45	4	Yes	Follicular adenoma

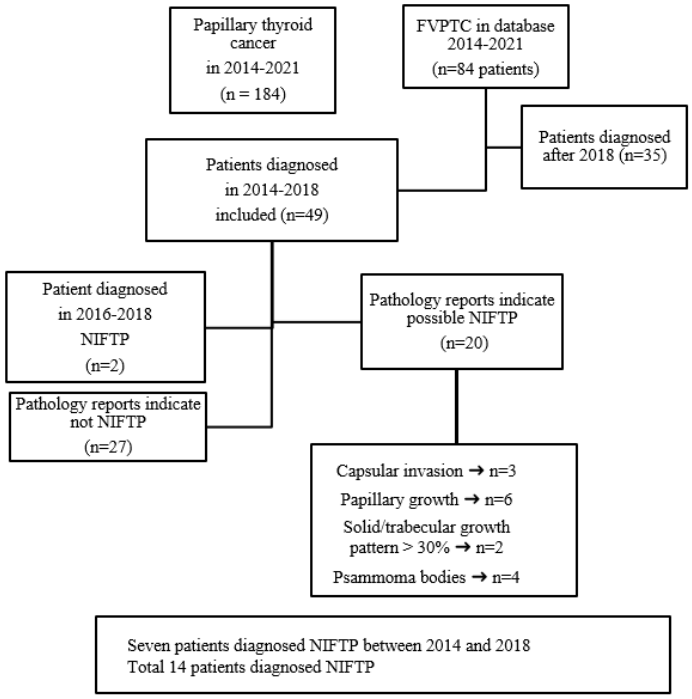
FNAC: Fine Needle Aspiration Cytology, RAI: Radioactive iodine treatment, F: Female, M: Male, * Patient with previous lobectomy, ** The other lobe tumor size 3 mm, #: Unknown

Clinicopathologic findings of NIFTP

A total of 14 patients were diagnosed with NIFTP between 2014 and 2021. The mean age of patients was 51 (8.0) years, and 78.6% of the patients were women. The mean size of the lesions was 18.2 (10.0) mm, the minimum tumor diameter was 8 mm, and the maximum was 45 mm. Six nodules were located in the right lobe and 8 in the left lobe.

Total thyroidectomy was performed in 11 (88,6%) patients, and lobectomy was performed in three patients (one had previously undergone a lobectomy on the contralateral side). The other two patients did not undergo a completion thyroidectomy to the contralateral lobe. Fine needle aspiration biopsy (FNAB) results had been obtained for 10 patients, and out of these results, three were undetermined significance, one had a follicular neoplasm, two were positive for PTC, and four had negative results.

Figure 1: Patient selection diagram. FVPTC, follicular variant of papillary thyroid carcinoma; NIFTP, noninvasive follicular thyroid neoplasm with papillary-like nuclear features



Outcome of NIFTP

The median follow-up of the NIFTP patients was 4.3 (1.86 [range 2–8]) years. Among them, seven (50%) had at least three years of follow-up (diagnosed before 2018), and no recurrence or metastasis was reported. Incidental concomitant micropapillary carcinoma focus on the contralateral lobe was observed in two patients and follicular adenoma was observed in one patient. It was observed that four patients who underwent total thyroidectomy were given radioactive iodine treatment (RAI) treatment. One of these patients had a concomitant papillary microcarcinoma focus, and the other had follicular adenoma.

Discussion

In the first study by Nikiforov et al. [9], 109 patients with a non-invasive encapsulated follicular variant of PTC (67 patients treated with only lobectomy without RAI ablation treatment) were alive with no evidence of disease at their follow-up periods (median of 13 years). Otherwise, an adverse event was seen in 12% of invasive E-FVPTC cases, including distant metastasis and disease-related mortality. Based on these results, the first time the term “non-invasive follicular thyroid neoplasm with papillary-like nuclear features” (NIFTP) was used, and this change also was adopted by the World Health Organization in 2017 [16]. This change in diagnostic terminology aimed to reduce overtreatment and eliminate the psychosocial issues associated with a cancer diagnosis.

Another contribution of this new modification is that less total thyroidectomy is required due to the indolent nature of NIFTP, and lobectomy is sufficient for these lesions. Our results demonstrate the aggressive treatment of patients with FVPTC who were reclassified as having NIFTP. In this study with its high rate of total thyroidectomy, all patients were treated with initial total thyroidectomy except two patients (one had contralateral lobe surgery). All tumor diameters were more than 1 cm. In the most extensive studies of NIFTP patients, approximately 25%–50% underwent thyroid lobectomy as the initial procedure [13, 17]. Given that a significant number of patients with NIFTP undergo total thyroidectomy for various reasons, the impact of this reclassification on the extent of surgery is less than expected. Compared to the results of a different study involving 500 thyroidectomy patients in our clinic, our total thyroidectomy rates (96.2%) were already high [18]. This result is probably associated with an increased incidence of goiter and thyroid nodules since we are living in an endemic goiter region. The 2015 American Thyroid Association guidelines recommend that lobectomy is sufficient for the low-risk patient with a well-differentiated thyroid malignancy defined as tumors > 1 cm and < 4 cm without extrathyroidal spread or evidence of lymph node metastasis [11, 19]. However, some studies report that 43% of patients with lobectomy will require a completion thyroidectomy [20].

Multifocality and contralateral lesions are other issues of discussion for NIFTP. Canberk et al. [14] reported detection of contralateral tumoral lesions in 13 (18%) of 74 total thyroidectomy cases for NIFTP, 11 of them were malignant, and the other two were NIFTP. Also, NIFTP and FVPTC cases had statistically similar incidences of contralateral tumors. Canini et al. [21] reported that 14.7% of 68 NIFTP patients were multifocal and approximately 10% were bilateral, and Turan et al. [22] also detected 17.9% multifocal NIFTP foci in 84 patients. No difference in survival between solitary and multifocal NIFTP was found [21, 22]. In our present study, no multifocal NIFTP focus was detected, but concomitant PTC was present, and no statistically significant difference in multifocality between FVTPC and NIFTP was noted (35.7% and 14.3%, respectively).

In addition, 57.1% of NIFTP patients in our study received RAI for residual thyroid tissue for quantitative thyroglobulin evaluation and clinical follow-up. RAI treatment rates in other published reports are 44%–47% [13, 23]. Using pathological evaluation with strict application of the NIFTP criteria, the patients in our study results had excellent outcomes. Contrary to our results, in the literature, Parente et al. [13] published five patients with nodal metastases and one distant metastasis (lung) over a mean follow-up of 5.7 years. Kim et al. [24] had nine patients with positive central neck lymph nodes

(over half of these patients had concomitant classic PTCs) among 74 NIFTPs. Cho et al. [25] also followed two patients with central lymph node metastases, but no distant metastases, over a median follow-up of 37 months. An overall lymph node metastasis rate of 1.8% (range: 0%–12%) and distant metastasis rate of 0.08% (range: 0%–1%) were demonstrated in a systematic review [22]. Consistent with the literature considering the 14.3% contralateral tumor rates detected in our study and also the significant heterogeneity in the overall lymph node metastasis rate in the literature, it is recommended that these patients should remain in the follow-up routine.

The incidence of NIFTP varies considerably in retrospective studies (despite the stringent criteria defined for diagnosis) and ranges from less than 1% to 28% of all thyroid neoplasms [26]. Won et al. [27] reclassified 71% of EFVPTC as NIFTP, and the overall percentage was 27% of all PTC. Kiernan et al. [23], in a review based on a consensus diagnosis involving three pathologists, reclassified 46% of 60 FVPTC as NIFTP, and Agrawal et al. [28] reclassified 40% of non-invasive EFVPTC as NIFTP by a single pathologist. Chung et al. [29] identified only 15 (13%) NIFTP among 110 FVPTCs as determined by two expert pathology specialists, a finding that was similar to our results. When evaluated according to the geographical distribution, NIFTP incidence was very similar in North America and Europe (9.3% and 9.6%, respectively) with a significantly lower overall rate in Asia (2.1%) [30]. In reclassification studies in our country, the incidence of NIFTP was found to be 2.4%–3.9% in PTCs [14, 22]. In the present study, the incidence of NIFTP was 7.6% in all PTCs, which was higher than the incidence rates in our country. However, our FVPTC rates were also high (45.7%) among all PTCs. Turan et al. [22] reclassified 84 (17.5%) of 481 patients with FVPTC as NIFTP, a finding similar to our results. In a study recently published in our country, the most common subtype was FVPTC with 247 (53.7%) among 460 PTC cases [31]. In another study, it was the third most common subtype (23.6%) after micropapillary and classical PTC [32].

Another controversial issue is the ethical situation since these cases had been diagnosed with cancer beforehand after which the patients were treated and followed accordingly. Some authors have recommended that pathology departments implement retrospective database reviews of tumors diagnosed as FVPTC for patients currently under surveillance. If the nodules are suitable for NIFTP diagnosis, the clinicians and patients should be alerted about the new diagnosis [33].

Limitations

This study has several limitations, most notably the small number of patients and its retrospective nature. Results may not reflect the entire NIFTP population. Moreover, we did not have the molecular profile of the tumors, and FNAB results were missing in some patients. Only a limited number of patients had a follow-up period of more than three years, and we think future studies should support the long-term follow-up results of these patients.

Conclusions

Due to our high thyroidectomy rates, our results do not contribute to the question of the adequacy of lobectomy in the treatment of NIFTP. According to the literature, although lobectomy seems sufficient in the treatment, unanswered issues remain regarding multifocality, concomitant PTC, and bilaterality for NIFTP. Also, no definitive recommendations for a follow-up routine have been put forth. In our results, no recurrence or metastasis in follow-up was found. Still, keeping these patients under active follow-up seems appropriate due to the question marks about NIFTP and no definitive recommendations/guidelines for a follow-up routine.

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Middle cerebral artery to uterine artery pulsatility index ratios in pregnancy with fetal growth restriction regarding negative perinatal outcomes

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Abstract

Background/Aim: Fetal growth restriction (FGR) causes a high risk of perinatal morbidity and mortality, and the timing of the correct delivery time decision remains controversial. Cerebroplacental ratio (CPR), umbilical artery, uterine artery (UA) and middle cerebral artery (MCA) Doppler studies are used to predict adverse perinatal outcomes in FGR. However, since there is insufficient reliability for each separately and together, the search for new methods continues. This retrospective study was conducted to determine the degree of neonatal morbidity in fetuses suspected of having FGR by evaluating the MCA to UA pulsatility index (PI) ratios together with frequently used Doppler examinations.

Methods: This was a retrospective cohort study conducted in a single-center hospital with the approval of the Medical Institutional Ethics Committee. A total of 424 pregnant women admitted to a tertiary hospital and diagnosed with FGR between July 2020 and December 2021 who were informed and approved were included in the study. Gestational age was confirmed by first trimester sonographic measurements of pregnancy. All pregnant women were examined by Doppler USG and umbilical artery, mean UA, fetal MCA, ductus venosus, CPR (MCA/umbilical artery pulsatility index ratio) and cerebrouterine ratio (MCA/UA) PI values were measured. Negative perinatal outcomes were recorded as blood gas level of the newborn at 7.2 and below, Apgar score of 7 and below at the fifth minute, and needing neonatal intensive care (NICU). Adverse perinatal and postnatal outcomes were recorded and compared with Doppler findings. If there were no signs of a negative perinatal outcome, it was considered a positive outcome. If at least one of the symptoms of adverse perinatal outcomes was present, it was considered a negative outcome

Results: Decreased CPR and decreased MCA to UA PI were significantly and positively associated with an increased likelihood of exhibiting negative perinatal outcomes in pregnancies with FGR ($P < 0.001$, $P < 0.001$, respectively). The receiver operating characteristic (ROC) curve analysis showed that the optimal cut-off value for MCA to uterine artery PI was 1.41 to predict FGR with 57.37% sensitivity and 62.50% specificity (AUC: 0.629; 95% CI: 0.581–0.675). When the CPR cut-off value was taken as 1.2069, the sensitivity was 42.86% and the specificity 83.93% in predicting negative perinatal outcomes in CPR values below this value ($P < 0.001$).

Conclusion: CPR is the most successful criterion in distinguishing between positive and negative perinatal outcomes. It has been demonstrated that the MCA to uterine artery PI ratio values after CPR can also be used for this distinction. MCA to UA PI ratio sensitivity was higher than CPR and umbilical artery. This situation shows that MCA to uterine artery PI ratio (alone or when evaluated together with PPV and NPV ratios) is a criterion that can be added to other Doppler examinations in predicting negative perinatal outcomes.

Keywords: Fetal growth restriction, Cerebroplacental ratio, Uterine artery pulsatility index, Middle cerebral artery pulsatility index, Doppler

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Introduction

Fetal growth restriction (FGR), defined as the failure of expected fetal growth, is an important cause of perinatal mortality and morbidity [1, 2]. It is known that more than half of stillbirths are associated with FGR due to the inability to detect FGR [3].

Early detection of fetuses at risk for adverse outcomes remains a challenge and is of great importance for clinicians and researchers to correct the abnormality and reduce perinatal mortality before permanent damage occurs. In current practice, the management of FGR aims to monitor fetal status and provide necessary prenatal support to optimally time labor induction.

Middle cerebral artery (MCA), uterine artery (UA), and umbilical artery Doppler measurements have been used for a long time to estimate negative perinatal consequences. In recent studies, Cerebroplacental ratio (CPR) has been suggested as a more reliable test with the advantage of its neuroprotective effect [4, 5]. Many CPR thresholds are recommended, but it is not yet accepted as an international reference gold standard, but is considered a useful method, and different accuracy rates have been reported in different studies [6-8]. Research is ongoing for a more precise method to estimate adverse perinatal outcomes.

This study aimed to reveal the ability of MCA, UA, umbilical artery, ductus venosus, and CPR to predict adverse perinatal outcomes in pregnant women diagnosed with FGR and to compare their reliability with MCA/UA pulsatility index (PI) ratios, which is an uncommon method.

Materials and methods

Study design and participants

A retrospective cohort study was conducted to evaluate the MCA/UA PI ratios in pregnancies with FGR between July 2020 and December 2021 in a tertiary hospital. Ethical Approval was obtained from Prof. Dr. Cemil Taşcıoğlu City Hospital's ethics committee (28.02.2022 / number E-2022/45). A total of 424 women aged 16–44 years, 27–40 weeks pregnant, diagnosed with FGR, who applied to the high-risk perinatology clinic were included in the study. As a result of data scans, routine laboratory, ultrasonographic measurements, and follow-up delivery data of the pregnant women who applied to the hospital were recorded. Gestational age was confirmed by first trimester sonographic measurements of pregnancy.

Gestational diabetes, cases with a fetal structural or chromosomal abnormality, history of hypertensive disease during pregnancy, history of chronic disease in the mother, smoking or drug use, fetal infections, and multiple pregnancies were determined as exclusion criteria from the study.

Criteria established by international consensus in the ISUOG Practice Guidelines were used to define FGR [9]. Negative perinatal outcomes were determined as fifth minute Apgar score <7, neonatal cord blood gas pH <7.2, and NICU requirement [10, 11]. If there were no signs of a negative perinatal outcome, it was considered a positive outcome. If at least one of the symptoms of adverse perinatal outcomes was present, it was considered a negative outcome.

All pregnant women were followed up, and negative perinatal and postnatal outcomes were determined and recorded. All patients underwent an ultrasonographic examination using a Mindray Resona 7 ultrasound (Mindray Bio-Medical Electronics Co., Ltd., Shenzhen, China), diagnostic apparatus with a 1.2–6 MHz convex abdominal probe by a fetal medicine specialist (HAS).

UA, umbilical artery, fetal MCA, and CPR Doppler examinations were performed as described in the ISUOG guidelines; CPR value was determined by proportioning the MCA PI value with the umbilical artery PI values; and the MCA/UA ratio was determined by dividing the MCA PI value with the mean UA PI value [12]. The Hadlock formula was used for estimated fetal weight (EFW) calculations [13].

Statistical analysis

Statistica 13.3.1 (TIBCO Software Inc. CA, USA) and the MedCalc demo version (MedCalc Software Ltd, Ostend, Belgium) were used for all analyses. The assumption of normality for numerical variables was examined with the Shapiro-Wilk test. Since the assumption of normality was not provided, the variables were summarized in terms of median and 25th Quarter-75th Quarter (min.-max.). The difference between the two independent groups was investigated with the Mann-Whitney U test. Visually presented with Raincloud Plot. ROC (receiver operating characteristic curve) analysis was used to calculate the cut-off point and the area under the curve, and sensitivity (95% CI), specificity (95% CI), PPV (95% CI) and NPV (95% CI) values were also given. Spearman correlation coefficient was used to investigate the relationship between continuous variables. A network graph has been drawn for the visualization of the correlation coefficients. $P < 0.05$ was accepted as a statistical significance level.

Results

The demographic and obstetric characteristics are shown in Table 1. According to the perinatal results that were accepted as negative, the newborn's fifth minute Apgar score was 7 and below in 100 of the 424 pregnant women who participated in the study. Cord blood pH values of 23 newborns were measured as 7.2 and below. Two-hundred-fifty-seven newborns were admitted to the NICU (1–102 days). Three-hundred-tweleve patients (73.6%) had at least one of the signs of adverse perinatal outcome. If there were no signs of a negative perinatal outcome, it was considered a positive outcome. If at least one of the symptoms of adverse perinatal outcomes was present, it was considered a negative outcome (Table 2).

Table 1: Demographic and obstetric characteristics

	Mean	Standard deviation
Maternal Age (years)	28.33	5.98
Body Weight	83.68	8.47
Height	162.09	49.07
Gestational age at US (weeks)	35.72	2.75
Gestational age at delivery (weeks)	31.98	2.87

Table 2: Number of perinatal results

Newborn	Number	Percent
Positive Outcomes	112	26.4
Negative Outcomes	312	73.6
Total	424	100

Adverse perinatal outcomes: blood gas level of the newborn 7.2 and below, Apgar score of 7 and below at the fifth minute, needing neonatal intensive care (NICU); Negative outcome: had at least one of the signs of adverse perinatal outcomes. Positive outcome: no signs of adverse perinatal outcomes

Doppler evaluations, measured PI values, and all positive and negative perinatal outcomes were compared, and statistical significance was found between Doppler values and results (Table 3).

Table 3: Statistical relationship between Doppler measurements-PI values and positive and negative perinatal outcomes

	Groups										
	Positive perinatal outcomes					Negative perinatal outcomes					*P*-value
	Median	Min	Max	Percentiles 25	75	Median	Min	Max	Percentiles 25	75	
Umb.A-PI	1.06	0.60	1.50	0.90	1.20	1.14	0.20	5.23	0.92	1.30	0.003
Ut.A-PI	1.01	0.48	2.11	0.83	1.26	1.09	0.50	2.89	0.90	1.34	0.025
MCA-PI	1.51	0.95	3.44	1.40	1.80	1.40	0.90	4.40	1.30	1.60	<0.001
Duc.V-PI	0.50	0.20	2.00	0.40	0.60	0.60	0.20	1.30	0.40	0.70	0.002
CPR	1.51	0.77	3.16	1.27	1.89	1.29	0.27	7.00	1.08	1.60	<0.001
MCA/Ut. A	1.56	0.67	4.60	1.23	2.08	1.33	0.44	5.27	1.03	1.73	<0.001

Min: Minimum, Max: Maximum, PI: Pulsatility Index, Umb.A: Umbilical Artery, MCA: Middle Cerebral Artery, Duc.V: Ductus Venosus, CPR: Cerebroplacental ratio

Separate ROC analysis results in terms of the relationship between umbilical artery PI, CPR and MCA/UA PI ratio Doppler measurements, and perinatal outcomes are given in Table 4.

The predictive and cut-off values of CPR PI (Figure 1a), MCA /UA PI (Fig. 1b), and umbilical artery PI (Fig. 1c) for negative perinatal outcomes were evaluated by applying ROC analysis. Decreased CPR and decreased MCA to uterine artery PI were significantly and positively associated with an increased probability of indicating a negative perinatal outcome (P < 0.001, P < 0.001, respectively). The optimal cut-off for MCA to UA PI was 1.4118 to predict FGR with 57.37% sensitivity and 62.50% specificity, demonstrated by ROC analyzes (AUC 1~4 0.629; 95% CI, 0.581–0.675).

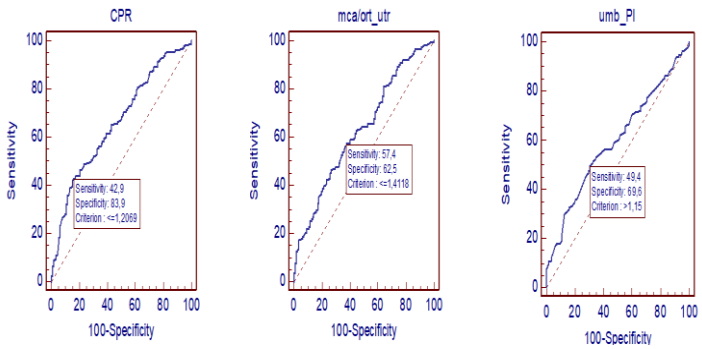
When the CPR cut-off value was taken as 1.2069, the sensitivity was 42.86% and the specificity 83.93% in predicting negative perinatal outcomes in CPR values below this value (P < 0.001). When the cut-off value for the umbilical artery was taken as 1.15, the sensitivity of umbilical artery PI values above this value was 49.35%, and the specificity was 69.64% to predict adverse perinatal outcomes (P = 0.003). When the cut-off value for the MCA/UA PI ratio was taken as 1.4118, values below this value had a sensitivity of 57.37% and a specificity of 62.50% (P < 0.001) in predicting negative perinatal outcomes (Table 4 and Figure 1).

Table 4: ROC results for CPR, MCA to uterine artery and umbilical artery

	CPR (MCA PI/UMB A. PI)	MCA PI/ Ut. A. PI	Umbilical Artery PI
AUC	0.661 (0.614-0.707)	0.629 (0.581-0.675)	0.596 (0.547-0.643)
	P < 0.001	P < 0.001	P = 0.001
Criterion	≤1.2069	≤1.4118	>1.15
Sensitivity	42.86	57.37	49.35
95% CI	37.3 - 48.6	51.7 - 62.9	43.6 - 55.1
Specificity	83.93	62.50	69.64
95% CI	75.8 - 90.2	52.9 - 71.5	60.2 - 78.0
PPV	88.0	81.0	81.7
95% CI	81.7 - 92.7	75.2 - 85.9	75.4 - 87.0
NPV	34.8	34.5	33.3
95% CI	29.1 - 40.8	28.0 - 41.5	27.3 - 39.8

CPR was calculated by dividing the MCA PI by the UA PI, and MCA/ uterine artery PI ratio by dividing the MCA PI by the uterine artery PI. AUC: area under the curve, CI: confidence interval, CPR: cerebroplacental ratio, MCA: middle cerebral artery, PI: pulsatility index, UA: umbilical artery, PPV: positive predictive value, NPV: negative predictive value.

Figure 1: ROC results for CPR, MCA to uterine artery and umbilical artery



Predictive and cut-off values of (a) CPR PI, (b) MCA/uterine artery PI, (c) UA PI for adverse perinatal outcomes in pregnancies with FGR by receiver operating characteristic curve. MCA PI, CPR and MCA/uterine artery PI were three predictors of adverse perinatal outcomes, with AUCs of 0.661, 0.629 and 0.596, respectively (P < 0.001, P < 0.001, P = 0.001). CPR was calculated by dividing the MCA PI by the UA PI, and MCA/ uterine artery PI ratio by dividing the MCA PI by the uterine artery PI. AUC: area under the curve, CI: confidence interval, CPR: cerebroplacental ratio, MCA: middle cerebral artery, PI: pulsatility index, UA: umbilical artery.

Discussion

FGR is a fetal developmental disorder in which the fetus fails to develop and grow adequately, which is a major cause of adverse perinatal outcomes, including stillbirths [14, 15]. The aim of FGR management is to make a timely decision for delivery, thereby minimizing fetal morbidity and mortality.

It is thought that uteroplacental circulatory failure plays a role most frequently in the etiology, and therefore, the fetus, which cannot receive nutrients and oxygen from the placenta, cannot develop and grow sufficiently [15]. While studies continue to provide information about the fetus's nutritional level and growth rate, many authors stated that Doppler assessments are a reliable, noninvasive predictor of adverse perinatal outcomes in high-risk pregnancies [16-18]. Hypoxemia, which develops as a result of uteroplacental circulatory failure, causes protective changes in vascular flow to protect vital organs, such as the brain, which is defined as the centralization of blood flow, which causes changes in Doppler blood flow resistances. Studies have shown that MCA and umbilical artery Doppler measurements are good indicators of negative pregnancy outcomes [19]. Our MCA and umbilical Doppler indexes are similar to the literature [19, 20].

CPR is a useful index of fetal stress and hypoxemia, combining increased umbilical artery and decreased MCA impedance [20, 21]. This index is significantly more sensitive than UA and MCA separately [22]. CPR is also more successful than other velocimetry measures alone in predicting adverse perinatal outcomes [20-23]. In our study, CPR was remarkably low in FGR with negative obstetric outcomes. In addition, similar to previous studies, CPR was the most accurate predictor of negative obstetric outcomes among the previously mentioned parameters [24, 25].

Despite many studies suggesting that they accurately predict negative perinatal outcomes, Doppler indices of the uterine artery are rarely used in clinical evaluation. Uterine artery Doppler changes are detected before the findings of the uteroplacental insufficiency clinic in the fetus [26]. Uterine artery Doppler findings are also accepted as predictors of true FGR and poor perinatal outcomes in small fetuses [27, 28]. Some similar studies have shown that uterine artery PI values in small for gestational age (SGA) fetuses can be accurate in predicting adverse perinatal outcomes [29]. A separate evaluation of Doppler indices with MCA PI or UA PI may not show some small alterations, but the calculation of the ratio may propose more possibilities to show small alterations in blood flow in a timely and accurate. The success of MCA/UA ratios in predicting adverse perinatal outcomes is not fully known. Except for a few studies that predicted perinatal outcomes in patients with preeclampsia, there is insufficient information in the literature about MCA/UA PI ratios. The MCA/UA PI ratio was proposed in another study as a good predictor of neonatal outcomes in third-trimester pregnancies with preeclampsia [30]. Another similar study showed that a low MCA/UA PI ratio was associated with negative obstetric outcomes in pregnancies with preeclampsia [31]. Similar results were obtained in the study of Zhou et al. in which they investigated MCA/UA ratios in term pregnant women, and it was suggested that MCA to UA ratios could help predict negative perinatal outcomes [32].

In our study, CPR is the most successful criterion in distinguishing between positive and negative perinatal outcomes. It has been demonstrated that the MCA to UA PI ratio values after CPR can also be used for this distinction. MCA to UA PI ratio sensitivity was higher than CPR and umbilical artery. This situation shows that the MCA to UA PI ratio (perhaps alone or when evaluated together with PPV and NPV ratios) is a criterion that can be added to other Doppler examinations in predicting negative perinatal outcomes.

Limitations and strengths of the study

The strengths of this study are that the Doppler studies were performed by a fetal medicine specialist and the use of simple, reproducible and validated ultrasound modalities. It is the first study to assess the MCA/UA PI ratio in FGR with negative perinatal outcomes.

A relatively small retrospective cohort, lack of a control group, and inability to perform consecutive Doppler follow-ups, especially in pregnancies with pathological Doppler values, are some of the limitations of our study. We believe more specific results can be achieved in larger series, especially in pregnancy with close Doppler and perinatal outcome follow-ups.

Conclusion

In this study, it was revealed that MCA/uterine artery ratios are a valuable criterion in predicting adverse perinatal outcomes in pregnant women with FGR, as well as frequently used CPR values. In conclusion, our study shows that MCA/UA PI ratio and CPR are two good indicators of negative obstetric outcomes. These two indicators may be useful in predicting negative obstetric results and supporting the timing of delivery to reduce morbidity and mortality caused by FGR.

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Single-center experience of COVID-19 vaccine in patients with inflammatory rheumatic disease: Real-life data

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Ethics Committee Approval
The study was approved by the ethics committee at Istanbul Health Sciences University, Umraniye Research and Training Hospital (Date: 21/04/2022 No.148).
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

Conflict of Interest
No conflict of interest was declared by the authors.

Financial Disclosure
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Abstract

Background/Aim: Patients with rheumatic disease are at high risk of infection complications, and vaccines are essential to prevent these diseases. Moreover, biologic disease-modifying/targeted synthetic anti-rheumatic drugs (b/tsDMARDs) have been shown to reduce the immunogenicity of vaccines, although their effectiveness, side effects, and effects on disease activity are not yet clear. In this study, we aimed to investigate the incidence of post-vaccine side effects, disease exacerbation, and COVID-19 infection despite vaccination in patients with inflammatory rheumatic disease; the difference in vaccination effects between patients who received and did not receive b/tsDMARD treatments.

Methods: Patients received b/tsDMARD (i.e., biologic group (BG)) (n = 194) who were admitted to the rheumatology outpatient clinic, were included in this study. All patients with inflammatory rheumatological disease, who did not receive b/tsDMARD (n = 185), but who applied to the rheumatology outpatient clinic during this time, were included in the non-biologic group (NG). Patients followed were included and evaluated cross-sectionally. Clinical and demographic characteristics, as well as type of COVID-19 vaccination, post-vaccine side effects, COVID-19 infection status before and after vaccination, and post-vaccine rheumatological disease exacerbation, were also evaluated.

Results: In BG, 92.2% of patients were vaccinated, but for NG, 82.7% were vaccinated against COVID-19 patients with BG, 46.2% were vaccinated with CoronaVac vaccine alone, 51.4% with Pfizer/BioNTech BNT162b2 vaccine alone, and 37.4% with a combination of CoronaVac and BNT162b2 vaccines. In the NG, 53.8% of patients were vaccinated with CoronaVac vaccine alone, 48.6% with BNT162b2 vaccine alone, and 36.2% with a combination of CoronaVac and BNT162b2 vaccines. There was a significant difference between groups, according to vaccine types (P = 0.040), as this difference was due to a larger number of patients vaccinated with the CoronaVac + BNT162b2 combination for BG. Adverse effects were detected in 99 patients (55.9%) with BG and 95 patients (62.5%) with NG post-vaccination. There was no difference between BG and NG vaccines (CoronaVac, BNT162b2, or their combination) for adverse effects (P > 0.05 for all). The vaccine with the most common adverse events was BNT162b2, for both BG and NG. The most common side effect was arm pain, significantly higher in BG (P = 0.014). Fever and rash were more common for NG (P = 0.017). Disease exacerbation was not observed with BG, whereas it was detected in 5 (1%) patients for NG that was different (P = 0.021). SARS-COV-2 infection was also significantly less common for BG vs. NG (15.3% vs. 20.3%) (P = 0.017). Despite COVID-19 vaccinations, 56 patients with BG and 62 patients with NG had COVID-19 (P = 0.005).

Conclusion: Standardized vaccination comparisons could not be achieved, as patients using b/tsDMARD were vaccinated for fewer COVID-19 infections. Additionally, COVID-19 vaccines are well-tolerated in patients with rheumatological disease, with vaccine-related disease activity at 1%, only seen in those not using b/tsDMARDs.

Keywords: COVID-19, Biologic / targeted synthetic DMARD, Conventional synthetic DMARD, Vaccination

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Introduction

COVID-19 disease is caused by the SARS-COV-2 virus, detected in Wuhan, China in December 2019, causing millions of deaths worldwide from developing acute respiratory failure syndrome. After the rapid spread of COVID-19, studies to create vaccines started in our country and globally. However, patients with inflammatory rheumatic diseases were not included in them. Information about vaccine efficacy and side effects in this group were obtained after their introduction. Vaccination is an effective tool to prevent infections for those with autoimmune and inflammatory rheumatic diseases, as well as the general population [1]. In autoimmune inflammatory rheumatic disease, the risk of infection may be higher due to underlying disease, chronic inflammatory processes, and immunosuppressive drug use. It is controversial if inflammatory rheumatic disease increases risk of severe COVID-19 [2-4]. Yet, those with inflammatory rheumatic diseases were at higher risk for hospitalization and severe COVID-19 vs. the general population: vaccination is critical in preventing disease [5]. Previous vaccinations showed that immunosuppressive therapy inhibited the humoral response to influenza and pneumococcal vaccines in rheumatologic patients [6]. Immunosuppressive therapy was shown to reduce and delay COVID-19 vaccine response in this patient group [7-9].

In one of the earliest studies conducted on 2,860 patients with a diagnosis of rheumatic disease, the first and most common side effects of vaccination were found to be fatigue, headache, muscle and joint pain, and fever. Also, side effects were similar in these patients vs. healthy population controls [10]. Another issue is post-vaccine disease exacerbation: there are studies about other routine vaccines, with disease exacerbation reported at a rate of 4.6 -5% after COVID-19 vaccines [10, 11].

When the current study was conducted, there were 2 different types of COVID-19 vaccines, one of which was CoronaVac, inactive for SARS-CoV-2, and the other Pfizer-BioNTech, a BNT162b2 messenger RNA (mRNA) vaccine. In most COVID-19 vaccine studies for rheumatic patients, we had Pfizer-BioNTech BNT162b2, viral vector vaccines, Oxford/AstraZeneca, and Janssen/Johnson & Johnson, plus Moderna mRNA. There is very little information about CoronaVac, one of the vaccines first used in our country, and the rheumatological patient group. Most patients used with the same type of vaccine, whereas many were inoculated with a combination of CoronaVac and BNT162b2 vaccines.

There is hesitation about vaccination for patients with inflammatory rheumatic diseases [12]. In the VAXICOV study [13], it was reported that 30% of patients with systemic autoimmune or inflammatory rheumatic diseases were hesitant to get vaccinated for COVID-19. In this cross-sectional study, considering apprehension about vaccination, we aimed to investigate the incidence of post-vaccine side effects, disease exacerbation, and COVID-19 infection despite vaccination in patients with inflammatory rheumatic disease; the difference was noted in effects between patients who received and those who did not receive biologic/targeted synthetic DMARD treatment.

Materials and methods

Study design and data collection

This is a single-center, cross-sectional, observational study conducted at the Ümraniye Training and Research Hospital. In our study, volunteers who applied to our rheumatology outpatient clinic between May 1-30, 2022 were diagnosed with inflammatory rheumatic diseases, such as rheumatoid arthritis (RA), ankylosing spondylitis (AS), psoriatic arthritis (PsA), connective tissue disease (systemic lupus erythematosus, Sjogren's syndrome, myositis), familial Mediterranean fever (FMF), Behcet's disease, and vasculitis – so were included. Patients refusing to participate or those with neuropsychiatric diseases preventing communication or those with mental retardation or pregnancy, were followed without medication and noninflammatory issues such as osteoarthritis and fibromyalgia, but were excluded. During data collection, 432 patients were evaluated and 53 were excluded, as they did not meet eligibility criteria. In total, 379 patients were included in the study.

Those included were split in two groups, i.e., receiving biologic/targeted synthetic disease-modifying anti-rheumatic drugs (b/tsDMARD), and the biologic group (BG), or the non-biologic group (NG) – vs, patients with BG, using TNFi, (etanercept, adalimumab, infliximab, golimumab, and certolizumab), Interleukin-6 inhibitor (IL-6- tocilizumab), Interleukin-17A inhibitor (IL-17A- secukinumab), CTLA4-Ig (abatacept), JAK inhibitor (JAKi-tofacitinib), Interleukin-1 antagonists (IL-1- anakinra, canakinumab), and CD20 inhibitors (rituximab). NG patients used conventional synthetic DMARDs (csDMARD): methotrexate, leflunomide, hydroxychloroquine, colchicine, azathioprine, mycophenolate mofetil, and sulfasalazine, or anti-inflammatory drugs (NSAIDs).

An evaluation about COVID-19 vaccination was used with patients in the study as clinic controls. This consisted of information regarding patient age, gender, disease duration, comorbidities (hypertension, diabetes mellitus, cerebrovascular diseases, cardiovascular diseases, chronic obstructive pulmonary disease (COPD), interstitial lung disease (ILD), asthma, malignancy). Their medications and steroid doses, if any, were noted.

Assessment of vaccination

The COVID-19 vaccine status, vaccination time, doses and type of vaccines were recorded. Those who passed more than three months after the last vaccine, those vaccinated less than twice, or those with disease in the first 14 days after vaccination were considered to not have completed the protocol. Side effects in the first 7 days after vaccination were grouped as local and systemic side effects, and evaluated for each dose.

Local side effects were redness, warmth, regional arm pain, and axillary lymphadenopathy. Systemic side effects were weakness, fatigue, drowsiness, headache-dizziness, joint-muscle pain, fever, allergic complaints (itching, rash, shortness of breath), nausea-vomiting, or loss of appetite. Additional side effects were classified as *"other effects."*

Increase of disease activity at more than 2 days post-vaccination was vaccine-related exacerbation [10]. Unvaccinated patients were evaluated for not being vaccinated, which was also noted. COVID-19 infection status before and after the vaccine

was assessed. Vaccination timing was noted for those with COVID-19 infection.

Symptoms of COVID-19 and treatments were also considered. Use of immunomodulatory or immunosuppressive medications for COVID-19 were questioned for all patients. Clinical characteristics of COVID-19, pharmacological therapy in treatment, presence of lung involvement, and clinical outcomes (hospitalization, intensive care admission, length of stay, use of noninvasive mechanical ventilation) were each evaluated.

Ethical approval

This study was approved by the Ethics Committee at Ümraniye Training and Research Hospital (Date 21/04/2022, No. 148) and conducted as per the Helsinki Declaration and Good Clinical Practices guidelines. All patients had given oral and written consent.

Statistical analysis

The Statistical Package for Social Science (IBM-SPSS Statistics, v. 20.0, Armonk, NY, USA) was performed with statistical analyses. Descriptive statistics were reported as means (SD) for continuous variables and as number and frequency for binary and categorical variables. Comparing these variables was conducted with the Mann-Whitney U test for continuous variables, as data distribution was non-parametric, while the Chi-square test was used for categorical variables: this test was performed to compare COVID-19, symptoms, and disease between BG and NG. $P < 0.05$ held statistical significance.

Results

Demographic and clinical characteristics

The mean age of 379 patients in the study was 48.22 (13.05) years, with 58.8% male. While 194 (51.2%) patients were in the BG, 185 (48.8%) were in the NG. Table 1 shows demographic and clinical data of the overall study population and its subgroups.

BG consisted of patients on only b/tsDMARD (n = 101, 52%) or the combination of b/tsDMARD and csDMARD (n = 94, 47.9%), whereas NG consisted of patients who received csDMARD (n = 158, 85.4%) and no DMARDs (n = 27, 14.5%). Distribution of biologic drugs were: TNFi, (n = 142, 73.2%), JAKi (n = 13, 6.7%), IL-17 inhibitors (n = 13, 6.7%), rituximab (n = 18, 9.3%), IL-1 inhibitors (n = 2, 1%), CTLA4 inhibitor (n = 1, 0.5%), and IL-6 blockers (n = 5, 2.6%). For 142 patients receiving TNFi, 7 (3.6%) were treated with infliximab, 26 (13.4%) with etanercept, 42 (21.6%) with adalimumab, 38 (19.6%) with golimumab, and 29 (14.9%) with certolizumab. Seventy-three patients (37.6%) for BG used steroids. Drug distributions in NG were: methotrexate (n = 62, 33.5%), leflunomide (n = 38, 20.5%), hydroxychloroquine (n = 46, 24.9%), sulfasalazine (n = 24, 13%), azathioprine (n = 6, 3.2%), mycophenolate mofetil (n = 2, 1%), colchicine (n = 20, 10.8%) and NSAIDs (n = 27, 14.5%): 88 NG patients existed on steroids (47.6%).

COVID-19 vaccination status

One-hundred seventy-nine patients (92.2%) in BG and 153 patients (82.7%) in NG were vaccinated against COVID-19 ($P = 0.005$). For the BG, 36 patients were vaccinated only with CoronaVac (46.2%) and 76 patients with BNT162b2 vaccine (51.4%). Sixty-seven patients (37.4%) were vaccinated with a combination of CoronaVac and BNT162b2. For NG, 42 patients (27.6%) were vaccinated with CoronaVac, 72 patients (47.4%) with only BNT162b2, and 38 (25%) with a combination of CoronaVac and BNT162b2.

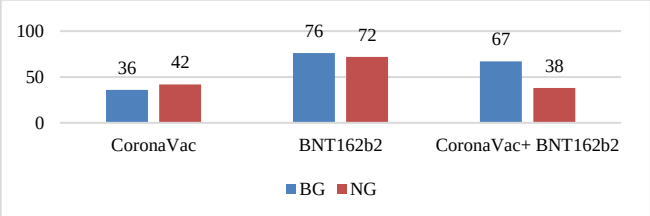
Table 1: Demographic and clinical data.

	Biologic group (n = 194)	Non biologic group (n = 185)	*P*-value	Overall (n = 379)
Female/Male (n)	98/96	58/127	<0.001	156/223
Age (years)	47.57 (12.19)	48.89 (13.89)	0.084	48.22 (13.05)
Disease duration (mo)	128.17 (81.60)	85.33 (84.95)	<0.001	107.32 (85.86)
Disease			<0.001	
Rheumatoid arthritis (n)	75	85	0.151	160
Spondyloarthropathies (n)	82	43	<0.001	125
Psoriatic arthritis (n)	31	13	0.007	44
Connective tissue disease (n)	7	21	0.004	28
FMF/BD/vasculitis (n)	8	20	0.013	28
Seropositivity (RF, anti-CCP + n)	42	52	0.050	94
HLAB27 positivity (+ / n)	45	24	0.596	69
Comorbidities (n)	70	70	0.723	140
Diabetes mellitus (n)	21	17	0.596	38
Hypertension (n)	37	34	0.863	71
Cerebrovascular accidents (n)	4	3	0.656	7
Cardiovascular disease (n)	12	8	0.418	20
COPD/Asthma/ILD (n)	11	10	0.910	21
Chronic Renal Failure (n)	2	5	0.227	7
Malignancy (n)	2	3	0.614	5
Inflammatory bowel disease (n)	10	2	0.024	12
Rheumatologic medications (n)			<0.001	
Only b/tsDMARD	101	0		101
b/tsDMARD + csDMARD	93	0		93
Only csDMARD	0	158		158
No DMARD	0	27		27

FMF: Familial Mediterranean Fever, BD: Behcet's disease, COPD: Chronic Obstructive Pulmonary Disease, b/tsDMARD: Biologic/ targeted synthetic disease-modifying anti-rheumatic drugs, csDMARD: conventional synthetic disease-modifying anti-rheumatic drugs. RF: rheumatoid factor, Anti-CCP: Anti-Cyclic Citrullinated Peptide

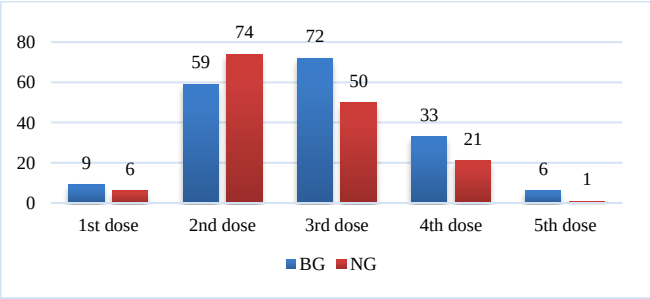
A significant difference was found between groups for vaccination rates ($P = 0.040$). There was no difference between patients vaccinated with only CoronaVac and only BNT162b2 ($P > 0.05$). However, there was a significant difference between groups vaccinated with a combination of CoronaVac and BNT162b2, only CoronaVac, or with only BNT162b2 ($P = 0.017$ and $P = 0.049$, respectively). This difference was ostensibly due to a combination of CoronaVac and BNT162b2 in BG (Figure 1A).

Figure 1A: Distribution of vaccine types among groups.



Vaccination doses are shown in Figure 1B. There was a significant difference between the two groups in terms of vaccine dose ($P = 0.035$). Pairwise comparison revealed that a significant difference was found between those who received 2 doses of vaccine and those who received 3, 4, and 5 doses ($P = 0.019$; $P = 0.038$; $P = 0.032$, respectively).

Figure 1B: Variation of vaccine dosages between groups



The rate of unvaccinated patients was 0.7% for BG, vs. 17.2% for NG (P = 0.005). No vaccination included fear of side effects (35.2%), not wanting to be vaccinated (18.9%), not trusting the vaccine (16.2%), indecisiveness about it (18.9%), and the disease (10.8%).

Side effects of COVID-19 vaccine

Post-vaccine side effects were detected in 99 (55.9%) patients in the BG Group and 95 (62.5%) patients in the NG Group. When side effects for all vaccines were evaluated, no difference was found between BG and NG. There was also no difference in side effects according to vaccine types (CoronaVac, BNT162b2, and the CoronaVac-BNT162b2 combination) for BG and NG (P > 0.05). The vaccine with the most common adverse events was BNT162b2 in both BG and NG. Evaluation of side effects due to each vaccine is shown in Table 2. The most common side effect, arm pain, was significantly higher in BG (P = 0.014). Fever and rash were more common in NG (P = 0.017), but there was no difference between BG and NG groups in terms of other side effects (P > 0.05).

Table 2: Vaccine side effects and their frequency.

	Biologic group	Non-biologic group
Rash-Swelling-pain the arm (n)	78	62
Lymphadenopathy (n)	4	4
Fatigue (n)	28	25
Headache-drowsiness (n)	12	10
Joint-muscle pain (n)	21	27
Fever-rash (n)	10	22
Anaphylaxis (n)	1	2
Nausea- vomiting- loss of appetite (n)	2	5
Runny nose (n)	1	0
Conjunctivitis (n)	0	1
Sore throat (n)	0	1
Chest pain (n)	1	0
Vaginal bleeding (n)	1	0

Among those with side effects in first dose vaccines (n=107), 73 (68%) had BNT162b2 while 34 (32%) had CoronaVac. Side effect rates were found to be significantly higher in patients vaccinated with BNT162b2 in BG and NG groups (P = 0.001 and P = 0.001, respectively). Distribution of side effects after the first vaccine dose were 49 (46.2%) local, 35 (33%) systemic, and 22 (20.8%) local + systemic.

Of those with side effects after the second vaccine dose (n = 104), 81 (77.9%) had BNT162b2 and 23 (22.1%) had CoronaVac. Side effects were significantly higher in BNT162b2 patients for BG and NG groups (P = 0.001 and P = 0.001, respectively). After the second vaccine dose, 48 (46.2%) of the side effects were local, 30 (28.8%) were systemic, and 26 (25%) were local with systemic side effects.

Among those with third vaccine doses (n = 80), 77 (96.3%) had BNT162b2 and 3 (3.6%) had CoronaVac. Side effects were significantly higher after BNT162b2 in BG and NG groups (P = 0.001 and P = 0.001, respectively). Side effects after the third vaccine dose were 46 (57.5%) local, 16 (20%) systemic, and 18 (22.5%) both local + systemic. Among those who had a fourth dose (n = 27), 24 (88.9%) had BNT162b2, whereas 3 (11.1%) had CoronaVac. There was no difference in side effects following the fourth dose of BNT162b2 and CoronaVac between BG and NG (P > 0.05). For vaccine effects after this dose, 15 (55.6%) were local, 5 (18.5%) were systemic, and 7 (25.9%) were local + systemic. Side effects after the fifth dose were noted in 2 patients (33%) for BG, but not for NG. Side effects were no different between BG and NG for vaccine doses, P > 0.05).

Disease exacerbation after COVID-19 vaccine

Disease exacerbation after vaccination was significantly different between BG and NG groups (P = 0.021). While no exacerbation of disease was observed in BG, it was detected in 5 patients (1%) for NG. The median disease duration with exacerbation was 172.80 (106.18) months: 2 patients with exacerbation had RA, 2 had AS, and 1 FMF. While 1 patient with RA was treated with methotrexate and steroids and the other was treated with leflunomide, patients with AS received NSAIDs, while those with FMF received colchicine. Vaccinations for exacerbated patients were BNT162b2 (n = 1), CoronaVac (n = 1), and 3 vaccinated with a combination of CoronaVAC and BNT162b2.

COVID-19 infection status

In the study, 135 patients (18%) were infected with SARS-COV-2. The frequency of infection was significantly lower in BG vs. NG (15.3 vs. 20.3%, P < 0.017). In patients with COVID-19, 96% with BG and 80.5% with NG were vaccinated (P = 0.005). Fifty-nine (18%) had at least one dose and 28 (8%) were fully vaccinated, even with COVID-19 infection. Headache and loss of smell, among clinical symptoms of COVID-19, were found to be significantly higher in BG (P = 0.012 and P = 0.023, respectively). Clinics with treatments for those with COVID-19 are listed in Table 3.

Table 3: Vaccination status of patients with COVID-19 with clinical characteristics and treatments for COVID-19.

	Biologic group (n = 58)	Non-biologic group (n = 77)	*P*-value
Vaccination (+ / n)	56	62	0.005
Post-vaccine COVID-19 positivity (+ / n)	30	29	0.389
Fully vaccinated (+ / n)	17	11	0.150
Interval time between the vaccine and COVID-19 infection (day)	92.93 (72.93)	81.40 (67.93)	0.740
Symptoms			
Asymptomatic (n)	8	9	0.723
Fever (n)	17	20	0.725
Dyspnea (n)	15	21	0.787
Cough/sputum (n)	20	29	0.620
Myalgia/weakness (n)	34	49	0.493
Loss of appetite (n)	13	20	0.574
Arthralgia (n)	20	23	0.628
Headache (n)	17	9	0.012
Sore throat (n)	7	9	0.989
Vomiting (n)	3	3	0.749
Diarrhea (n)	6	2	0.062
Loss of smell (n)	21	14	0.023
Loss of taste (n)	17	16	0.303
Lung involvement (n)	13	9	0.138
Hospitalization (yes, n)	10	11	0.680
Duration of hospital stay (day)	6.45 (4.50)	5.29 (4.44)	0.546
ICU stay (yes, n)	1	0	0.253
Non-invasive mechanical ventilation	1	0	0.363
COVID-19 Medication			
Favipiravir (n)	25	25	0.377
Hydroxychloroquine (n)	3	2	0.815
Azithromycin (n)	1	2	0.444
Steroids (n)	5	7	0.321
Anti-cytokine-IVIG (n)	1	0	0.363
Antibiotics	8	6	0.864

Data are presented as mean (SD). ICU: Intensive Care Unit, IVIG: intravenous immunoglobulin

The rate of patients with COVID-19 despite being vaccinated, were no different between BG (n = 30) and NG (n = 29) (P > 0.05). One patient completing the vaccination scheme was hospitalized with severe COVID-19 infection: this was a 56-year-old woman with a diagnosis of RA on rituximab and methotrexate. She did not need intensive care with hospitalization. Data regarding treatment for all patients with COVID-19 infection and those with COVID-19 after at least one vaccine are shown in Table 4. Fifty-nine patients (15.5%) with COVID-19 were infected after at least one vaccine dose, whereas

28 patients (7%) had the infection despite completing the vaccination regimen. There was no statistically significant difference between treatments for those with COVID-19 in both BG and NG ($P > 0.05$).

Table 4: Treatment for people with COVID-19 infection.

	All COVID-19 positive (n = 135)	COVID-19 positive + vaccinated (n = 59)
Biologic group	58 (43)	30 (50.8)
TNFi, n (%)	43 (74.1)	24 (80)
JAKi, n (%)	3 (5.2)	1 (3.3)
IL-17A inhibitor, n (%)	5 (8,6)	2 (6.7)
Rituximab, n (%)	6 (10.3)	2 (6.7)
IL-1 inhibitor, n (%)	-	-
CTLA4 Ig, n (%)	-	-
IL-6 inhibitor, n (%)	1 (1.7)	1 (3.3)
Non-biologic group	77 (57)	29 (49.2)
Methotrexate, n (%)	24 (31.2)	12 (41.4)
Leflunomide, n (%)	13 (16.9)	7 (24.1)
Hydroxychloroquine, n (%)	20 (26)	13 (44.8)
Sulfasalazine, n (%)	13 (16.9)	3 (10.3)
Azathioprine, n (%)	3 (3.9)	3 (10.3)
Colchicine, n (%)	10 (13)	2 (6.9)
Steroids, n (%)	35 (45.5)	14 (48.3)
NSAID, n (%)	10 (13)	-
Mycophenolate mofetil, n (%)	-	-

TNFi: Tumor Necrosis Factor Inhibition, JAKi: Janus Kinase Inhibitor, IL: Interleukin, NSAID: Non-steroidal anti-inflammatory drugs

Discussion

In our study, vaccination was more frequent for BG. There was no difference between BG and NG with all vaccine side effects. However, when side effects were evaluated, arm pain was more common with BG, whereas fever and rash were more common with NG. Side effects were higher in BNT162b2 vaccine with BG and NG. Vaccine-related disease exacerbation was seen at a rate of 1% for NG. Frequency of those infected with COVID-19 was lower for BG, but no difference was found in those vaccinated, or those who still had COVID-19 for BG and NG.

In a survey study conducted in those with inflammatory rheumatic disease, rates of vaccination for COVID-19 were 54% [12]. It was observed that they were willing to be vaccinated to protect themselves and their relatives, even without severe COVID-19. This increased to 67% if the doctor recommended it [13]. We saw a high vaccination rate of 92% in BG and 82% in NG. We suspect this was due to briefing about the vaccination for BG patients with close follow-up. Patients with BG were commonly vaccinated with a combined vaccine, and BNT162b2 was more common in NG. Patients on biological treatment had priority with vaccinations, with only CoronaVac vaccine available then.

In the literature, side effects ranging from 30 to 89% related to mRNA vaccine were reported, but most were not serious. The most common were pain and fatigue at the injection site [11, 14-17]. In the phase 4 immunogenicity study, conducted with Coronavac for inflammatory rheumatic patients, adverse effects were seen in 50% of inflammatory patients, with induration, headache, malaise, and sweating in the inflammatory group for the first dose (higher in the inflammatory group than the control group [18]). We found the frequency of vaccine side effects at 58%, which did not reach a significant difference between BG and NG in terms of side effects. Along with our study, another conducted in a pediatric patient group from Turkey, still no difference was found between BG, NG, and healthy control groups for all side effects [19]. Apart from these findings, analysis of side effects revealed that arm pain was more common in BG, while fever and rash were more common in NG. This may pertain to how treatments for BG can reduce fever. As stated, there are few studies evaluating differences between BG and NG in the literature.

The rate of side effects for vaccines shows differences between studies. In those given both mRNA and CoronaVac vaccines, side effects were more frequent after the first dose, with the rate decreasing after the second dose [15, 18]. In another study with a small number of patients, side effects were found at a higher rate after the second vs. the first dose [20]. In our study, there were patients with up to five doses of vaccine. We did not detect any differences in side effects after each dose between BG and NG. In a study of 225 patients with autoimmune rheumatic disease [21], side effects of 6 different COVID-19 vaccines were assessed: BNT162b2 was one with the highest frequency vs. the number of patients vaccinated, and localized pain was the most common. Side effects were less common in CoronaVac [21]. When BNT162b2 and CoronaVac were compared, the rate of side effects was highest in BNT162b2 for all doses. We did not find differences between groups with local, systemic, and local + systemic side effects evaluating doses individually, but local side effects were reported to be more common in the literature [16].

Inflammatory rheumatic diseases were excluded from the initial phase 3 studies of COVID-19 vaccines; this created hesitation in patients and clinicians. Vaccine reactogenicity was studied in inflammatory rheumatic diseases, and although this was higher, it was not more severe than healthy controls [14]. Our study included only patients with inflammatory rheumatic disease, with side effects from all vaccines; this profile was found to be similar to studies of both BNT162b2 and CoronaVac groups [22, 23].

Different results were found in studies for disease activity after vaccination. In a series of 1,101 patients [24], most were vaccinated with mRNA vaccines, but 15% reported worse rheumatologic disease status, experienced as moderate to severe. In another study of 5,121 patients [11], 4.4% of disease was detected, so treatment was changed in 1.5% of patients. There are smaller-scale studies in which there is no increase in disease activity after vaccination, or those with less than 1% activity [25, 26]. In our cohort, we identified 1% with increased disease after vaccination, and all were in the group not receiving BG.

In studies investigating indecisiveness about getting vaccinated, no consensus was established about a common cause. The far most common seemed to be fear of the vaccine, fear of side effects, and worsening of the disease, as well as safety concerns about the speed of vaccine production and doubts about its effectiveness [12, 27]. In this study, 11.8% of patients were not vaccinated, the most common reason being fear of side effects. The ratio of patients with COVID-19 infection after full dose vaccination was 0.6% in a systemic review [16]. In another multicenter study of 5121 patients, the rate of infection after full vaccination was 0.7% in patients with inflammatory rheumatological disease, as per the previous study [11]. In our study, the rate of COVID-19 infection was higher after one dose of vaccination or completing the vaccination scheme vs. in other studies. This may be due to the cross-sectional nature of our study, lack of standardization of the interval between doses,

variety of vaccines, and initial use of inactivated vaccine in our country.

It was reported that more than half the patients with rheumatological disease, hospitalized for COVID-19 after a full dose vaccination, used mycophenolate mofetil and B cell depletion therapy [28]. We did not detect any difference between treatment of patients with COVID-19 after vaccination with BG and NG. This may be the inhomogeneity of treatment in our population. For example, the number of patients using rituximab in BG and mycophenolate mofetil in NG was lower than other medications. These groups may not be complete enough to reach adequate sample size: 1 patient who received a full dose was hospitalized for severe COVID-19 after rituximab, a B-cell depletion therapy. In the NG group, about half the patients vaccinated with COVID-19 received steroid therapy, with a similar proportion taking hydroxychloroquine and methotrexate. No one in this group received mycophenolate. In accord with the data, when the antibody response after a single dose of BNT162b2 was assessed, it was shown that steroid and methotrexate can affect the vaccine response in chronic inflammatory diseases [29].

Limitations

This study has some limitations. Vaccination doses and intervals between doses were not standard in our country. In this study, people were vaccinated with different doses, from one to five. The CoronaVac was the only option available in our country when vaccination was given to patients receiving biological therapy for the first time. Since the BNT162b2 vaccine became available later, patients with BG were largely vaccinated with a combination of CoronaVac and BNT162b2. Since the interval time between doses depended on the person having it, it could not be standardized. Another limitation was a difference in the distribution of diseases and genders for BG and NG. We included all patients with inflammatory diseases who applied to our clinic at a certain time interval, as per our study design. Another limitation was that vaccine evaluation was performed only in clinical cases of COVID-19, as humoral and cellular immune response could not be evaluated in the laboratory. Disease activity was not evaluated for activity indices in the initial stage, with disease activity post-vaccine, not assessed for activity indices.

There are predominantly studies on mRNA vaccines in the literature, with the number of studies for other types of vaccines continuing to increase. Our study is important for evaluating two different groups of vaccines, or for groups with combined vaccinations, with real-life results, as well as to assess differences between BG and NG.

Conclusion

Patients with inflammatory rheumatic disease on b/tsDMARDs were vaccinated more often than others, and side effects were not different between BG and NG. Adverse effects were common in BNT162b2. Despite that COVID-19 infection occurred less in BG independent of vaccination, there was no difference between BG and NG in post-vaccine COVID-19 infection. Vaccine-related disease activity was as low as 1% in this study.

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Extracorporeal shock wave lithotripsy for urinary tract stones in pediatric patients: Our 11 years of experience

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Ethics Committee Approval
Ethical approval was obtained from the Ethics Committee of Harran University Faculty of Medicine (HRU/22/04/17).
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

Conflict of Interest
No conflict of interest was declared by the authors.

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Abstract

Background/Aim: Urinary system stone disease creates a significant burden on the health system. Many treatment methods are available, including extracorporeal shock wave lithotripsy (ESWL), endourological procedures, and open and laparoscopic procedures. In recent years, in parallel with technological developments, endourological devices have become more usable in the renal system. For this reason, urologists are opting for endourological procedures more frequently. ESWL is the least invasive procedure for urinary system stone disease, and it has a higher success rate in pediatric patients than in adults. In this retrospective cohort study, we analyzed the data from the pediatric cases in which we used ESWL treatment in our clinic. We aimed to reveal the effectiveness of ESWL and the factors that will increase the success rate of this procedure in light of the current literature.

Methods: The files of patients aged 16 years and under who underwent ESWL at the Urology Clinic of University of Health Sciences Sanliurfa Mehmet Akif Inan Training and Research Hospital between January 2010 and December 2021 were retrospectively reviewed. Age, gender, stone area, stone localization, number of sessions, energy and frequency used, complete stone-free status, and secondary intervention requirement were recorded. The absence of stone fragments or the presence of fragments smaller than 3 mm only in imaging after ESWL was considered a success.

Results: This study included 433 pediatric patients. The mean age of the patients was calculated as 12.02 (4.67) (range: 1–16) years. The most important factors affecting the number of residual stones were stone localization ($P = 0.045$) and size ($P < 0.001$). When stone localization was compared according to patient age, the older patients were found to have a significantly higher rate of stones in the proximal ureter than in the lower calyx of the kidney ($P = 0.045$) and renal pelvis ($P = 0.048$).

Conclusion: Although there are continual advances in other minimally invasive surgical methods today, ESWL is a treatment method that can be safely applied in pediatric patients. Stone size and stone localization are the two most important factors affecting its success rate.

Keywords: Pediatric urolithiasis, Lithotripsy, Shock wave, Urinary stone

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Introduction

Urinary system stone disease is a common and important health problem. It is rarer in the pediatric age group than in adults. This disease is endemic in Turkey, Pakistan, and some South Asian, African, and South American countries [1]. However, in recent years, the incidence of stones has been increasing across the world, especially in the adolescent age group. There is an annual increase of 4–10% in the incidence of stone disease [2, 3]. Urinary system stone disease creates a significant burden on the health system. Many treatment methods are available, including extracorporeal shock wave lithotripsy (ESWL), endourological procedures, and open and laparoscopic procedures. ESWL is the least invasive compared to other procedures [4]. ESWL has been shown to be an effective and safe procedure in the treatment of stones smaller than 20 mm in all childhood age groups, including infants [5]. In the pediatric age group, it was first performed in 1986 by Newman et al. [6]. The success rate seems to be higher in children than in adults [7]. The higher success rate in pediatric patients has been associated with relatively softer stone composition, smaller stone volume, smaller body structure allowing better transmission of shock waves, and easier stone passage due to increased ureteral compliance [8]. As in all stone treatments, the aim of ESWL is to provide complete stone-free status. In the last two decades in particular, the frequency of endoscopic procedures has increased with developments in endoscopic equipment. However, current guidelines still recommend ESWL as the first-choice treatment method in kidney stones of <20 mm [9].

Although the literature contains large-case series on ESWL treatment in adults, there are only a few large series on the treatment in children [9]. In this retrospective study, we aimed to reveal the efficacy of ESWL in pediatric patients and factors that will increase the success rate of this procedure by evaluating the data from pediatric cases in which we performed ESWL treatment over 11 years at our clinic and discussing our findings in light of the literature.

Materials and methods

This study was conducted at the Urology Clinic of University of Health Sciences Sanliurfa Mehmet Akif Inan Training and Research Hospital. Following the Declaration of Helsinki, consent was obtained from the Ethics Committee of Harran University Faculty of Medicine before the study (HRU/22/04/17). A total of 433 patients aged 16 years and younger who underwent ESWL at our clinic between January 2010 and December 2021 were included in the study. Patient files were retrospectively screened. Age, gender, stone area, stone localization, number of sessions, energy and frequency applied, complete stone-free status, and secondary intervention requirement were recorded. All of the patients were evaluated in terms of full urinalysis, hemogram, blood biochemistry, bleeding, and coagulation time before ESWL. They also underwent ultrasonography (USG) and direct urinary system graphy (DUSG) before and after ESWL. Stone size and localization and the presence of urinary system anomalies were evaluated before ESWL. Patients with solitary kidneys, congenital urinary system anomalies, bleeding disorders, and urinary tract infections were excluded from the study. All patients under the age of 12 years were evaluated by an anesthesiologist the day before the procedure to determine the American Society of Anesthesiology (ASA) score, and all had a score of either ASA 1 or ASA 2. Intravenous analgesics (paracetamol 10–15 mg/kg) were administered to the patients over 12 years of age, while general anesthesia or sedoanalgesia (ketamine 1.5 mg/kg and midazolam 0.05 mg/kg) were administered to those 12 years and under.

With the patients placed in the supine position, the procedures were performed using the Richard Wolf piezoelectric ESWL (Richard Wolf, Knittlingen, Germany). Fluoroscopic and ultrasonographic methods were used for focusing. The amplitude of shock waves was gradually increased and maintained at the point where the stone started to break. A maximum of 1,200 shocks were applied in the voltage range of 7–12 kilowatts (kW) in patients under the age of 5 years, a maximum of 2,000 shocks in the range of 12–18 kW in those aged 5–10 years, and a maximum of 2,500 shocks in the range of 12–18 kW in those over 10 years. One week later, the patients returned for a control session, during which simultaneous USG and DUSG were performed, and cases showing an absence of stone fragments or a presence of fragments smaller than 3 mm only on imaging were considered a success [10]. In the presence of fragments of 4 mm or larger, ESWL was applied again, leaving at least two weeks between the sessions. A maximum of three sessions of ESWL were undertaken. All the patients were evaluated one month after the last ESWL session. At the end of the three sessions, follow-up was recommended for clinically insignificant stones, while other endoscopic procedures were planned for patients requiring clinical intervention.

Statistical analysis

For the statistical analysis of the data obtained from the study, we used IBM SPSS Statistics v. 22.0. While evaluating the study data, the compatibility of the parameters with the normal distribution was checked with the Kolmogorov-Smirnov test. In addition to descriptive statistical methods (mean [standard deviation]), the unpaired t-test was used to evaluate quantitative parameters with a normal distribution, and Fisher's exact test was used for the evaluation of parameters that did not show a normal distribution. Significance was evaluated at the $P < 0.05$ level.

Results

This study included 433 pediatric patients, 229 boys and 204 girls, aged 16 and under. The mean age of the patients was calculated as 12.02 (4.67) (range, 1–16) years. The number of stones, stone side (right/left), stone localization, stone area, stone length, number of ESWL sessions performed, whether ureterorenoscopy (URS) was performed, residual stone status after ESWL, applied kW, and total number of shocks applied were determined. Table 1 presents the results of parameters that contributed to the presence of residual stones. The most important factors affecting residual stone status were stone localization ($P = 0.045$) and stone size ($P < 0.001$).

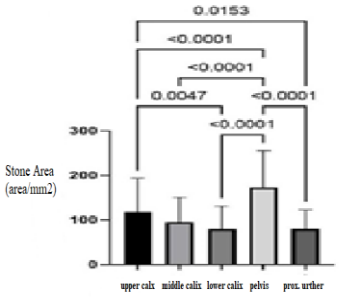
Table 1: Parameters affecting the presence of residual stones

	Total (n)	Residual stone (n)		*P*-value
		Absent	Present	
Number	433	370	63	
Age (years)		12.14 (4.644)	11.26 (4.84)	0.17*
Gender				
Male	229	199	29	0.32**
Female	204	171	33	
Stone localization				
Upper calyx of the kidney	65	59	6	<0.001**
Middle calyx of the kidney	115	107	8	
Lower calyx of the kidney	79	73	6	
Renal pelvis	119	82	37	
Proximal ureter	54	49	5	
Stone side				
Right	251	217	34	0.58**
Left	182	153	29	
Stone area (mm^2)		100.9 (59.4)	201.0 (100.1)	<0.001*
Number of sessions				
1	61	60	1	<0.001**
2	210	206	4	
3	162	107	58	
kw		16.61 (2.7)	16.95 (2.4)	0.35*
Total number of shocks		1862 (353.8)	1939 (372.1)	0.12*
JJ -	370	328	42	<0.001**
stent +	63	41	22	

*Unpaired t-test, **Fisher's exact test, kw: kilowatt

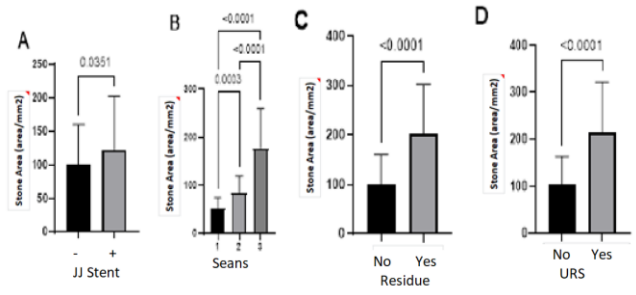
Considering the distribution of stone burden according to stone localization location, it was determined that the stones with the highest burden were located in the renal pelvis (Figure 1).

Figure 1: Results of Tukey's multiple comparison test showing the distribution of stone burden according to stone localization (*P*-values are given on the graph)



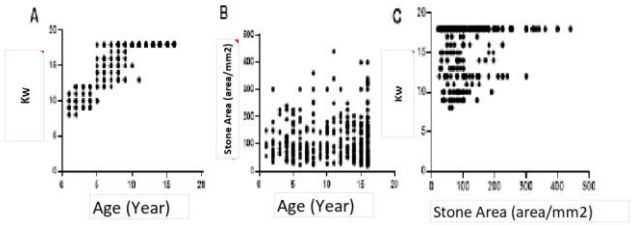
When the stone burden of the patients was compared according to their JJ stent requirement after ESWL, it was found that the stone burden of the patients with JJ stenting (122.4 (80.1) area/mm^2) was significantly higher than those without this requirement (100.9 (59.4) area/mm^2) (P = 0.035; Figure 2A). The stone burden of the patients also significantly differed according to the number of sessions (one session [51.96 (22.8) areas/mm^2] vs two sessions [84.82 (35.2) areas/mm^2], $P < 0.001$; one session vs three sessions [177.6 (82.1) area/mm^2], $P < 0.001$; and 2 sessions vs 3 sessions, $P < 0.001$, Figure 2B). As the stone burden increased, the residual stone rate ($P < 0.001$) and URS requirement also increased ($P < 0.001$). It was also determined that as the number of stones increased, the number of patients requiring a JJ stent increased (P = 0.02, Fisher's exact test) (Figure 2).

Figure 2: Comparison of the patients' stone burden according to JJ stent requirement after ESWL (unpaired t-test) (A), number of sessions (Tukey's multiple comparison test) (B), residual stone status (unpaired t-test) (C), and URS requirement (unpaired t-test) (D)



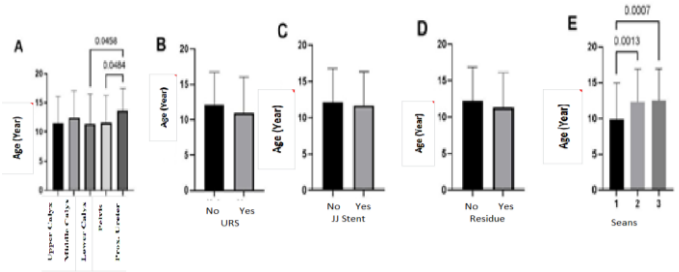
The applied kW value increased (positive correlation) as the patient age increased (r = 0.7065, $P < 0.001$). There was no correlation between patient age and stone burden (r = -0.0097; P = 0.84). A positive correlation was found between stone burden and kW (P = 0.017; r = 0.013) (Figure 3).

Figure 3: Correlation analysis. A; correlation between age and kw, B; correlation between patient age and stone burden, and C; correlation between stone burden and kw (Pearson's r correlation analysis method was used)



When URS and JJ stent requirements and residual stone status were compared according to patient age, no significant difference was found (Figures 4A, C, and D). However, the patients with a higher age were found to have a significantly higher rate of stones in the proximal ureter than in the lower calyx of the kidney (P = 0.045) and renal pelvis (P = 0.048). There was no other significant difference between the remaining groups (Figure 4).

Figure 4: Comparison of stone localization (Tukey's multiple comparison test) (A), URS requirement (unpaired t-test) (B), double-J stent requirement (unpaired t-test) (C), residual stone status (unpaired t-test) (D), and number of sessions (Tukey's multiple comparison test) (D) according to patient age (years)



As the stone area increased, the presence of residual stones increased ($P < 0.001$). However, the total number of shocks and kWs were not found to be associated with the presence of residual stones (Figure 5).

According to stone localization, the highest rate of residual stones was found in the renal pelvis ($P < 0.001$) (Figure 6).

Figure 5: Comparison of residual stone status according to stone area (A), kw (B), and total number of shocks (C) (unpaired t-test)

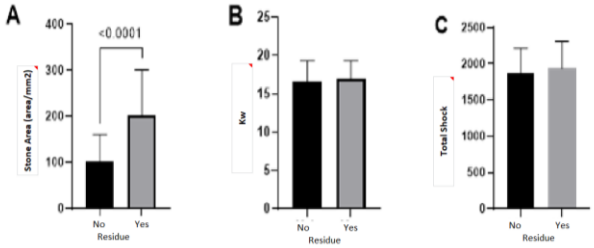
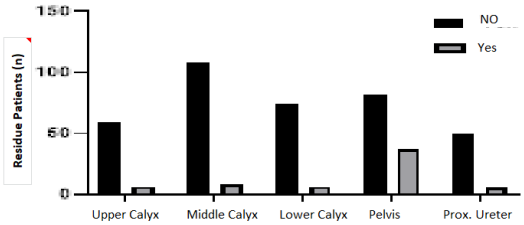


Figure 6: Comparison of stone localization according to the patients' residual stone status after ESWL



Discussion

In this study, the experiences of 433 patients aged 16 years and under who underwent ESWL due to urinary tract stones were evaluated. Although our stone-free rate (SFR) was low in the first session, it increased in the repeated sessions. We found that the need for a JJ stent increased as the stone size increased. We did not experience any major complications after ESWL. We compared our 11 years of data with the literature.

Pediatric nephrolithiasis is a rare urinary system disease, but the incidence of kidney stones in children has increased in the last decade, with a dramatic rise of 10% per year [11]. Stone disease prevalence studies report the prevalence of stone disease at 5.2% in patients under 18 years and 1–3% in the pediatric age group [12]. The 2019 pediatric urology guidelines of the European Association of Urology (EAU) recommend ESWL as the first-line treatment option for 10–20 mm kidney stones due to the minimally invasive nature of this intervention [13]. Among early complications are steinstrasse, decreased oral intake, pain requiring parenteral analgesics, gross hematuria, fever, and hematomas [14]. No statistically significant change has been reported in terms of renal parenchymal scarring in dimercapto-succinic acid screening or diethylenetriamine pentaacetic acid screening and glomerular filtration rate for up to six months after ESWL. Therefore, ESWL is considered to be a safe method for the treatment of kidney stone disease in children aged up to 16 years, with no adverse effects being observed on long-term kidney function [15].

ESWL is a procedure in which shock waves are used to break down kidney and ureteral stones into smaller pieces, which can then be spontaneously expelled from the urinary system. It is known that many factors, including patient selection, stone size, localization and composition, lithotripter type, operator experience, total number of shocks, energy delivered, and shock frequency, affect the final outcomes of ESWL [14, 15]. The average number of shock waves per ESWL session is approximately 1,800 to 2,000 (up to 4,000 if needed), and average power settings range from 14 kW to 21 kW. The use of USG and digital fluoroscopy has significantly reduced radiation exposure, and it has been shown that children are exposed to significantly lower radiation doses compared to adults [16–20]. Increased shock frequency and energy level have been associated with lower stone clearance among adults [21]. A retrospective cohort study of children treated with ESWL reported similar stone clearance and complications for the shock frequencies of 60 and 90 per minute [22]. In our study, the amplitude of shock waves was increased gradually until the stone started to break. It was determined that the kW value increased (positive correlation) as the patient age increased. There was no correlation between patient age and stone burden. A positive correlation was observed between stone burden and kW. When the groups with and without residual stones were compared, no statistically significant difference was found in relation to kW and the total number of shocks. We believe that this is related to the lack of a homogeneous distribution among the age groups. We attributed the increase in the kW value as the patient age increased to the shorter stone-skin distance in younger age groups. Since there is no study using a similar application method in the literature, we were not able to make a comparison in this regard.

When the three studies evaluating the largest series in the literature are examined, it is seen that the complete SFRs vary between 70 and 89.2% after repeated sessions of ESWL. The success rate after the first session was found to be between 50.5 and 82.4% in the same studies [21–25]. In our study, SFR was 13% after the first session and 85.9% after repeated sessions. While our results after multiple sessions were similar to those in the literature, our first session success rate was found to be much lower. This may be related to the higher mean age and higher stone area of our cohort.

In children, it is widely preferred to perform ESWL under general anesthesia to achieve a better focus and reach high shock frequencies. The rate of stone clearance after a single session of ESWL is low and depends on the location and size of the stone. Therefore, repeated procedures mean repeated general anesthesia and further associated risks [26]. In our clinical practice, we accepted the age of 12 years as the limit and applied general anesthesia or sedation in younger children and muscular or intravenous analgesics in older patients. Of the patients that received anesthesia, 20% (n = 36) were stone-free after a single session, and 48% (n = 87) achieved stone-free status after the second session.

In a meta-analysis published by Lu et al. [14] in 2015, it was found that ESWL was statistically significantly more successful in stones smaller than 10 mm than in those larger than 10 mm. When evaluated according to stone burden, the mean stone area of 85.9% (n = 370) of the patients without residual stones was 100.9 (59.4), while the mean stone area of 14.1% (n = 63) of those with residual stones was 201.0 (100.1). Our results on stone burden are consistent with those in the literature.

One of the important factors in breaking a stone is the localization of the stone. The infundibular angle between the lower calyx and the proximal ureter is an especially effective factor in stone removal. Lower calyx stones have the lowest clearance, while the highest stone-free rates are in the renal pelvis and ureteropelvic junction [27]. In a study published by Srisubat et al. [28] in 2009, ESWL success rates were found to be 86–89% in the renal pelvis, 71–-83% in the upper calyx, 73–84% in the middle calyx, and 37–68% in the lower calyx, according to stone locations. In our study, the rates were 68.9%, 90.7%, 93% and 92.4%, respectively. We believe that our complete stone-free rate in the renal pelvis is lower than that in the literature because of our high renal pelvis stone load.

Stone density and composition are other factors affecting the success of ESWL. While calcium apatite stones and struvite stones are sensitive to ESWL, cystine stones are resistant [29]. Stone density can be found by calculating the HU value on non-contrast tomography [30]. However, due to the high radiation risk, non-contrast tomography is not recommended for pediatric patients unless it is necessary [31]. Since stone analysis and non-contrast tomography were not performed in the patients in our study, stone composition and HU values could not be determined. This is an important limitation of our study.

The update on urinary system stone disease in children published by Silay et al. [32] in 2017 stated that pediatric ESWL complications ranged from 1.5% to 35%. Mild complications,

such as skin ecchymosis, hematuria, infection, and renal colic, are frequently observed [7, 31]. In our series, skin ecchymosis and infection were not observed in any of the patients. Steinstrasse is another important complication that requires intervention. The rate of stenting before ESWL is 15.4% in the literature [32-34]. In our study, we did not apply a JJ stent to any of the patients before the procedure. A JJ stent was used in cases that developed steinstrasse or had colic pain after the procedure. Our rate of JJ stent insertion was 14.5% after the procedure, and we also determined that the JJ stent requirement was statistically significantly higher in the group with residual stones. Fedulo et al. [35] reported that 26.25% of patients who developed steinstrasse required endoscopic intervention. In another study, Badawy et al. [25] performed endoscopic intervention on 1.2% of patients. Habib et al. detected steinstrasse in 6.66% of patients and treated 20% of these patients endoscopically [23]. Our results on the post-ESWL intervention requirements are in agreement with the literature.

Conclusion

The success of ESWL in the pediatric age group depends on factors such as stone size, type, and localization, characteristics of the device used, distance of the stone to the skin, and operator experience. Based on 11 years of pediatric ESWL experience in our clinic, our SFR increased compared to the first years we performed this procedure. In light of our experience, we recommend that the applied energy and the number of shocks be kept within ideal limits. Despite advances in laser lithotripsy technology and micro and ultra-thin endoscopic instruments developed for stone intervention, ESWL can still be safely recommended as the first-choice treatment in stones smaller than 20 mm.

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The effects of moderate-intensity step-aerobics, spinning, and educational game exercise programs on plasma dopamine and oxytocin levels in women in the menopausal transition period

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Ethics Committee Approval
Ethics Committee approval was obtained from the Niğde Ömer Halisdemir University Non-Interventional Clinical Research Ethics Committee (Date: December 24, 2020, No: 2020/81).
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

Conflict of Interest
No conflict of interest was declared by the authors.

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Abstract

Background/Aim: Menopausal transition (MT) is defined as the transition from reproductive to post-reproductive life. Oxytocin has beneficial effects on health problems, such as sexual activity disorder, vaginal atrophy, cardiovascular system diseases and acceleration in bone mass loss, which may develop due to changes in reproductive hormone levels during the MT period. During exercise, which can be used as adjuvant therapy for most of these health problems, a temporary increase in catecholamine levels is required for response to exercise-induced stress. However, the effects of exercise programs applied during the MT period on plasma dopamine (pDA) and plasma oxytocin (pOT) levels are unknown. The aim of this study was to investigate the effects of three different types of exercise on plasma dopamine (pDA) and plasma oxytocin (pOT) levels in sedentary women in the MT period.

Methods: Twenty-six sedentary healthy participants in the MT period whose fitness levels in the standard maximal exercise treadmill test were at a level that would complement the exercise programs in our study, were included in the study. Participants with the following conditions were excluded from the study: physical disability that would not allow exercise, systemic disease, unilateral oophorectomy, or history of smoking. In addition, participants who could not complete any of the exercise programs for any reason were excluded from the study. Three different exercise programs at moderate intensity [maximum heart rate (HR) = 50%-60%] of 60 minutes duration were performed by the participants at one-week intervals: (i) step-aerobics (SA), (ii) spinning (SP) and (iii) station work in the form of recreational educational games (EG). pOT and pDA levels were measured using the enzyme-linked immunosorbent assay method in venous blood samples taken from participants before the exercise and during the last five minutes of the exercise. pOT and pDA levels measured before the exercise and in the last five minutes of the exercise were compared.

Results: The median age was 45 (41-45) and the body mass index (BMI) was 29 (27-34). There was a significant increase in mean pDA levels during exercise compared to pre-exercise in all three activities of moderate-intensity, SA, SP, and EG (P = 0.008, P = 0.001 and P = 0.030, respectively). The mean pOT level increased significantly during moderate-intensity SA and EG (P = 0.003 and P = 0.001, respectively). When the relationships between pDA and pOT levels and the variables of age, BMI, pulse rate, and maximum HR during all three exercises were evaluated, there was a significant positive correlation between pOT levels and maximum HR only during EG (r = 0.439, P = 0.028).

Conclusions: This study showed that SA and EG applied in women in the MT period increased both pDA and pOT levels, while SP only increased the pDA level significantly. Therefore, SA and EG exercises can contribute positively to the quality of life of women with health problems due to low pOT levels during the MT period.

Keywords: Menopausal transition period, Woman, Exercise, Dopamine, Oxytocin

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Introduction

Menopausal transition (MT) is defined as the transition from reproductive to post-reproductive life [1], and the average duration of the MT period is approximately four years [2]. During this period, depending on the changes in reproductive hormonal patterns, various changes including vasomotor and genitourinary symptoms, mood, temporary cognitive function and sleep disorders, decrease in sexual desire, and increase in bone absorption rate can be observed [1]. This period is also associated with the development of central adiposity [3]. Aerobic exercises (AEs), which are characterized by high repetition and low resistance demands during skeletal muscle contraction, play a homeostatic role in the regulation of energy production, blood flow, and substrate use in response to movement. They are also influential on obesity, coronary artery disease, Alzheimer's disease, and depression and anxiety disorders. Exercise can be used as adjuvant therapy for various diseases, such as osteoporosis and some types of cancer [4].

Dopamine (DA) is a member of the catecholamine family. In the synthesis of catecholamines, phenylalanine or L-tyrosine are sequentially converted to 3.4-dihydroxyphenylalanine (L-DOPA), DA, norepinephrine (NE) and epinephrine (E) by enzymatic biotransformation [5]. Although catecholamines are mainly produced in the adrenal medulla and postganglionic fibers of the sympathetic nervous system [5], DA cannot cross the blood-brain barrier [6] and approximately 50-90% of plasma DA (pDA) originates from sympathetic noradrenergic (sympathoneural) nerves [5]. Less than 2% of the total pDA is in free form, but free pDA concentration is approximately equivalent to E concentration and is around 20% of NE concentration [7]. DA has established roles in the regulation of cognitive functions in the central nervous system (CNS), in the control of movement and coordination [6], and in various tissues outside the CNS, including the cardiovascular system (CVS) and the endocrine pancreas [8]. Oxytocin (OT), a classical neuropeptide, is produced mainly in hypothalamic OT neurons, in extrahypothalamic brain regions, and scattered OT cells in peripheral regions [9]. Locally produced OT, in addition to its local effects, influences central and peripheral functions in relation to brain OT [10]. OT generally facilitates social cognition and behaviors, such as social memory, attachment, sexual activity, maternal behavior, inter-couple bonding and trust [9]. It also protects the CVS [10], exerts anabolic effects on bone tissue [11], and can reverse vaginal atrophy when applied topically [12].

Blood OT levels decrease gradually starting at middle age [11] and decreased OT secretion during aging can cause various health problems, including CVS diseases, osteoporosis, urinary incontinence, sexual dysfunction, obesity, and a decrease in postmenopausal metabolic rate [9]. During exercise, which can be used as adjuvant therapy for most of these health problems [4], a temporary increase in catecholamine levels is required for response to exercise-induced stress [5]. However, the effects of exercise programs applied during the MT period on pDA and pOT levels are unknown. This study aims to evaluate the effects of moderate-intensity exercise programs, including step-aerobics (SA), spinning (SP), and educational games (EG), on pOT and pDA levels during the MT period of sedentary women.

Materials and methods

This prospective study was conducted between March 1, 2021 and August 1, 2021, as a cooperative research between the Gynecology & Obstetrics and Medical Biochemistry Departments and the Sport Sciences Faculty at Niğde Ömer Halisdemir University. All study steps were carried out at the Training and Research Hospital of the same university. Local Ethics Committee approval was obtained before the study (Niğde Ömer Halisdemir University Non-Interventional Clinical Research Ethics Committee, Date: December 24, 2020, No: 2020/81).

Participants

Twenty-six healthy participants between the ages of 41-45 years who were in the MT period completed this study. Subjects were selected from women who applied to the NOHU Training and Research Hospital Gynecology and Obstetrics Polyclinic between March 1, 2021 and May 11, 2021.

A detailed anamnesis of all women with menstrual cycle disorders was taken in the Gynecology and Obstetrics Clinic, followed by physical and genital examinations, transvaginal ultrasonography, and diagnostic laboratory tests. Blood urea, creatinine, glucose, high-density lipoprotein, low-density lipoprotein, triglyceride, hemoglobin, follicle stimulating hormone, luteinizing hormone, thyroid stimulating hormone, and free thyroxine levels and blood pressure were measured in all participants who agreed to participate in the study. The Stages of Reproductive Aging Workshop plus Ten Simplified Bleeding Criteria were used to determine the menopausal status of women with menstrual cycle disorders. Early MT was defined as a persistent (recurring within ten cycles) difference of seven or more days in length of consecutive cycles. Late MT transition was defined as the occurrence of amenorrhea for 60 days or more, or detection of greater than 25 IU/L of follicle-stimulating hormone (FSH) in a randomly taken blood sample [13]. Physical inactivity was defined as insufficient physical activity (PA) level less than 150–300 minutes of moderate-intensity, or 75–150 minutes of vigorous-intensity PA, or some equivalent combination of moderate-intensity and vigorous-intensity aerobic PA per week [14]. The PA readiness questionnaire test [15] was administered to all participants to determine the safety or potential risks of exercise. In addition, the standard maximal graded exercise treadmill test [16] was applied to determine the fitness levels of the participants.

The study included healthy sedentary participants who were in the early or late MT period, did not have any diagnosed disease, did not receive medical treatment, did not actively engage in any sports, and whose fitness levels in the standard maximal exercise treadmill test were at a level that would complement the exercise programs in our study.

The following patients were excluded from the study: those with physical disability or systemic disease (cardiovascular, respiratory, musculoskeletal, metabolic-endocrine system, cancer and psychological disease) that would not allow exercise, malnutrition, anemia, ovarian pathology, oophorectomy, hysterectomy, medical treatment history, and a

history of smoking. In addition, participants who could not continue the research for any reason or could not complete any of the exercise programs in our study were also excluded.

Protocols of exercise interventions

Before starting the exercise, the participants' height, weight, resting heart rate (HR) and blood pressure were measured. BMI (kg/m²) was calculated by dividing body weight (kg) by height squared (m2). Maximum HR was calculated by subtracting age from 220. The target HR of the participants was calculated according to the Karvonen formula: Target HR range = [resting HR + (maximum HR - resting HR) × exercise intensity%] [17].

The daily activities of the participants were restricted on the days in which the exercise programs were scheduled, but not on other days. Participants were subjected to 60-minute moderate-intensity (50-60% of maximum reserve HR) SA, SP, and EG exercise programs at one-week intervals under supervision. In each type of exercise, seven minutes of warm-up exercises were completed before starting the exercise, and five minutes of stretching and cooling exercises were performed to rest the muscles after the exercise was completed. The heart rate during exercise was determined by calculating the average heart rate, as recorded on a Polar watch (Polar S810i, Finland) every five minutes.

The step-aerobics exercise plan consisted of the following movements: 24 counts of march and step touch; 16 counts (each side) of step heel, step touch, toe touch, toe jack, heel jack, knee lift, leg curl, leg reach, grapevine, heel lift, soft jog, and basic step.

The spinning exercise plan comprised pedaling movements for 20 minutes each, by sitting (holding and clapping) and standing (climbing and sprinting position) according to the rhythm of the music.

Educational game and station plan: Since the educational games were in a competitive format, the participants were divided into two equally-sized groups, A and B. Following the hoop game (Hula Hoop), each participant performed five counts of the following: trunk curl on a mat, mounting and dismounting a bosu ball while standing, rope jumping, and slalom movement. The educational games were completed by touching a pre-specified agility cone and then passing a hoop through the slalom bar once. Finally, the groups made a contest of popping the balloon on the chair for two minutes by sitting down.

Blood sample collection and measurement of oxytocin and dopamine levels in plasma

Before the exercise and during the last five minutes of the exercise, 5 ml of venous blood samples were taken from all participants in tubes with ethylenediaminetetraacetic acid, and plasma was obtained by centrifuging at 3000 rpm for ten minutes. The obtained plasma samples were stored at -80 °C until ELISA analysis. pDA and pOT levels were measured using ELISA kits (Cloud-Clone Corp., USA) according to the manufacturer instructions. The minimum detectable dose of OT (Catalog No: CEB052Ge) and DA (Catalog No: CEA851Ge) were typically less than 4.99 pg/mL and 4.71 pg/mL respectively. The intra-assay coefficient of variation (CV) and inter-assay CV for both OT and DA were < 10% and < 12%, respectively. Human DA and OT standard curve graphs were obtained by plotting the absorbance values obtained against their standard concentrations. The least squares method was used in this graph. Concentration values corresponding to the absorbance values determined from each sample were calculated by using the relevant correlation coefficient and the resultant linear equation.

Statistical analysis

The Statistical Package for Social Sciences (SPSS) version 15 (SPSS Inc., Chicago, IL, USA) was used for statistical analyses. Normal distribution was determined by examining the Shapiro-Wilk tests. If continuous data did not show normal distribution, descriptive statistics were provided with median and interquartile range values; whereas mean and standard deviation were used to describe data with normal distribution. When continuous variables were not normally distributed, comparisons of two dependent groups were performed using the Wilcoxon test, and when they were normally distributed, comparisons were performed with the paired samples t test. The Kruskal-Wallis test was used for intergroup comparisons to detect the differences in the amount of change in hormone levels during exercise. Spearman's correlation coefficients were calculated to determine directional relationships between continuous variables. Two-tailed *P*-values with an alpha significance level of less than 0.05 were considered statistically significant.

Results

The median age of the 26 women included in the study was 45 (41-45) years and subjects had a median BMI of 29 (27-34).

When the moderate-intensity SA, SP and EG exercise types were compared in terms of median HR and maximum HR before and during exercise, no significant difference was found (Table 1).

Table 1: Pulse rates before and after exercise programs

Exercise programs	Pulse rate		Maximum
	Before exercise Mean (SD) Median (min-max)	During exercise Mean (SD) Median (min-max)	Heart rate Mean (SD) Median (min-max)
Step-Aerobics	76.15 (2.89) 76 (70-84)	132.96 (1.93) 132 (130-136)	175.23 (1.27) 174 (174-178)
Spinning	75.02 (1.69) 75 (70-79)	123.77 (1.82) 123 (120-126)	174.73 (2.24) 175 (171-180)
Educational Game	75.81 (1.76) 75 (70-80)	138.88 (1.44) 140 (137-142)	172.77 (2.36) 172 (170-178)
P-value	0.94	0.065	0.96

SD: Standard deviation, min-max: The minimum and maximum values of the dataset

Compared with the mean plasma levels before exercise, there was a significant increase in mean pDA levels during all three exercise programs and in mean pOT levels during the EG and moderate-intensity SA programs (Table 2).

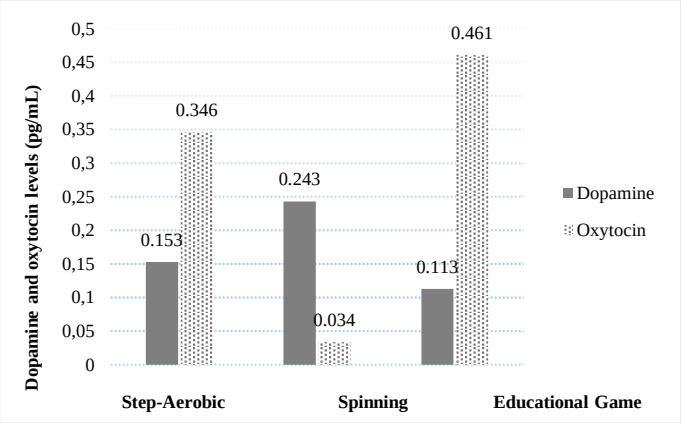
Table 2: Comparison of mean plasma dopamine and oxytocin levels (pg/mL) before and after step-aerobics, spinning, and educational game exercise programs

Hormones	Step-Aerobics Mean (SD)	Spinning Mean (SD)	Educational Game Mean (SD)
Dopamine			
Before Exercise	2.00 (0.19)	2.03 (0.15)	2.01 (0.17)
After Exercise	2.16 (0.31)	2.28 (0.29)	2.13 (0.19)
P-value	0.008	0.001	0.030
Oxytocin			
Before Exercise	2.16 (0.40)	2.66 (0.32)	2.04 (0.56)
After Exercise	2.51 (0.43)	2.69 (0.21)	2.50 (0.53)
P-value	0.003	0.808	0.001

SD: Standard deviation

Although pDA level increased during all three exercise programs, there was no significant difference between the three programs in terms of the amount of change in pDA (P = 0.083). The amount of increase in pOT level during exercise was found to be significantly higher in both the SA and EG groups compared to the SP group (P = 0.025), while levels were similar in the SA and EG groups (P = 0.714) (Figure 1).

Figure 1: The effects of moderate-intensity step-aerobics, spinning, and educational game exercise programs on plasma dopamine and oxytocin levels



When the relationships between pDA and pOT levels measured during exercise and age, BMI, pulse rate and maximum HR were evaluated, there was a significant positive correlation only between pOT levels during EG and maximum HR (r = 0.439, P = 0.028).

Discussion

In this study, the changes in pDA and pOT levels were investigated by applying moderate-intensity acute SA, SP and EG exercise programs to sedentary women in the MT period at one-week intervals. Our first important finding in the study was that all three exercise programs caused a significant increase in pDA level. Our second important finding was that SA and EG, which were the other two exercise programs apart from SP, caused a significantly greater increase in pOT levels.

The effect of exercise on pDA level has been investigated in several studies that differ in participant characteristics and methodological aspects [18-21]. In one study, an increase in plasma DA, NE and E levels was found in non-athlete healthy women in their 20s (compared to controls) after high-intensity standard team sports, light athletics, gymnastics, and outdoor runs performed with the interval method [18]. Another study found that there was a significant increase in plasma levels of DA, NE and E in participants with dilated cardiomyopathy with a mean age of 49.7 years after cardiopulmonary exercise (upright graded bicycle exercise) [19] A significant increase in urinary DA level four weeks after the application of bicycle ergometer exercise in participants aged 42-63 with essential hypertension has been reported [20]. Furthermore, in a study involving women with depression who were aged 18-65 years, exercise was found to significantly increase the pDA level compared to the control group (16 weeks of low-intensity warm-up, aerobics and low-intensity cooling down exercises); however, interestingly, plasma NE and E levels did not change [21]. In another study, it was found that NE and E concentrations in arterial and venous blood increased, but DA concentrations did not change after dynamic forearm exercises at submaximal and maximal intensities in healthy men [22]. Although comparison of our results with the literature was limited due to a lack of similarly designed studies, we found that there was a significant increase in pDA level after the application of moderate-intensity SA, SP, and EG exercises in sedentary women in the MT period. Interestingly, in a previous study, it was found that blood DA level did not change with dynamic forearm exercises at submaximal and maximal intensities [21], but increased in two other studies in which relatively larger muscle groups were activated, which was similar to the results found in our study [18, 19]. These findings suggest that the size of muscle groups activated during exercise may be related to the change in blood DA level.

The effect of acute exercise on pOT levels in healthy non-athletes was investigated in only one previous study involving nine participants aged 25-42 years [23]. In this study, a 20-minute treadmill run was planned with gradual increase of intensity until 90% of the maximum oxygen consumption (VO2) of each subject was reached in the last five minutes of the exercise. It was determined that the pOT concentrations did not change in either the early follicular phase or the midluteal phase of the menstrual cycle [23]. Another study reported that 11 weeks of lower-body pressure supported exercise, which was applied to obese non-diabetic women aged 18-56 years by use of 60% body weight for half an hour 2-3 times a week, had no effect on pOT concentrations [24]. When the effects of a maximum fatigue test, 60-minute treadmill and a 56-km ultramarathon, were evaluated among seven runners (five males and two females), the ultramarathon was found to be the only running exercise that caused a significant increase in pOT value [25]. It has also been reported that female athletes between the ages of 14-21 have lower nocturnal pOT levels compared to non-athletes [26]. When literature data are evaluated overall, it can be said that the effects of different types and intensities of exercise programs on pOT levels may vary depending on the age groups and fluctuating PA levels of participants. In our study, we found that the pOT level increased significantly during moderate-intensity SA and EG exercise programs in women in the MT period, but did not change during SP. Our findings show that, although different types of exercise performed among sedentary women in the MT period have similar duration and intensity, their effects on pOT levels may vary.

Adaptive changes that occur during exercise to meet the demands of increased metabolic rate include increased VO2, HR, and respiratory rate, altered blood flow to active muscles, secretion of stress hormones (e.g., adrenocorticotropic hormone, cortisol, catecholamines), and increased body temperature [27]. Conflicting results have been reported in studies evaluating the potential effects of DA on PA and exercise capacity in healthy participants. It has been reported that when DA infusion was given to male participants during exercise, breath-taking ventilation (VE), carbon dioxide production (VCO2) and oxygen consumption (VO2) did not change [28], but the VE / VCO2 slope decreased [29]. In studies in which DA inhibitor drugs were administered, it was found that the acute administration of a dual dopamine/noradrenaline reuptake inhibitor to athletes increased performance under hot conditions [30], while pulmonary gas exchange improved with DA receptor blockade in

healthy young adults, but with decreased exercise performance [31]. Our study showed no significant correlation between pDA level and heart rate and maximum heart rate during the three exercises. Although it is not possible to determine whether there is a relationship between pDA level and exercise performance with these findings, our findings suggest that there may not be a direct relationship between pDA level and HR in women in the MT period.

Little is known about the role of OT in PA. One study determined that there was a positive correlation between fasting serum OT level and resting energy consumption in athletes, but this correlation was not found in non-athletes, and it was concluded that OT may play an important role in regulating energy intake and use, especially in determining an energy deficit [32]. In addition, it has been reported that OT increases stress-related oxygen consumption to facilitate muscle movement for active stress coping behaviors under stressful conditions [33, 34]. This can have a supportive role for various hormones (arginine vasopressin and atrial natriuretic peptide), which are known to have a role in the regulation of fluid balance, together with brain natriuretic peptide, under extreme physical stress conditions [25]. As a result, OT may cause cardiovascular effects through its effects on the central nervous system and with other mediators such as atrial natriuretic peptide, nitric oxide, and alpha 2-adrenoreceptors [35]. Our study observed that pOT levels increased significantly in both moderate-intensity SA and EG during exercise, but there was a positive and significant correlation between maximum HR and pOT level during EG. These findings suggest that, unlike SA, various mechanisms may be activated during EG, which may be influential on pOT levels and could have an effect on maximum HR.

Limitations

One of the limitations of our study is that we could not compare our findings with the literature due to methodological differences. Our study comprised female participants in MT, limiting the possibility of generalizing our findings to other populations. In addition, only pDA and pOT levels were measured both before and after exercise in our study. Therefore, the relationship between other hormones whose secretion can change with exercise and pDA or pOT could not be evaluated. In addition, once the participants were included in the study, their daily activities were restricted on the days of the exercise programs, but daily activities were not restricted on other days. Therefore, differences in baseline activities may have affected the results of the study.

More studies are needed to reveal the potential bidirectional relationships between acute or chronic exercise programs and pDA or pOT levels in women experiencing MT and the long-term clinical outcomes of these relationships.

Conclusion

This study showed that SA and EG applied in women in the MT period increased both pDA and pOT levels, while SP was only associated with a significant increase in pDA level. Therefore, SA and EG exercises that increase pOT levels might contribute positively to the quality of life of women with health problems, which may be a result of low pOT levels during the MT period.

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Effects of low-dose, short-duration periods of asymmetric radiation on colony formation of C6 glioma cell cultures

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Abstract

Background/Aim: Previous studies on fractionation in radiation therapy have been mainly based on applying equal doses over at least 6 h. The main purpose of fractionation is to increase normal tissue tolerance rather than tumor sensitivity. Thus, one can apply higher doses to the tumor. In contrast, new molecular studies indicate that high and low doses of radiation act by different mechanisms. This study was conducted to investigate the radiobiological effect of asymmetrical radiation doses.
Methods: This is an experimental study done *in vitro* with a G6 glioma cell line to investigate the responses when C6 glioma cells are irradiated with single doses of 30 and 230 cGy using an orthovoltage therapy device or doses split into 30 and 200 and 115 and 115 cGy within periods of 15 and 30 min. A total of 5×10^3 cells were transferred to polyethylene culture flasks for colony formation. A cluster containing more than 30 cells was considered a new colony.
Results: A single dose of 230 cGy caused a 56.8% reduction in colony formation. However, when 230 cGy was divided over 15- and 30-min periods in fractions of 30 and 200 cGy, colony formation was significantly reduced compared to the control group (68.13% and 52.64%, $P = 0.030$, respectively). This effect continued when the radiation dose was divided into equal fractions (115 and 115 cGy) with periods of 15 and 30 min (42.60%, $P = 0.021$ and 20.77%, $P = 0.008$, respectively).
Conclusion: According to these results, (i) short interval (15 and 30 min) fractionation significantly reduces colony formation compared to a single equal dose; and (ii) the protective mechanisms activated in cell response probably vary at different radiation doses and different fractions.

Keywords: C6 glioma cells, Fractionation, Radiation, Interval, Low dose

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Introduction

Radiation therapy is one of the main modalities used in cancer treatment. Shortly after the discovery of ionizing radiation, it began to be used to treat many diseases [1]. Although radioactive irradiation was quickly introduced to clinical application, its mechanism of action is not fully understood even today, and the evolution of treatment protocols and doses is empirical [2]. Soon, it was recognized that splitting the dose into parts (fractionation) did not harm tumor control while increasing the tolerance of normal tissues, and the concept of fractionated treatment was born [3].

As generally accepted, (i) cells that divide rapidly are more affected than those that divide more slowly. (ii) Dividing the dose into daily fractions ensures normal tissue tolerance, and (iii) the maximum dose that could be applied depends on dose-limiting tissues. As a further attempt, the procedures of hyperfractionization (daily dose divided into two or three equal loads) and continuous hyperfractionization (no interval at weekends) were tested for this purpose. However, they did not provide the expected benefit and remained experimental in daily treatment practice [4].

Studies that aim to optimize the therapeutic ratio of radiation therapy are based on the principle of applying multiple doses in a day within a minimum interval of 4 h. However, emerging data in molecular biology demonstrated that cellular response to irradiation in the first minutes differs from those after hours. There are numerous studies on the effects of low-dose irradiation on biological and gene expression [5]. Studies revealed a protective priming effect in low doses of radiation on the cell if a second dose is given after hours. A study on human lymphoblasts indicated that a 5 cGy low dose irradiation, followed by a standard dose of 2 Gy, upregulates genes for protein synthesis while genes for metabolism are inactivated [6]. Research indicated that irradiations activate early stress genes and large molecular panels confirm that many more genes are involved than expected [7, 8]. As a result of molecular mechanisms, an adaptive response emerges in the cells, which differs in radiation sensitivity [9, 10]. Mechanisms activated on low-dose radiation trigger apoptosis, while conventional doses abolish this effect with further molecular rearrangements [11, 12].

Most of these studies were conducted considering the genetic pattern of molecular expression in a time interval of at least 3 or 4 h between fractions. Almost all molecular mechanisms are activated within minutes and return to the initial level within hours [13]. For instance, low-dose priming irradiation causes cell proliferation and generally reduces radiation response [14]. Almost all studies divided the daily dose into equal fractions at least 6 h apart. The effect of a large fraction after a first low dose can provide a biological additive gain. This study investigates irradiation with priming doses given in asymmetric fractions in short periods on colony formation in C6 glioma model cells.

Materials and methods

Cell culture

A C6 glioma cell lineage (American Cultural Collections) was used in the study. C6 glioma cell colony formation is a well-defined cell culture model that has been successfully used in radiobiology due to its adhesion to culture flasks, which allows colony counting. Briefly, cells were incubated at 37°C in a 95% O_2, 5% CO2 condition containing 10% fetal bovine serum; 1% L-glutamine and 1% essential amino acid, supported by 10,000 units of penicillin and 10 mg/ml of streptomycin solution DMEM (Sigma Chemicals Co., In St Louis, Missouri). During the experimental phase, the cells were treated with trypsin-EDTA, separated from the culture environment, washed after being turned into a single cell suspension, and re-suspended in a full nutrition medium. A total of 500,000 cells were transferred to polyethylene culture flasks with areas of 25 cm^2 and 5 ml volume in an attempt for colony formation. The passages were checked twice per week using an inverted microscope [15]. These steps were carried out at the Department of Histology and Embryology of Istanbul University, Istanbul Medical Faculty.

Irradiation procedure

An orthovoltage teletherapy device (Stabilipan) was used for radiation exposure [16]. Before the experiment, a preliminary study was conducted to determine the radiation sensitivity of the C6 glioma cell line to standardize the dose. Cells were irradiated with a single fraction of 50, 100, 200, 400, and 800 cGy to determine the LD50 dose in colony formation. The results were plotted on a graph, and the dose that inhibits colony formation by 50% was extrapolated from the logarithmic scale, thus reaching a dose of 230 cGy. This dose was divided into two fractions as low and conventional doses of 30 and 200 cGy. In contrast to the 6-h exposure interval in classical hyperfractionation, the time between fractions was shortened to 15 and 30 min. In this way, we aimed to investigate the biological effect of a low priming dose followed by a conventional fraction size at short intervals.

Determination of colony-forming

Culture flasks were transferred to the Institute of Oncology of Istanbul University in thick styrofoam containers to avoid temperature differences. Culture flasks were placed in the center of the 20 × 20 cm field, and radiation was applied. Care was taken to ensure that all culture dishes were homogeneously affected by temperature change. After irradiation, containers were rapidly transferred to the Department of Histology and Embryology of the Istanbul Medical Faculty. A cell count per mm^3 was performed by applying trypsin to the cell culture within an hour. One thousand cells from each sample were transferred to the new culture medium and incubated for 7 days. Flasks were evaluated under an inverted microscope; a cluster containing more than 30 cells was considered and counted as a new colony.

Statistical analysis

The experiment results were compared between groups using ANOVA with the Bonferroni test. Differences were considered significant at *P*-values less than 0.05. All results are expressed as mean SEM calculated from triplicate data.

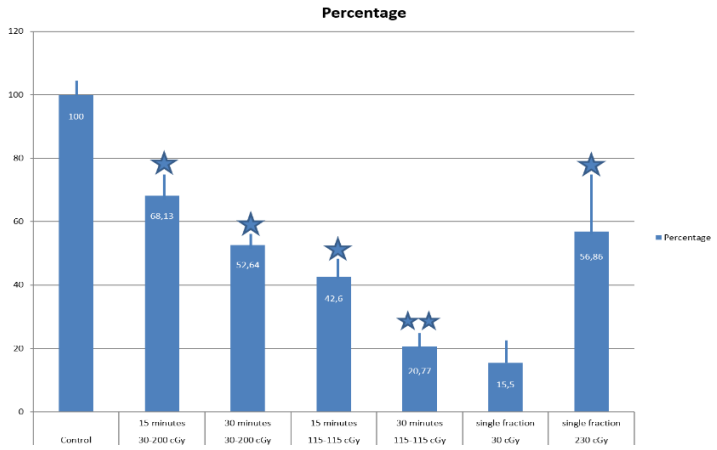
Results

The different irradiation procedures on C6 flioma cell lineage data are shown in Table 1. Single-dose 30 cGy irradiation reduced colony formation by 15.50%, but there was no significant difference compared to the control group. In contrast, a single dose of 230 cGy caused a decrease in colony formation close to the calculated value in the preliminary study (56.8%). However, when 230 cGy was divided into 30 and 200 cGy fractions over 15- and 30-min periods, it significantly reduced colony formation compared to the control group. (68.13% and 52.64%, P = 0.038, respectively). This effect was also detected when the radiation dose was divided into equal fractions (115 and 115 cGy) with periods of 15 and 30 min (42.60%, P = 0.021 and 20.77%, P < 0.01, respectively; Figure 1).

Table 1: Data of different irradiation procedures on the C6 flioma cell lineage.

Execution	Control	30-200 cGy 15 min	30-200 cGy 30 min	115-115 cGy 15 min	115-115 cGy 30 min	30 cGy single fraction	230 cGy single fraction
mean	568	387	299	242	118	480	323
(SD)	(20.97)	(62.63)	(20.42)	(24.84)	(3.84)	(35.42)	(156.37)
Percentage	100	68.13	52.64	42.60	20.77	15.50	56.86
P-value		0.030	0.038	0.021	0.008	0.0913	0.0363

Figure 1: Effects of different dose and fraction periods on colony formation percent inhibition in C6 glioma cells.



Discussion

Discussion

This study indicates that fractionated equal doses of radiation with short periods of 15 and 30 min reduce the statistically significant ability of cells to create colonies more than the administration of the total dose in a single fraction. Dividing the dose into two doses over a short period, even if the fraction sizes are different, causes more colony inhibition than the application of the same dose in a single fraction. This effect persists when the dose is given in two loads of 115 and 115 cGy. These results differ from previous studies using longer fraction intervals [17, 18]. It can be argued that the colony-forming inhibition effects of irradiation depend not only on the dose but also on the function of the period between the fractions.

In conventional radiotherapy schedules, hyperfractionation aims to increase normal tissue tolerance. It is generally accepted that treatment periods should be at least 6 h. However, another reason to prefer 6-h periods is that this is likely the maximum feasible period in clinical practice. In daily treatment procedures, the so-called “set-up” (laying the patient in treatment position) takes 80% of the total treatment duration before irradiation. Thus, after positioning, most devices complete radiation application within seconds. For this reason, previous studies, even experimental, have been based on intervals of at least 4 h [19]. However, these results indicate that 15- and 30-min fraction intervals can provide a further advantage in colony-forming ability.

Recently published research has revealed that multiple mechanisms modulate cell response to radiation in terms of temporal aspect [20]. The radiation exposure initiates a stress response that becomes active within seconds [21]. In contrast, the effects of this acute response are short-lived. If programmed cell death does not occur, it has no impact on cell survival [22]. The radiation dose that leads to programmed cell death is much lower than the doses used in clinical practice. Thus, increasing doses abolishes the priming effect of low-dose irradiation.

For this reason, it is important to evaluate whether the biological effects of a conventional fraction will provide an additional therapeutic advantage if applied split by minutes (asymmetric low-high) without changing the total dose [23]. This study demonstrated that a low priming dose followed by a standard dose divided into two asymmetric fractions reduced the colony-forming property of C6 cells. A possible explanation of this phenomenon is molecular changes triggered after low priming dose administration. For instance, it has been demonstrated that even a low dose of 0–22 cGy in human A549 lung adenocarcinoma, T98G glioma, and MCF7 breast carcinoma cell lineages has an inhibitory effect on the p53 gene expression [24]. In a further study, it has been shown that when radiation was administered at a low dose rate (72–168 h) on HeLa Hep2 cells, the expression of early response genes was induced [25]. Another explanation for the additive suppression of colony formation by short-period radiation is the synchronization of cell cycles. Thus, within minutes after the first irradiation, cells go to the synchronous division stage, reinforcing the effect of the following second dose [26, 27]. Previous studies have confirmed that conventional single fraction significantly reduces colony formation [28]. In accordance with our study, if 230 cGy doses are divided into two equal fractions, the additive effect differs. Furthermore, extending the interval between fractions from 15 min to 30 min strengthens the effect. However, this research is a preliminary experimental study. It could not cover the exact limit of the optimal dose-time interval, and its clinical interpretation may be completely controversial.

Conclusions

This result supports other studies that have used initial irradiation with a low priming dose, followed by a conventional dose, effectively reducing colony-forming ability in the C6 glioma cell line. A limitation of this study is that no other molecular markers were used besides fractionation. The C6 glioma cell line is an appropriate model for evaluating the effects of radiation therapy. In contrast, unlike the general approach, asymmetric fractions were applied within short periods in this study. However, since the study aimed to test a hitherto untested approach, it confirms that dividing the dose into parts produces a separate effect.

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Immunohistochemical evaluation of glucose transporter protein-1 density in the placenta in preeclampsia patients and its association with intrauterine growth retardation

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Ethics Committee Approval
The study protocol was approved by the Ethics Committee of Erciyes University Medical Faculty Hospital (Date of Issue/Number: 2021/258). All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

Conflict of Interest
No conflict of interest was declared by the authors.

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Abstract

Background/Aim: Preeclampsia (PE) complicates 2–8% of all pregnancies worldwide. Placental malperfusion and dysfunction are observed in PE. The supply of glucose, the main energy substrate for the fetus and placenta, is regulated by placental expression and activity of specific glucose transporter proteins (GLUTs), primarily GLUT1. GLUT1 expression is affected by uteroplacental malperfusion and oxidative stress, which are important components of PE. Very few studies have investigated GLUT1 expression in preeclamptic placentas. In this study, we aimed to compare GLUT1 staining intensity in the terminal villi of the placenta in healthy subjects and patients with E-PE or L-PE and determine whether there was a relationship between GLUT1 staining intensity and IUGR.

Methods: This case-control study was carried out in our hospital's gynecology and obstetrics clinic, a tertiary center for perinatology cases. A total of 94 placentas, 47 of which were preeclamptic and 47 were from uneventful pregnancies (controls), were included in the study. PE was diagnosed according to the American College of Obstetrics and Gynecologists 2019 diagnostic criteria for gestational hypertension and PE. Placentas in the control group were obtained from pregnancies without maternal, placental, or fetal pathology and resulted in spontaneous idiopathic preterm or term delivery. The PE group was divided into two subgroups as early onset PE (E-PE [$\leq 33^{+6}$ gestational week]) and late-onset PE (L-PE [$\geq 34^{+0}$ gestational week]), according to the gestational week of PE onset. Sections prepared from placental tissues were stained for GLUT-1 by immunohistochemical method. Slides were evaluated by light microscopy, and each slide was scored from 0 to 4 to determine the staining intensity. The results were compared between the control and PE group/PE sub-groups.

Results: GLUT1 scores were significantly higher in both early- and late-onset PE subgroups compared to controls ($P < 0.001$ for both). In the late-onset PE subgroup, GLUT1 scores were significantly higher in those with severe PE features than those without them ($P = 0.039$). While intrauterine growth restriction (IUGR) was not found in any cases in the control group, IUGR was present in 11 (23.4%) of 47 pregnant women with PE, including eight (53.3%) of the 15 pregnant women with early-onset PE and 3 (9.38%) of the 32 pregnant women with late-onset PE. GLUT1 scores were similar in placentas obtained from pregnant women who had PE with and without IUGR ($P = 0.756$). In the late-onset PE subgroup, GLUT1 scores were correlated negatively with maternal body mass index ($r = -0.377$, $P = 0.033$) and positively with placental weight-to-fetal weight ratio ($r = 0.444$, $P = 0.011$).

Conclusions: Our findings show that GLUT1 expression might be increased due to placental adaptation to new conditions in PE and, thus, is unlikely to be the main factor in PE-related IUGR.

Keywords: Preeclampsia, Placenta, Glucose transporter proteins, Glucose transporter protein-1, Intrauterine growth retardation

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Introduction

Preeclampsia (PE) is defined as the development of hypertension with proteinuria or hypertension with thrombocytopenia, renal failure, liver dysfunction, or pulmonary edema, which begins after the 20th week of pregnancy [1]. PE complicates 2–8% of all pregnancies worldwide [1] and is responsible for 12% of all maternal deaths [2]. Although the pathophysiology of PE has not been clarified, a clinical subclassification with early-onset ($<34^{+0}$ weeks of gestation) and late-onset ($\geq 34^{+0}$ weeks of gestation) [3] has been increasingly accepted [4, 5]. Early onset PE (E-PE) has been predominantly associated with defective placentation during the first few weeks of pregnancy, indicating a pathophysiological similarity to intrauterine growth restriction (IUGR). Unlike E-PE, late-onset PE (L-PE) is suggested to develop in the presence of maternal cardiovascular risks and a discrepancy between placental supply and demand, ultimately leading to oxidative changes in the placenta [4]. Although the underlying causes and timing can differ, placental malperfusion and dysfunction are observed in both E-PE and L-PE [6].

Among the nutrients provided by the maternal circulation, glucose is the main energy substrate for the fetus [7, 8] and the placenta [9]. The main regulatory factors in the maternal-fetal glucose exchange process are the placental expression and activity of specific glucose transporter proteins (GLUTs) [8]. In the placenta, GLUT1 is the primary isoform found in syncytiotrophoblasts [10, 11], which are cells that function as the primary barrier for the transfer of nutrients from the mother to the fetus. The GLUTs that mediate glucose transfer are expressed in both the maternal-facing microvillous membrane (MVM) and the fetal-facing basal plasma membrane (BM) of syncytiotrophoblasts [9]. While GLUT1 expression remains unchanged in the MVM throughout pregnancy, its expression in the BM increases around 2-fold between the 16–22 and 27–30 gestational weeks and remains stable after this period. There is significantly higher GLUT1 expression in MVM syncytiotrophoblasts compared to BM syncytiotrophoblasts [12]. Thanks to its large surface area and high GLUT density, the MVM has a high capacity for glucose transport. This property facilitates the maintenance of a glucose gradient between the syncytiotrophoblast cells and the fetal capillary, which is necessary for net glucose transport to the fetus. In addition, this discrepancy enables the placenta to receive a sufficient amount of energy for its consumption, which corresponds to approximately one-third of total placental glucose uptake [9].

Few studies [13-15] have investigated GLUT1 expression in preeclamptic placentas or its relationship with IUGR. Considering the importance of placental adaptation for fetal and maternal health and that BM GLUT1 levels change considerably during periods coinciding with PE onset, we hypothesized that placental GLUT1 concentrations could be associated with PE or IUGR. Therefore, in this study, we aimed to compare GLUT1 staining intensity in the terminal villi of the placenta (MVM+BM) in healthy subjects and patients with E-PE or L-PE and also, to determine whether there was a relationship between GLUT1 staining intensity and IUGR.

Materials and methods

This case-control study was carried out in our hospital's gynecology and obstetrics clinic, a tertiary center for perinatology cases. The study protocol was approved by the ethics committee of the Non-invasive Clinical Research Ethics Committee of Erciyes University (date: April 7, 2021, decision no: 2021/258). All participants were informed in detail about the purpose(s) of the study, and informed consent forms were obtained before inclusion.

Study population and exclusion criteria

First, placentas in the study group were obtained according to the inclusion/exclusion criteria. Although it was noted that GLUT1 expression did not change during pregnancy in MVM and remained stable after 30 weeks of gestation in BM [12], placentas matching those complicated by PE in terms of the gestational week at birth were included in the control group. Placentas in the control group were obtained from pregnancies without maternal, placental, or fetal pathology and resulted in spontaneous idiopathic preterm or term delivery. PE was diagnosed according to the American College of Obstetrics and Gynecologists (ACOG) 2019 diagnostic criteria for gestational hypertension and PE [1]. Pregnant women with PE who had any of the following characteristics were excluded from the study: a systemic disease diagnosis before pregnancy, any complications other than PE, such as gestational diabetes mellitus or hypertension during pregnancy, smoking and alcohol use, multiple pregnancies, or fetal or placental anomalies. The final PE group was divided into two subgroups, E-PE ($\leq 33^{+6}$ gestational week) and L-PE ($\geq 34^{+0}$ gestational week), according to the gestational week of PE onset. Additionally, patients who had PE with severe features were also determined according to the presence of any of the following: severe blood pressure elevation occurring twice at least 4 h apart (systolic ≥160, diastolic ≥110 mm Hg), new-onset cerebral or visual problems, hepatic abnormality, thrombocytopenia (<100,000 per ul), renal abnormality, or pulmonary edema.

Data collection

At the first visit, a detailed anamnesis of all participants was taken, and physical and obstetric examinations were performed. Maternal age, gravidity, parity, last menstrual period (LMP), and other relevant examination results were recorded. Gestational age was primarily calculated based on the last menstrual dates reported by the participants and was confirmed by crown-rump length (CRL) measurements performed within the first trimester, whereas, in patients who did not know their last menstruation date, CRL measurements were used to calculate the gestational age. Intrauterine growth restriction (IUGR) was defined according to ACOG 2013 diagnostic criteria [16] and confirmed at postpartum.

The maternal body mass index (BMI) values were calculated before delivery. Blood and urine samples were also taken from all participants for the measurement of complete blood count and biochemical and urine analyses. Analysis results were obtained from the hospital automation system. Glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), total and direct bilirubin, blood urea nitrogen (BUN), creatinine, urinary protein levels, hemoglobin (Hb), and platelet count (Plt) were recorded.

Gestational week at delivery, type of delivery, APGAR scores (1st and 5th minute), placental weight (PW), and fetal weight (FW) were obtained from newborn cards. The PW/FW ratio was calculated. In patients with PE, we recorded the mean of the last two systolic and diastolic blood pressure measurements before starting magnesium sulfate for eclampsia prophylaxis.

Placental specimen acquisition and preparation

After delivery, the placentas were randomly numbered and transferred to the Pathology Laboratory in a plastic container. All the placentas included in the study were processed in the same manner. The fetal membranes on the surface of the placenta and the umbilical cord at the insertion site were cut off. Superficial fetal vessels were drained from all blood, conjoined blood clots on the maternal surface were removed, and the placentas were weighed (gram) on a calibrated digital device. Horizontal and vertical incisions were made through the placenta to determine macroscopic intraparenchymal pathology, and a total of three tissue samples (1×1×1 cm) were taken from different parts of each placenta. Tissue samples were fixed in 10% formaldehyde for 12–24 h and embedded in paraffin blocks.

Immunohistochemical evaluation

Final tissue sections used for immunohistochemical (IHC) analysis of GLUT1 were prepared similarly to those previously described [17]. Briefly, serial 5-µm thick tissue sections of formalin-fixed, paraffin-embedded tissue samples were used. Tissue sections were deparaffinized twice in xylene (5 min applications), rehydrated through graded ethanol solutions to distilled water, and washed in 1X phosphate buffered saline (PBS; Lonza, Basel, Switzerland). Antigenic determinant or epitope retrieval was carried out by treating the slides in a PT Link (Dako, Santa Clara, CA, USA). Endogenous peroxidase was inhibited with a peroxidase-blocking solution (REAL™ Peroxidase-Blocking Solution; Dako, Denmark) for 15 min, and non-specific binding sites were blocked with an additional protein block (DAKO) for 20 min. Afterward, sections were immunostained with the anti-glucose transporter GLUT1 antibody (ref: ab15309; Abcam, Cambridge, UK) and incubated for 30 min at room temperature. Next, sections were incubated with the secondary antibody (goat anti-rabbit, Ref: P0448, Dako), and the 3,3-diaminobenzidine (DAB; Dako) chromogen was used. Finally, sections were counterstained with hematoxylin (Sigma-Aldrich, San Luis, AZ, USA), dehydrated in alcohol, cleared with xylene, and mounted for analysis. The positive control specimens for the analyses were invasive ductal carcinoma sections (breast tissue). Negative controls were obtained by using blocking serum instead of primary antibody.

Quantitative analysis and scoring of immunohistochemical staining

The staining intensity for GLUT1 was evaluated independently on coded slides by the same pathologist (HA) via light microscopy (Nikon DS-Fi2, Tokyo, Japan). Four different regions (×40 magnification) of each of the three slides prepared from the same placenta were analyzed. The intensity of GLUT1 staining in both the MVM and BM of the syncytial barrier was evaluated by a single pathologist. Staining intensity was determined as absent (0 points), trace (1 point), mild (2 points), moderate (3 points), and intense (4 points). The final score was accepted as the rounded whole number closest to the arithmetic mean of the scores obtained from four different regions of the three specimens obtained from each placenta. Microscopic images showing the presence and absence of GLUT1 staining in the trophoblastic cells of the terminal villi in a preeclamptic placenta are shown in Figure 1.

Statistical analysis

All analyses were performed on SPSS v21 (SPSS Inc., Chicago, IL, USA). For the normality check, the Shapiro-Wilk test was used. Data are given as mean (standard deviation [SD]) or median (1st quartile - 3rd quartile) for continuous variables according to the normality of distribution and as frequency (percentage) for categorical variables. In the analysis of normally distributed variables, the independent samples t-test was used to compare two groups, and the one-way analysis of variance (ANOVA) was used between more than two-group comparisons. In the analysis of non-normally distributed variables, the Mann-Whitney U test was used to compare two groups, and the Kruskal-Wallis H-test was used to compare more than two groups. To assess directional relationships between continuous variables, Pearson's correlation coefficient was considered for normally distributed variables, and the Spearman correlation coefficient was used for non-normally distributed variables. $P < 0.05$ values were accepted as statistically significant results.

Results

A total of 94 placentas, obtained from 47 women with PE-complicated pregnancy and 47 pregnant women with a well-matched gestational week at delivery (to the PE group), were included in this study. Among the 47 pregnant women with PE, 37 (78.7%) used antenatal magnesium sulfate, and 15 (31.9%) used antenatal corticosteroids. The comparison of the PE group and the controls in terms of maternal demographic characteristics and clinical data are shown in Table 1.

Table 1: Comparison of the control and PE groups in terms of maternal demographic characteristics and clinical/laboratory data.

Parameters	Groups		
	Controls (n = 47)	Preeclampsia (n = 47)	*P*-value
MA (years), median (min - max)	28 (20 - 38)	29 (19 - 41)	**0.035**
Gravidity, median (min - max)	2 (1 - 3)	2 (1 - 4)	0.401
Parity, median (min - max)	1 (0 - 2)	1 (0 - 3)	0.594
M-BMI (kg/m^2), mean (SD)	27.66 (2.57)	30.36 (5.47)	**0.044**
SBP (mmHg), median (min - max)	110 (100 - 120)	160 (150 - 170)	**<0.001**
DBP (mmHg), median (min - max)	70 (60 - 80)	100 (90 - 110)	**<0.001**
Glucose (mg/ dL), median (min - max)	90 (76 - 111)	88 (73 - 108)	0.915
AST (U/L), median (min - max)	19 (15 - 24)	24 (19 - 49)	**0.005**
ALT (U/L), median (min - max)	10.5 (9 - 12)	15 (11 - 40)	**0.006**
LDH (U/L), median (min - max)	406.5 (369.5 - 473)	547 (437 - 754)	**0.001**
T-Bil (mg/dL), median (min - max)	0.55 (0.5 - 0.9)	0.6 (0.5 - 1)	0.562
D-Bil (mg/dL), median (min- max)	0.3 (0.2 - 0.4)	0.3 (0.2 - 0.4)	0.777
Proteinuria, n (%)	0 (0.00%)	47 (100.00%)	**<0.001**
BUN (mg/dL), median (min- max)	9 (7 - 11)	11 (9 - 16)	**0.013**
Creatinine (mg/dL), median (min- max)	0.7 (0.55 - 0.7)	0.8 (0.7 - 1)	**0.002**
Hemoglobin (g/dL), mean (SD)	12.15 (1.18)	12.77 (1.71)	0.092
Plt (×10^3 cells/mm^3), mean (SD)	220.93 (93.14)	211.99 (103.26)	0.740
GW at delivery (weeks), median (min-max)	36 (32 - 39)	36 (32 - 39)	0.967
Type of delivery			
Vaginal, n (%)	40 (85.11%)	18 (38.30%)	**0.001**
Caesarean section, n (%)	7 (14.89%)	29 (61.70%)	
IUGR, n (%)	0 (0.00%)	11 (23.40%)	**0.026**
PW/FW ratio, mean (SD)	0.20 (0.02)	0.20 (0.05)	0.656

P-values in bold indicate statistical significance ($P < 0.05$). MA: maternal age at delivery, M-BMI: maternal body mass index, SBP: Systolic blood pressure, DBP: diastolic blood pressure, AST: aspartate aminotransferase, ALT: alanine aminotransferase, LDH: lactate dehydrogenase, T-Bil: total bilirubin, D-Bil: direct bilirubin, BUN: blood urea nitrogen, Plt: platelet count, GW: gestational week, IUGR: intrauterine growth retardation, PW/FW ratio: placental weight/fetal weight ratio.

Figure 1: Microscopic images showing lack of GLUT1 staining in the trophoblastic cells of the terminal villi in a preeclamptic placenta (H&E × 200, Scale bar: 5 µm).
A) GLUT-1 score 1: Placenta of a 25-year-old case diagnosed with preeclampsia at 32 weeks of gestation, with normal fetal development and severe preeclampsia features (H&E × 200).
B) GLUT-1 score 2: Placenta of a 29-year-old case diagnosed with preeclampsia at 32 weeks of gestation, with fetal growth retardation and severe preeclampsia features (H&E × 200).
C) GLUT-1 score 3: Placenta of a 31-year-old case diagnosed with preeclampsia at 37 weeks of gestation, with normal fetal development and severe preeclampsia features (H&E × 200).
D) GLUT-1 score 4: Placenta of a 30-year-old case diagnosed with preeclampsia at 36 weeks of gestation, with fetal growth retardation and severe preeclampsia features (H&E × 200).

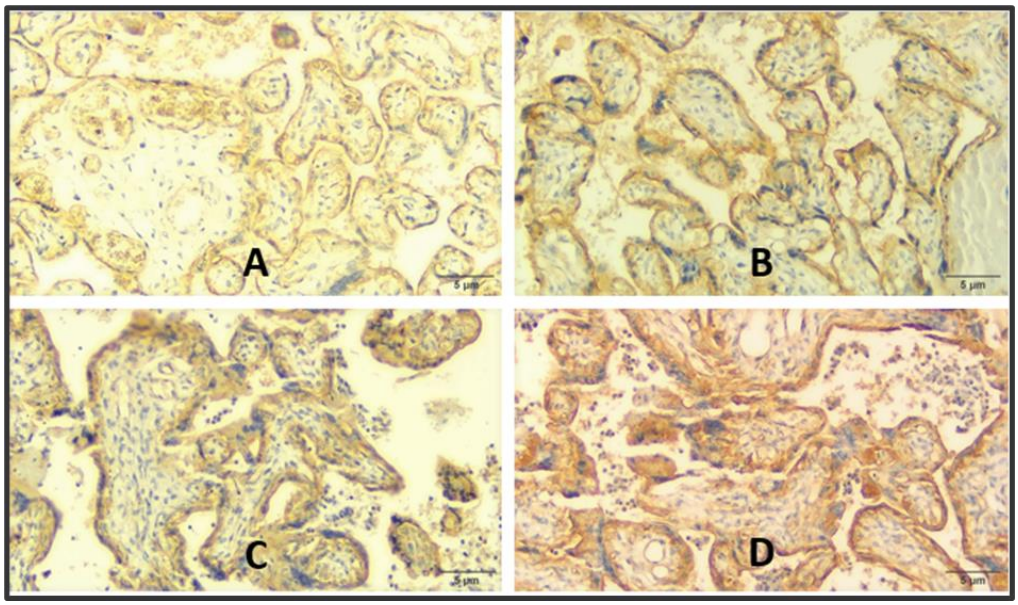


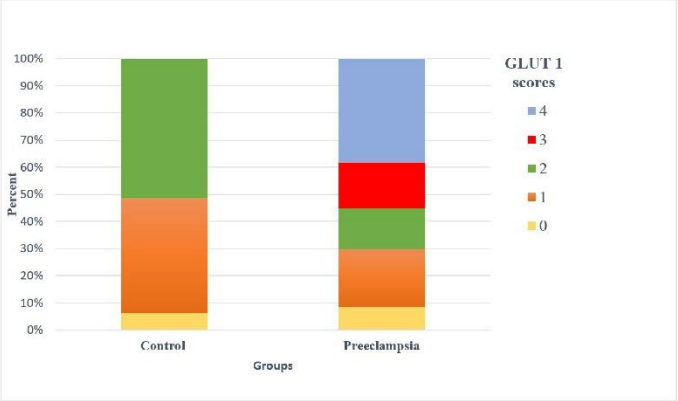
Table 2: Comparison of clinical and laboratory data between the control, E-PE, and L-PE subgroups.

	Control (n = 47) Mean (SD)	E-PE (n = 15) Mean (SD)	L-PE (n = 32) Mean (SD)	Test Statistic	*P*-value
MA (years)[1]	28 (20 - 38)	27 (20 - 39)	29 (19 - 41)	2.384 *	0.100
Gravidity	2.10 (1.17)	2.80 (1.90)	2.88 (2.32)	0.725	0.696
Parity	0.95 (0.89)	1.40 (1.68)	1.31 (1.53)	0.287	0.866
M-BMI (kg/m^2)	27.66 (2.57) [a]	29.01 (5.68) [a,b]	30.99 (5.34) [b]	6.479	**0.039**
SBP (mmHg)	113.00 (9.38)	165.67 (13.48)	158.55 (21.26)	57.520 *	**<0.001**
DBP (mmHg)	67.75 (9.80)	105.33 (13.02) [a]	98.91 (16.10) [a]	39.024	**<0.001**
GLUT1 score[1]	1.45 (0 - 4)	2.47 (0 - 4) [a]	2.72 (0 - 4) [a]	23.720	**<0.001**
Glucose (mg/ dL)	95.05 (32.21)	93.40 (22.82)	97.66 (35.59)	0.028	0.986
AST (U/L)	20.42 (7.27) [a]	184.33 (379.05) [b]	38.34 (39.90) [a,b]	10.397	**0.006**
ALT (U/L)	13.15 (8.73)	107.20 (202.90) [a]	23.88 (26.25) [a]	11.076	**0.004**
LDH (U/L)	464.9 (167.1) [a]	1192.9 (1305.5) [b]	706.5 (612) [a,b]	12.951	**0.002**
T-Bil (mg/dL)	0.69 (0.27)	1.29 (1.98)	0.74 (0.38)	0.155	0.925
D-Bil (mg/dL)	0.50 (0.83)	0.71 (1.58)	0.32 (0.16)	0.609	0.738
BUN (mg/dL)	12.86 (18.71) [a]	14.60 (6.33)	11.75 (4.53) [a]	7.982	**0.018**
Cr (mg/dL)	0.62 (0.19) [a]	0.90 (0.30) [b]	0.79 (0.23) [a,b]	10.043	**0.007**
Hb (g/dL)	12.15 (1.18)	13.02 (1.88)	12.66 (1.65)	1.357 *	0.265
Plt (×10^3/mm^3)	218.23 (99.22)	189.21 (105.96)	222.38 (102.53)	0.564 *	0.571
PW/FW ratio	0.20 (0.02) [a,b]	0.23 (0.06) [a]	0.18 (0.04) [b]	11.293	**0.004**

P-values in bold indicate statistical significance in the three-group comparison. [1] Values are presented as median (min - max). [a,b] Same letters illustrate statistical similarity between the denoted groups in post-hoc comparison(s). * One-way Analysis of Variance (ANOVA), other >2-group comparisons performed with the Kruskal-Wallis H-test. E-PE: early-onset preeclampsia, L-PE: late-onset preeclampsia, MA: maternal age at delivery, M-BMI: Maternal body mass index, SBP: systolic blood pressure, DBP: Diastolic blood pressure, AST: aspartate aminotransferase, ALT: alanine aminotransferase, LDH: lactate dehydrogenase, T-Bil: total bilirubin, D-Bil: direct bilirubin, BUN: blood urea nitrogen, Cr: creatinine, Hb: hemoglobin, Plt: platelet, PW/FW ratio: placental weight/ fetal weight ratio.

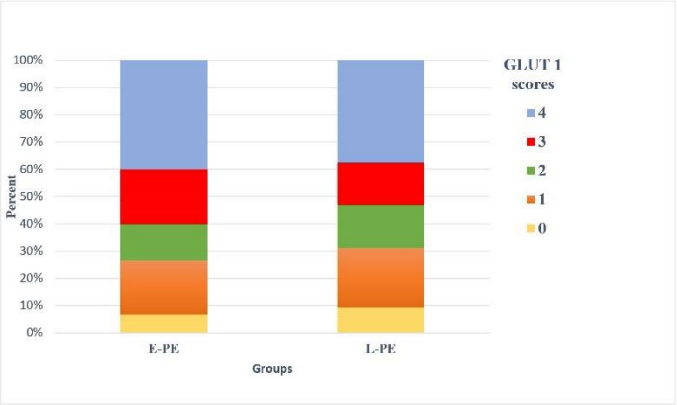
GLUT1 IHC scores were determined to be "0" in 3 (6.38%), "1" in 20 (42.55%), and "2" in 24 (51.06%) of the 47 placentas in the control group. In patients with PE, scores were determined to be "0" in 4 (8.51%), "1" in 10 (21.28%), "2" in 7 (14.89%), "3" in eight (17.02%) and "4" in 18 (38.3%) of the 47 placentas. GLUT1 scores were found to be significantly higher in the PE group compared to those with uneventful pregnancies ($P < 0.001$) (Figure 2).

Figure 2: Distribution of placental GLUT1 scores with regard to the control and preeclampsia groups.



PE was early-onset in 15 (31.9%) and late-onset in 32 (68.1%) of the 47 pregnant women in the PE group. Both antenatal magnesium sulfate and corticosteroid prophylaxis were applied to all the 15 pregnant women (100%) in the E-PE subgroup. In the L-EP subgroup, 22 (68.75%) of 32 pregnant women had received antenatal magnesium sulfate prophylaxis, while none had received antenatal corticosteroids. The frequency of those who received antenatal magnesium sulfate prophylaxis was significantly higher in the E-PE subgroup than in the L-PE subgroup ($P < 0.001$). GLUT1 IHC scores were determined to be "0" in 1 (6.67%), "1" in 3 (20%), "2" in 2 (13.33%), "3" in 3 (20%), and "4" in 6 (40%) of the 15 placentas in the E-PE subgroup. In patients with L-PE, scores were determined to be "0" in 3 (9.38%), "1" in 7 (21.88%), "2" in 5 (15.63%), "3" in 5 (15.63%), and "4" in 12 (37.5%) of the 32 placentas. GLUT1 scores were significantly higher in both the early-onset and late-onset PE subgroups compared to the control group ($P = 0.001$ and $P < 0.001$, respectively). The comparison of data between the control, E-PE and L-PE groups is shown in Table 2. However, there was no significant difference in GLUT-1 scores between the E-PE and L-PE subgroups ($P = 0.792$) (Figure 3).

Figure 3: Distribution of placental GLUT1 scores with regard to the early-onset and late-onset preeclampsia subgroups.



PE with severe features was identified in 37 (78.72) of the 47 PE patients, including all 15 (100%) women in the E-PE subgroup and 22 (68.8%) of the 32 pregnant women in the L-PE subgroup. The frequency of PE with severe features was significantly higher in the E-PE subgroup than in the L-PE subgroup (P = 0.019). GLUT1 scores were similar in placentas obtained from pregnant women with PE with and without severe features (P = 0.126). However, GLUT1 scores were significantly higher in placentas obtained from pregnant women with L-PE with severe features than in pregnant women without severe features (P = 0.039).

In our study, while IUGR was not found in any cases in the control group, IUGR was present in 11 (23.4%) of 47 pregnant women with PE, including eight (53.3%) of the 15 pregnant women with E-PE and three (9.38%) of the 32 pregnant women with L-PE. The frequency of IUGR in pregnant women with E-PE was significantly higher than in pregnant women with L-PE (P < 0.001). GLUT1 scores were similar in placentas obtained from pregnant women who had PE with and without IUGR (P = 0.756).

Finally, in each of the groups (including the E-PE and L-PE subgroups), the directional relationships between GLUT1 scores and other continuous variables, including parity, maternal age, BMI, systolic and diastolic blood pressure, blood glucose, AST, ALT, LDH, BUN, creatinine, total and direct bilirubin, hemoglobin, platelet levels, 1st and 5th minute APGAR scores, and the PW/FW ratios, were evaluated and compared. GLUT1 scores demonstrated moderate inverse correlations between maternal BMI in the L-PE subgroup (r = -0.377, P = 0.033) and moderate positive correlations with PW/FW ratio (r = 0.444, P = 0.011). A statistically significant moderate positive correlation was found between GLUT1 scores and hemoglobin levels in the PE group and the L-PE subgroup (r = 0.397, P = 0.006 and r=0.395, P = 0.025, respectively).

Discussion

In this study, the intensity of IHC staining for GLUT1 in trophoblastic cells of the terminal villi was significantly higher in PE placentas compared to the placentas of the control group, but scores were similar in the E-PE and L-PE subgroups. IHC staining intensity was similar between those with and without severe PE features in the PE group and those without IUGR; however, it was significantly higher among those with severe PE features in the L-PE group compared to those without severe PE features.

Although the pathogenesis of PE is not fully understood, the current paradigm focuses on placental malperfusion [6] and oxidative stress in trophoblasts that form the epithelial lining of placental villi in direct contact with maternal blood [18, 19]. In the perfused human placenta model, it is stated that placental glucose transport is reduced when maternal blood flow is reduced [20], but there is little reduction in glucose transfer on the fetal side until fetal blood flow rate reduces to ~100 mL/min from the maximum rate of 300 mL/min [12]. Oxidative stress has been shown to downregulate GLUT1 transcription in the human placenta, resulting in reduced glucose uptake [21], and also, it was determined to reduce glucose accumulation in syncytiotrophoblasts due to increased transepithelial permeability – without a change in the mRNA expression of GLUT1 [22]. On the other hand, it was found that hypoxia caused a significant increase in phosphofructokinase activity, one of the key enzymes of glycolysis, in the trophoblast cell line (BeWo cells), and this was associated with a significant increase in GLUT1 transcript levels [23]. *In vitro* studies have also demonstrated that hypoxia can upregulate placental glucose transporter expression via HIF-1 [24-26].

In a study by Luscher et al. [13] in which placentas were analyzed by Western blotting, it was found that, compared to normal term placentas, GLUT1 expression in preeclamptic placentas decreased by 60% in the MVM while remaining unchanged in the BM, and total membrane isolation showed a significant upregulation. In our study, the intensity of IHC staining for GLUT1 in trophoblastic cells in terminal villi (MVM+BM) was significantly higher in PE placentas compared to control placentas. Adaptation to new conditions, such as placental malperfusion and hypoxia, in PE is essential for the placenta. Considering the villous changes associated with maternal malperfusion in PE, such as distal villous hypoplasia and villous infarctions, it is not surprising that GLUT1 density is increased in the remaining villi to provide adequate glucose for the placenta and the fetus.

As established previously, E-PE is predominantly associated with defective placentation, while L-EP appears to be driven by oxidative changes in the placenta induced by a discrepancy between maternal perfusion and fetoplacental demands, particularly in the presence of maternal predisposition to cardiovascular or metabolic disease [4]. Systemic PE symptoms develop due to ischemic damage to maternal organ systems caused by soluble antiangiogenic agents released from the hypoxic placenta [27]. In a study by Dubova et al. [15], which also used IHC staining, placentas were grouped as controls, moderate PE, severe PE, and severe PE combined with IUGR. The authors found that GLUT1 syncytial expression in the terminal villi of severe PE cases (both with and without IUGR) was significantly lower than in controls. In our study, GLUT1 staining intensity in the terminal villi of PE placentas was similar between the E-PE and L-PE subgroups and those with and without severe PE features. However, GLUT1 staining intensity was significantly higher in those with severe PE features in the L-EP group than those without, and values demonstrated a moderate inverse correlation with maternal BMI. Therefore, our findings suggest that increased GLUT1 levels in the terminal villi of preeclamptic placentas may be associated

with placental and maternal changes resulting from PE rather than the development and onset of PE. In addition, GLUT1 scores were inversely correlated with maternal BMI in the L-PE subgroup. This suggests that GLUT1 upregulation in the terminal villi of the placenta in L-PE may be associated with obesity-related maternal metabolic factors.

Fetal growth depends on nutrient availability, which in turn is related to various factors, including maternal diet, uteroplacental blood supply, placental villous development, and the capacity of the villous trophoblast and fetoplacental circulation to transport these nutrients [28]. Therefore, a well-functioning placenta is essential for favorable fetal growth, and previous studies have suggested a placental weight-to-fetal weight (PW/FW) ratio as an appropriate indicator of placental function [29].

The pathophysiologies of placental-derived IUGR and PE are very similar, and placental damage is more severe in those with IUGR-related PE than those with IUGR alone, consistent with the greater degree of maternal vasculopathy [28]. In a study by Pribadi et al. [14], in which GLUT1 levels in placentas complicated with PE were measured by ELISA, it was found that GLUT1 levels were lower in the smaller-for-gestational-age (SGA) group than in the non-SGA group and showed a positive correlation with birth weight in the E-PE group. In the study by Dubova et al. [15], it was reported that GLUT1 syncytial expression in the terminal villi was significantly lower in both the severe PE and the severe PE combined with IUGR groups when compared to healthy controls, while the PW/FW ratio was similar to control group values in all three PE subgroups (moderate PE, severe PE and severe PE combined with IUGR). However, no GLUT1 expression comparisons were made between those with and without IUGR in the severe PE group. In a recent study by Shen et al. [30], researchers found that blood pressure (one of the general measures of maternal disease severity) and chronic villitis (determined in placental pathology) had a relationship with IUGR in patients with E-PE.

The former had a negative correlation, while the latter showed a positive correlation with IUGR [30]. In our study, unlike Pribadi et al. [14], GLUT1 staining in terminal villi in PE placentas was similar in patients with and without IUGR. The PW/FW ratio was similar between the PE and control groups, in line with Dubova et al.'s [15] results. In addition, there was a moderate positive correlation between GLUT1 levels and PW/FW ratio in the L-PE subgroup. Although insufficient placental glucose transport has been considered a pathophysiological mechanism in IUGR, it has been shown that GLUT1 intensities do not change in placentas with term and preterm IUGR [31] and that the GLUT1 expressions of the MVM and BM (and resultant D-glucose uptake) are not affected by IUGR [32]. In an *in vivo* study involving human term pregnancies, uterine and umbilical blood flow was determined by Doppler ultrasonography and glucose, and insulin levels were measured in the maternal radial artery, uterine vein, and umbilical artery and vein. The researchers also measured GLUT1 expression in isolated syncytiotrophoblasts from the BM and MVM. This study determined that fetal and placental glucose consumption were inversely proportional, but neither was associated with placental GLUT1 expression.

Additionally, it was stated that fetal glucose consumption was balanced against placental glucose requirements, and placental glucose consumption was a key modulator of maternal-fetal glucose transfer [33]. Of note, previous studies have shown that placenta-mediated fetal growth restriction occurs through chronic fetal hypoxia owing to poor placental perfusion [34]. Although our findings suggest that GLUT1 expression in terminal villi is not one of the main factors in the development of PE-related IUGR, it is clear that more research is needed to determine whether the GLUT1 changes observed in PE-exposed placentas have a role in the development of IUGR.

Limitations

The main limitations of the current study are that we did not determine GLUT1 staining intensities in MVM and BM separately and that we did not stain for other GLUT types. In addition, while we investigated relationships between GLUT1 expression and IUGR in PE-afflicted placentas, we did not include placentas obtained from cases with placenta-related IUGR, which PE did not complicate. This can also be cited as another limitation of the study. Finally, the low number of cases with IUGR may have reduced the study's statistical power with respect to this subgroup analysis.

Conclusion

The intensity of IHC staining for GLUT1 in trophoblastic cells in terminal villi was significantly higher in PE placentas compared to control-group placentas, but values were similar between the E-PE and L-PE subgroups. Furthermore, in the PE group, GLUT1 staining was similar among those with and without severe features and those with and without IUGR. These results suggest that the changes in GLUT1 expression result from placental adaptation to the new environment caused by PE rather than having a causative relationship. Similarly, it appears that GLUT1 expression is not a primary factor contributing to PE-related IUGR development. Nonetheless, the results of this study demonstrate the need for further research to determine whether GLUT1 expression contributes to PE development and/or its relationship with IUGR in PE-afflicted placentas.

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Exploring the relationship between preeclampsia and human epididymis protein 4

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Ethics Committee approval was taken from the Atatürk University Clinical Research Ethics Committee, May 30, 2019 (numbered: B.30.2.ATA.0.01.00/251).
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

Conflict of Interest
No conflict of interest was declared by the authors.

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Abstract

Background/Aim: The level of human epididymis protein 4 (HE4), a glycoprotein and protease inhibitor, increases under many malignancies and inflammatory conditions. HE4 is also associated with cell invasion, migration, and adhesion. In this study, we compared the HE4 protein levels in pregnant patients with preeclampsia to healthy pregnant and non-pregnant individuals with the aim of finding a biomarker that can be used to recognize preeclampsia.
Methods: Our study is a prospective case control study and included 20 pregnant women with preeclampsia, 20 pregnant women without preeclampsia, and 20 healthy non-pregnant women (the control). The participants' serum HE4 levels were analyzed statistically.
Results: Data analysis revealed that the mean HE4 levels were significantly lower in the preeclampsia group than in the other two groups (P = 0.002). Mean HE4 protein levels were also lower in the non-pregnant women than in the pregnant women without preeclampsia; however, this difference was not significant.
Conclusion: It is difficult to predict preeclampsia, and there is not any sensitive or specific biomarker for determining the condition. This study may support that HE4 protein may be useful and significant in predicting preeclampsia. The results we achieved provide proof that HE4 levels could be a potential biomarker for preeclampsia. Many more comprehensive studies are needed to support the association between HE4 protein and preeclampsia.

Keywords: HE4 protein, Preeclampsia, Protease inhibitor

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Introduction

Preeclampsia is one of the leading causes of morbidity and mortality during pregnancy and the postpartum period [1] and develops in approximately 4.6 % of pregnancies worldwide [2]. As the pathogenesis and etiology of preeclampsia are not yet fully understood, it is not always possible to predict [1, 3]. Although several mechanisms have been proposed to explain the development of preeclampsia, one of the most commonly accepted theories for its pathogenesis is abnormal placental formation due to defective cytotrophoblast invasion [3, 4]. During normal placental formation, cytotrophoblasts invade the uterus and the muscular layer of the spiral arteries [3]. Investigations of the etiopathogenesis of preeclampsia have primarily revealed the pathology of impaired cytotrophoblast invasion and defects in the molecules that facilitate this invasion, including surface adhesion molecules, such as integrins and molecules that aid invasion, such as metalloproteinases [4]. In particular, metalloproteinases facilitate the movement of cytotrophoblasts across the basement membrane; these cytotrophoblasts then invade the muscular layers of the uterine wall and spiral arteries—like a cancerous invasion—which is one of the most important stages of placental formation. Experimental studies have shown that various matrix metalloproteinase inhibitors disrupt cytotrophoblast invasion [5].

Human epididymis protein 4 (HE4) is a protease inhibitor, first isolated from human epididymal epithelial cells, which is known to be associated with many diseases [6]. Several studies have been conducted to determine the role of HE4 in gynecological malignancies, such as ovarian and endometrial cancers, or in benign gynecological diseases, such as endometriosis, which are known to progress upon organ invasion [6-8]. HE4 is also associated with several organ malignancies, such as breast adenocarcinomas, pulmonary cancers, and mesotheliomas [8, 9].

HE4 is actually a whey acidic protein (WAP), and studies have shown that it acts as a protease inhibitor, like other WAPs. It is also known to play a role in regulating cell migration, adhesion, and invasion [8, 10, 11]. Thus, HE4 may be responsible for impaired cytotrophoblast invasion during the development of preeclampsia. Moreover, because it is a protease inhibitor, it may cause impaired cytotrophoblast invasion (an important mechanism in the development of preeclampsia) by inhibiting the metalloproteinases needed for the process.

Studies have shown that cancer antigen (CA) 125 values increase during pregnancy and in cases of preeclampsia [12]. Considering that HE4 has been associated with many diseases, such as cancer and endometriosis, diseases that are also associated with increased CA 125 levels, it is necessary to investigate the relationship between preeclampsia and HE4 [6-8]. Thus, we investigated HE4 levels in patients with preeclampsia with the aim of identifying a new biomarker to predict the disorder.

Materials and methods

Study design

This study was designed as a prospective case control study. Three groups were involved in the study: the preeclampsia group, the healthy pregnant group, and the control group. Inclusion criteria for the preeclampsia group entailed having a gestational age >34 weeks, no chronic systemic disease, a preeclampsia diagnosis during this pregnancy period, and being between 18-45 years of age. Inclusion criteria for the healthy pregnant group entailed having a gestational age >34 weeks, no chronic systemic disease, no preeclampsia diagnosis during this pregnancy period, no pregnancy problems, and being between 18-45 years of age. Inclusion criteria for the control group entailed being between 18-45 ages, no chronic systemic disease, and not being pregnant.

According to our power analysis ($\alpha = 0.05$; 1-β [power] = 0.080), we required at least 20 participants for each group sample. An appropriate sample size, given the population size and specified combination of precision, confidence, and variability, was 45.

This study included 20 preeclamptic pregnant women with gestational ages of >34 weeks (preeclampsia group), 20 healthy pregnant women with gestational ages of >34 weeks (healthy pregnant group), and a control group comprising 20 healthy non-pregnant women (non-pregnant group). All visited the outpatient clinic of our university hospital between January 1, 2020, and January 1, 2021 and all participants were included in the study after obtaining their verbal and written informed consent. The study was initiated following the approval of Atatürk University Clinical Research Ethics Committee (numbered: B.30.2.ATA.0.01.00/251). The preeclampsia diagnosis was determined according to the criteria set by the American College of Obstetricians and Gynecologists (ACOG): new-onset hypertension occurring after 20 weeks of gestation or in the postpartum period in a woman with previously normal blood pressure (systolic blood pressure of ≥ 140 mmHg and/or diastolic blood pressure of ≥ 90 mmHg in the left lateral decubitus position measured at least twice and at least four hours apart) that is accompanied by proteinuria and/or end-organ damage [13]. Proteinuria is defined as having protein levels > 300 mg/L in 24-hour urine samples. Additionally, the ACOG has defined preeclampsia in patients without proteinuria as the presence of elevated blood pressure with at least one of the following disorders: renal failure (serum creatinine levels of > 1,0 mg/dL or doubling of creatinine concentration), liver involvement (liver transaminase levels of > 40 IU/L), pain in the right upper quadrant of the abdomen, neurological complications (eclampsia, stroke, visual scotoma, and/or severe headaches), hematological complications (thrombocytopenia with ≤ 100,000 platelets/mm^3), and pulmonary edema [13].

Patients who had conditions that met the definition of preeclampsia (e.g., multiple pregnancies, fetal anomalies, suspected hepatitis A, B, or C or other infectious hepatitis, or renal disease before or during pregnancy) or who had gestational hypertension, chorioamnionitis, premature rupture of membranes, diabetes mellitus, chronic hypertension, a multisystem disease, hemolysis, elevated liver enzymes, low platelet count (HELLP) syndrome, or those who smoked were excluded from the study. Pregnant women without any medical or obstetric pathology before or during pregnancy were included in the healthy pregnant women group. The non-pregnant group

consisted of healthy women who had visited the outpatient clinic for routine gynecological examinations.

Serum samples and laboratory assays

Venous blood samples were collected to measure the HE4 protein levels. After centrifugation, the blood serum was separated, frozen at −80 °C, and stored. After all samples were collected, HE4 levels were measured using an enzyme immunometric assay kit (Fujirebio Diagnostics, Inc., Malvern, Sweden) following the manufacturer's instructions.

Statistical analysis

SPSS v20 was used for the statistical analysis (SPSS Inc., Chicago, United States). The normality of the parameters was assessed using the Kolmogorov–Smirnov test. One-way ANOVA and post hoc tests were used to compare normally distributed data (i.e., the comparison of HE4 levels among pregnant women with preeclampsia, pregnant women without preeclampsia, and healthy non-pregnant women). Kruskal–Wallis tests were used to compare non-normally distributed data. Data were recorded as the mean (standard deviation [SD]), and $P < 0.05$ was considered to be significant.

Results

The clinical, biochemical, and obstetric characteristics of the groups are shown in Tables 1 and 2. There was no statistically significant difference between the three groups in terms of age, number of pregnancies, pregnancy end week, hemoglobin or hematocrit values, or international normalized ratios. However, the preeclampsia group had the highest systolic ($P = 0.001$) and diastolic ($P = 0.002$) blood pressures, urogram ($P < 0.001$) and 24-hour urine protein levels ($P < 0.001$), aspartate aminotransferase ($P < 0.001$) and alanine aminotransferase ($P < 0.001$) levels, creatine levels ($P < 0.001$), and lactate dehydrogenase levels ($P < 0.001$), and these levels were statistically significant. Platelet values were lowest in the preeclampsia group and these were also statistically significant ($P = 0.007$).

Table 1: Comparison of the demographic characteristics among the groups

Descriptive Characteristics mean (SD) (min-max)	Preeclampsia group (n = 20)	Healthy pregnant group (n = 20)	Non-pregnant group (n = 20)	*P*-value
Age (years)	27.25 (6.27) (18-39)	33.65 (6.63) (24-44)	30.45 (6.00) (20-40)	0.010
Number of pregnancies (gravida)	2.65 (1.98) (1-8)	3.50 (1.93) (1-8)	3.45 (2.58) (1-10)	0.277
Pregnancy end week	36 (1.52) (34-39)	36.10 (2.07) (34-40)	-	0.890
Systolic blood pressure at admission (mm Hg)	165.50 (18.77) (140-190)	112.00 (12.81) (90-135)	109.50 (13.46) (90-135)	0.001
Diastolic blood pressure at admission (mm Hg)	99.00 (13.33) (70-120)	68.50 (9.33) (50-80)	64.5 (8.87) (50-80)	0.002

Min: Minimum, Max: Maximum, SD: standard deviation

The statistical analysis showed that HE4 levels were significantly lower ($P = 0.002$) in women with preeclampsia (38.76 pmol/L) than in pregnant women without preeclampsia (64.40 pmol/L) and healthy non-pregnant women (59.98 pmol/L) (one-way ANOVA). Although the healthy non-pregnant women's HE4 levels were lower than the healthy pregnant women's levels, the finding was not significant ($P = 0.477$). The comparison of HE4 levels among the three groups is shown in Table 3.

Table 2: Comparison of the laboratory findings among the groups

Descriptive Characteristic mean (SD) (min-max)	Preeclampsia group (n = 20)	Healthy pregnant group (n = 20)	Non-pregnant group (n = 20)	*P*-value
Urogram protein (+1 - +4)	1.80 (1.15) (0.00-4.00)	0.30 (0.57) (0.00-2.00)	0.25 (0.55) (0.00-2.00)	<0.001
Urogram protein n (%)				
0	3 (15.0%)	15 (75.0%)	16 (80.0%)	<0.001
+1	5 (25.0%)	4 (20.0%)	3 (15.0%)	
+2	6 (30.0%)	1 (5.0%)	1 (5.0%)	
+3	5 (25.0%)	0 (0.0%)	0 (0.0%)	
+4	1 (5.0%)	0 (0.0%)	0 (0.0%)	
24-hours urine protein (g/L)	1.76 (1.34) (0.30-5.10)	0.23 (0.16) (0.10-0.60)	-	<0.001
Hemoglobin (g/dL)	12.21 (2.27) (7.8-16.6)	11.45 (2.05) (7.0-15.0)	11.8 (0.85) (10.0-13.7)	0.757
Hematocrit (%)	35.0 (5.50) (25.9-45.6)	33.57 (6.00) (21.7-43.4)	35.14 (2.10) (30.5-38.7)	0.630
Platelet (µL)	164,900 (92,109) (47,000-365,000)	209,000 (68,372) (110,000-365,000)	249,850 (59,823) (161,000-365,000)	0.007
Aspartate aminotransferase (IU/L)	269.55 (293.44) (72-1113)	30.30 (13.06) (10-58)	19.60 (6.09) (10-35)	<0.001
Alanine aminotransferase (IU/L)	159.80 (105.10) (51-481)	31.40 (10.65) (11-50)	15.80 (5.90) (10-31)	<0.001
Lactate dehydrogenase (IU/L)	1069.45 (676.16) (512-3051)	220.15 (44.60) (132-284)	235.15 (61.04) (158-384)	<0.001
International normalized ratio (INR)	0.99 (0.15) (0.76-1.37)	1.10 (0.32) (0.85-2.16)	0.99 (0.09) (0.87-1.19)	0.722
Creatine (mg/dL)	0.97 (0.90) (0.50-4.18)	0.74(0.27) (0.49-1.62)	0.47 (0.14) (0.29-0.85)	<0.001

Table 3: Comparison of human epididymis protein 4 levels among the groups

	Preeclampsia group (n = 20)	Healthy pregnant group (n = 20)	Non-pregnant group (n = 20)	*P*-value
Mean HE4 level mean (SD) (min-max)	38.76 (12,01) (17.65-65.34)	64.40 (12.69) (29.39-89.15)	59.98 (11.15) (41.76-81.75)	0.002

HE4 protein level: pmol/L, n: number of patients

Discussion

The present study is the first to investigate this topic. We aimed to determine the role of the HE4 protein in the pathogenesis of preeclampsia. We found that the mean HE4 levels were lowest in pregnant women with preeclampsia compared to healthy pregnant women and non-pregnant women. Our literature search revealed a lack of comparative research. Only a few studies have determined HE4 levels during pregnancy, and although a recent study evaluated HE4 protein levels in pregnant women with HELLP syndrome [14], there are currently no studies that have evaluated HE4 levels in women with preeclampsia. Furthermore, it should be noted that because of the variable range in the HE4 protein levels between studies caused by differences in the kits and devices used for measurement, the results were not comparable among studies.

We found that HE4 levels were higher in the healthy pregnant group than in the non-pregnant group; however, this difference was not significant. In a study by Gucer et al. [15], no significant differences were reported between pregnant and non-pregnant patients. In another study including 1,101 healthy non-pregnant and 67 pregnant women, Moore et al. reported that serum HE4 levels were significantly lower in pregnant women than in premenopausal, non-pregnant women, a finding contrary to ours. They also found that serum HE4 levels were significantly lower in premenopausal women than in postmenopausal women [16]. Age had no effect on our study results, however, as there was no significant difference in the mean age among our groups.

In another study, Uslu et al. [11] found that HE4 levels decreased in the first and second trimesters, but there was no significant difference in the levels of HE4 protein in the third trimester compared to pre-pregnancy levels. Unlike Uslu et al., we found that HE4 protein levels were higher in the healthy pregnancy group than in the non-pregnant (pre-pregnancy) group. Wang et al. [17] reported that HE4 levels did not change significantly in the first and second trimesters compared with the levels in non-pregnant patients. They further indicated that HE4 levels were significantly higher in the third trimester compared with the levels in non-pregnant women. Our results were similar to these, but the difference we found when comparing the HE4 protein levels between the healthy pregnant group and the non-pregnant group was not significant.

Gasiorowska et al. [18] found that HE4 levels significantly changed with age, which is supported by the literature [16, 18, 19], and they found a significant correlation between smoking and serum HE4 levels [18]. We excluded smokers from the study, so we could not assess that variable. In the same study, Gasiorowska et al. [18] compared HE4 protein levels among the first, second, and third trimesters and reported that while there was no significant difference between the first and second trimesters, there was a significant increase in the third trimester.

The literature supports the idea that HE4 protein levels are affected by age and gestational length. However, neither of these affected our results because all pregnancies in our study were over 34 weeks, and age was not statistically different among all participants. Thus, our study was not affected by any factor that could affect HE4 protein levels, such as age and gestational week.

Kurdoglu et al. [20] researched laminin receptor 1, an extracellular matrix protein, to see if it played a role in the pathophysiology of preeclampsia. Many studies have shown that laminin receptor 1 is involved in cell adhesion, invasion, and migration and is effective on protease activity (like the HE4 protein); in addition, it was shown to have a role in malignancies and tumor metastases [20-22]. Kurdoglu et al.'s [20] results were statistically significant and showed that laminin receptor 1 was less expressed in preeclamptic placentas, but the severity of the disease was not related.

In a recently study conducted by Cam et al. [14] HE4 protein levels in pregnant women with HELLP syndrome, an extremely advanced form of preeclampsia were compared, with healthy pregnant women. Similar to our study, they found that the mean HE4 protein levels in HELLP syndrome patients were lower, but in contrast to our study, the result was not statistically significant.

Limitations

In this study, we did not investigate the relationship between the severity of preeclampsia and HE4 protein levels, nor did we measure placental HE4 protein levels. This is a limiting factor; thus, further extensive studies are required.

Conclusion

To adequately understand preeclampsia and predict its complications, the identification of high-risk women could help minimize undesirable fetomaternal consequences. Considering that the diagnostic criteria for the condition are frequently reviewed, we believe that a predictive biochemical marker would help improve pregnancy outcomes. Our study suggests that a decrease in HE4 protein levels can be used as a new biomarker to predict the development of preeclampsia. However, much more comprehensive research is needed in the future, as well as studies with a much higher number of patients.

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Bladder filling test, cystoscopy, or both for checking bladder perforation in tension-free-vaginal tape operations

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Ethics Committee Approval
This study was approved by the Acibadem Mehmet Ali Aydınlar University, Medical School, Board of Ethics (No: ATADEK 2020/26). A written informed consent was obtained from each participant.
All procedures in this study involving human participants were performed in accordance with the 1964 Helsinki Declaration and its later amendments.

Conflict of Interest
No conflict of interest was declared by the authors.

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Abstract

Background/Aim: Tension-free-vaginal tape (TVT) has been widely used for treatment of stress urinary incontinence as a mid-urethral sling operation. Cystoscopy is routinely performed during tension-free-vaginal tape operations to check for bladder perforation or injury. This study aims to check the applicability and accuracy of the bladder filling test for predicting bladder perforation in tension-free-vaginal tape operations.
Methods: Between 2015 and 2020, 285 women who had TVT operations were subject to evaluation. Out of 285 cases, 23 cases were suspected subjectively by the operating surgeons to have visible or occult bladder perforation during the TVT procedure. A routine cystoscopy was performed at the end of all operations. Additionally, before the routine cystoscopy, in cases suspected of a visible or occult bladder perforation, the bladder was filled with 500 ml saline or diluted methylene blue dye through a urinary catheter (bladder filling test) to check for occult bladder perforation that might not be visualized by cystoscopy. Any fluid leakage through the paraurethral dissected canals or from the abdominal incisions was observed for the possibility of bladder perforation. The accuracy of the bladder filling test was compared to cystoscopy to diagnose bladder perforation in suspected cases. In addition, all cases were followed up for three months to record any cases with late or occult bladder perforations missed in the diagnosis using cystoscopy or the bladder filling test perioperatively.
Results: Out of 23 cases suspected subjectively by the operating surgeons to have visible or occult bladder perforation, 11 had visible bladder perforations (3.9%) confirmed by both cystoscopy and the bladder filling test. After the filling test, leakage at the abdominal incision site and/or para-urethral dissected canal was observed in all cases with bladder perforation. No leakage was observed in the remaining patients (n = 12) suspected of, but not diagnosed with bladder perforation by cystoscopy. The bladder filling test did confirm the same diagnosis revealed by cystoscopy in all suspected cases.
Conclusion: The bladder filling test was found to be very sensitive in predicting bladder perforation at tension-free-vaginal tape operations compared to cystoscopy. This test can decrease the need for routine cystoscopy at tension-free-vaginal tape insertion, and cystoscopy can be limited to cases with leakage in the bladder filling test.

Keywords: Stress urinary incontinence, Tension-free-vaginal-tape, Bladder perforation, Cystoscopy, Bladder filling test

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Introduction

Stress urinary incontinence (SUI) is a common worldwide disorder affecting 15-80% of women [1]. When conservative strategies fail, the preferred treatment is surgery. Mid-urethral sling operations are the gold standard for the treatment of SUI with long-term cure rate of 77-90% [2, 3]. Since first described by Ulmsten and Petros [4], tension-free-vaginal tape (TVT) has been widely used for SUI with satisfactory long-term outcomes [3, 5]. As a routine procedure, cystoscopy is performed during TVT operations to check for bladder perforation (BP), which is one of the main complications occurring at a rate of 4.5% [6]. If unrecognized, BPs may lead to serious postoperative complications, such as hematuria, irritative bladder symptoms, pelvic pain, recurrent urinary tract infections, bladder stones, and sinus tract formation [7-10]. Although cystoscopy is an effective procedure for diagnosing bladder pathologies, it is operator-dependent, and occult BP during mid-urethral sling procedures may not always be visualized by cystoscopy [11, 12]. Inadequate filling of the bladder during cystoscopy and/or using 30° lenses instead of 70° can lead to a false normal cystoscopic evaluation. The improper placement of a trocar through the submucosa with no visible tape at cystoscopy may later turn into an occult BP. This possibly results from trauma by the rough edge of the tape when the trocars are extracted [11].

Cystoscopy is not a difficult or complex procedure, but it increases the costs, prolongs the surgical time, and requires access to a camera stack, light source, and cystoscope. Despite being a minimally invasive procedure, cystoscopy also has risks like urethral stricture or bladder injury in addition to its minor complications like urinary tract infection, hematuria, and dysuria [13].

This study aimed to reveal the applicability and diagnostic accuracy of a fast and simple method that can be performed routinely to predict the BPs during the TVT operations and to decrease the need for routine cystoscopy for each patient at TVT insertion.

Materials and methods

Two hundred eighty-five women who had a TVT procedure with or without coexistent surgery from 2015 to 2020 were subject to evaluation. This study was approved by the Acıbadem Mehmet Ali Aydınlar University, Medical School, Board of Ethics (No: ATADEK 2020/26). A written informed consent was obtained from each participant. The study was conducted in accordance with the principles of the Declaration of Helsinki.

The surgeries were performed in the Egemed Hospitals in Aydın, and Acıbadem Health Group Hospitals in Istanbul by two surgeons experienced in urogynecological surgery (UK and MTE). The TVT procedures without coexisting surgeries were performed under spinal anesthesia. A 10x450 mm polypropylene mesh kit produced by Düzey Medikal Cihazlar San. Tic. Ltd. Şti, Istanbul, Turkey was used. The pre-surgical data of age, body mass index (BMI), parity, smoking, menopausal status, hormonal replacement therapy, previous hysterectomy, and relevant systemic diseases with medications were recorded.

The preoperative assessment included the Pelvic Organ Prolapse Quantification (POP-Q) System, cough stress test, Bonney's test, Q-tip test, post-void residual volume, urine analysis, and pelvic floor ultrasound. For cases with POP > stage 2 and/or for those having urgency symptoms or post-void residual volume > 150 cc, urinary stress incontinence was urodynamically confirmed (a stable detrusor, maximum urethral closure pressure (MUCP) < 30 cm H2O, Valsalva leak point pressure (VLPP) < 90 cm H2O, maximum flow rate (Qmax) > 17 ml/s). For rating urinary incontinence symptoms in the last four weeks, patients were asked to complete a Turkish validated ICIQ-SF form [14]. The cases diagnosed with urinary tract infection were re-assessed after the proper antimicrobial treatment. All cases received cefazolin 1 gr + metronidazole 500 mg iv as prophylaxis 30 minutes before the surgery.

Out of 285 cases, 23 cases were suspected subjectively by the operating surgeons to have visible or occult BP during the TVT procedure. A routine cystoscopy was performed at the end of all TVT operations. Additionally, before routine cystoscopy, in cases suspected of a visible or occult BP, the bladder was filled with 500 ml saline or diluted methylene blue dye through a urinary catheter (bladder filling test) to check for occult BP that might not be visualized by cystoscopy. Any fluid leakage through the paraurethral dissected canals or from the abdominal incisions was observed.

The urethral catheters were withdrawn six hours postoperatively unless a BP occurred. Duration of catheterization was 10 to 14 days for the patients with BP. The patients with postoperative voiding difficulty were re-catheterized and evaluated after three and ten days of re-catheterization.

In all cases, the duration of the TVT and cystoscopy procedures were recorded separately. Co-existing surgeries if present, duration of hospital stay, and perioperative and postoperative complications were also recorded.

The patients were evaluated one week, one month, and three months after the surgery unless they had any complaints and/or complications existed. The early post-operative period was defined as one week after surgery. The subjective cure rates were self-evaluated by the patients one and three months after the surgery according to the schedule proposed, and designated as cured, improved, failed, or worsened.

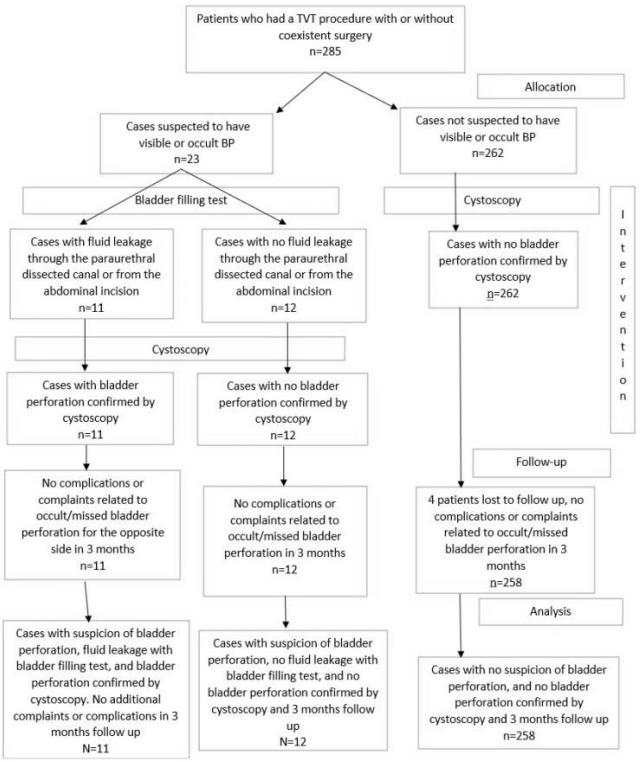
Statistical analysis

Statistical analysis was performed using the SPSS version V23 software (IBM Corp., Armonk, NY, USA). Descriptive data were expressed in mean, standard deviation (SD), median (minimum-maximum) for quantitative data and in frequency (percentage) for qualitative data. Distribution of the variables was analyzed using the Shapiro-Wilk test. Analysis of quantitative independent variables was carried out using the Two-Sample Independent t-Test and Mann-Whitney U test depending on distribution of the data. The chi-square test and, when the assumptions of the chi-square test were not met, Fisher's exact test was performed for the analysis of qualitative independent data. Comparison of the dual categorized variables according to dual time was performed by McNemar test and for the comparison of more than two categorized variables, McNemar test was used. A *P*-value of <0.05 was considered statistically significant.

Results

Out of 285 women who had a TVT procedure with or without coexistent surgery, 23 were allocated for evaluation with a preliminary diagnosis of occult or visible BP. After the bladder filling test, 11 suspected cases had fluid leakage through the paraurethral dissected canals and/or from the abdominal incisions, and 12 of the suspected cases had no leakage. Routine cystoscopy that was performed after the bladder filling test revealed BP in all cases with fluid leakage and no BP in any of the cases without leakage. No complications or complaints related to occult/missed BP were recorded at the three-month follow-up period (Figure 1).

Figure 1: Flow diagram of case series.



Mean age and BMI of the suspected cases were 56.83 (13.49) and 26.92 (5.34), respectively. The characteristics of the cases are presented in Table 1.

There were no intraoperative complications other than BP that occurred in 11 patients (3.9%). Early postoperative complications other than BP were subcutaneous hematoma (n = 1), urgency (n = 2), urinary tract infection (n = 2) and voiding difficulty (n = 12). Subcutaneous hematoma and urgencies resolved spontaneously in the follow-up period. Urinary tract infections were treated with proper antibiotic administration. Voiding difficulties were resolved by short-term (three to ten days) catheterizations and none of the patients required a mesh release intervention. None of these complications occurred in the patients with BP.

There were no correlations between the occurrence of BP or early post-operative complications, and the characteristics of cases, such as age, BMI, parity, number and type of deliveries, menopausal status, smoking, chronic systemic illnesses, medication, hormone therapy, previous hysterectomy, and co-existing surgery (Table 1).

Table 1: The characteristics of the cases.

	Cases with bladder perforation n = 11	Cases with no bladder perforation n = 12	*P*-value
Age	55.27 (13.93)[‡]	58.25 (13.53)[‡]	0.609[t]
BMI	25.88 (6.30)[‡]	27.87 (4.34)[‡]	0.385[t]
Parity	2.00 (1.00 - 5.00)[†]	2.00 (0.00 - 4.00)[†]	0.880[U]
Normal deliveries	2.00 (1.00 - 5.00)[†]	1.00 (0.00 - 4.00)[†]	0.190[U]
Cesarean deliveries[*]			
No	10 (90.9)	8 (66.7)	0.317[F]
Yes	1 (9.1)	4 (33.3)	
Menopause[*]			
No	3 (27.3)	4 (33.3)	1.000[F]
Yes	8 (72.7)	8 (66.7)	
Smoking[*]			
No	8 (72.7)	8 (66.7)	1.000[F]
Yes	3 (27.3)	4 (33.3)	
Systemic diseases[*]			
No	3 (27.3)	4 (33.3)	1.000[F]
Yes	8 (72.7)	8 (66.7)	
Medication[*]			
No	4 (36.4)	3 (25)	0.667[F]
Yes	7 (63.6)	9 (75)	
Hormone Therapy[*]			
No	10 (90.9)	11 (91.7)	1.000[F]
Yes	1 (9.1)	1 (8.3)	
Previous hysterectomy[*]			
No	9 (81.8)	10 (83.3)	1.000[F]
Yes	2 (18.2)	2 (16.7)	
Co-existing surgery[§, *]			
Anterior colporrhaphy	7 (87.5)	5 (83.3)	0.672[X]
Pectopexy	2 (25)	1 (16.7)	
Posterior colporrhaphy	2 (25)	2 (33.3)	
Laparoscopic subtotal hysterectomy	0 (0)	1 (16.7)	
Sacrocolpopexy	0 (0)	1 (16.7)	

[t]: Independent-samples t-test. [U]: Mann-Whitney U test [X]: Chi-square test. [F]: Fisher's Exact test. [†]: Median (Minimum-Maximum); [‡]: Mean (Standard Deviation). [§]: Multiple response. [*]: Number of patients (percentage)

Comparative analysis of data demonstrated that only total operation time ($P < 0.001$), duration of cystoscopy ($P < 0.001$), and mean hospitalization time ($P = 0.007$) increased in the cases with BP (Table 2).

Table 2: Preoperative, intraoperative, and postoperative evaluation of the cases.

	Cases with bladder perforation n = 11	Cases with no bladder perforation n = 12	*P*-value
ICIQ-SF score at first visit	15.45 (1.75)[‡] 15.00 (12.00 - 18.00)[†]	15.17 (1.99)[‡] 15.50 (11.00 - 17.00)[†]	0.880[U]
Preoperative post-void residual volume (ml)	107.27 (51.98)[‡] 80.00 (60.00 - 200.00)[†]	115.83 (48.14)[‡] 100.00 (60.00 - 200.00)[†]	0.608[U]
Cough stress test[*]			
Negative	1 (9.1)	4 (33.3)	0.317[F]
Positive	10 (90.9)	8 (66.7)	
Bonney's test[*]			
Leakage	2 (18.2)	5 (41.7)	0.371[F]
No leakage	9 (81.8)	7 (58.3)	
Q tip test angle[*]			
≤30	4 (36.4)	4 (33.3)	1.000[F]
>30	7 (63.6)	8 (66.7)	
Preoperative urgency symptoms[*]			
No	10 (90.9)	9 (75)	0.590[F]
Yes	1 (9.1)	3 (25)	
Preoperative urine analysis[*]			
Normal	11 (100)	12 (100)	N/A
Patients undergone for preoperative urodynamic[*]			
No	6 (54.5)	6 (50)	1.000[F]
Yes	5 (45.5)	6 (50)	
Surgical time for TVT (minutes)	43.45 (4.89)[‡] 44.00 (36.00 - 52.00)[†]	34.58 (3.99)[‡] 33.50 (28.00 - 40.00)[†]	<0.001[t]
Surgical time for cystoscopy (minutes)	11.27 (1.10)[‡] 11.00 (10.00 - 13.00)[†]	8.25 (1.60)[‡] 8.50 (6.00 - 10.00)[†]	<0.001[U]
Hospitalization time (hours)	56.09 (9.06)[‡] 54.00 (45.00 - 74.00)[†]	41.33 (14.00)[‡] 36.50 (27.00 - 74.00)[†]	0.007[t]
Subjective cure of SUI at 1st month[*]			
Cure	10 (90.9)	12 (100)	N/A
Failure	1 (9.1)	0 (0)	
Subjective cure of SUI at 3rd month[*]			
Cure	9 (81.8)	12 (100)	0.217[F]
Improvement	2 (18.2)	0 (0)	

[t]: Independent-samples t-test. [U]: Mann-Whitney U test. [F]: Fisher's Exact test. [‡]: Mean (Standard Deviation). [†]: Median (Minimum-Maximum); [*]: Number of patients (Percentage), N/A: not applicable

Subjective cure rates at the first and third months were described as cured, improved, or failed, and these are presented in Table 2. There was no statistically significant difference between subjective cure rates of the cases with BP and no BP at the first month and third month evaluations. Only two patients

presented with de novo urgency symptoms postoperatively, and cystoscopy of these patients was normal with no submucosal mesh.

The observed accuracy of the bladder filling test to diagnose BP was high. All patients without any observed leakage during the bladder filling test fell outside the BP complication confirmed by the following cystoscopy (Table 3).

Table 3: The observed accuracy of the bladder filling test.

		Bladder perforation diagnosed by cystoscopy*		*P*-value	Kappa (P)
		No	Yes		
Leakage after bladder filling test*	No	12 (100)	0 (0)	1.000[M]	1.000 (<0.001)
	Yes	0 (0)	11 (100)		

[M]: McNemar test. *: Number of patients (Percentage)

Discussion

In this study, the evaluation of the case series with bladder perforation in TVT operations revealed that the bladder filling test was highly accurate in predicting bladder perforation.

SUI is a common worldwide disorder affecting 15-80% of women [7] and one in three women over the age of 18 will be affected by it at some point in their lifetime [6]. TVT has a high success rate of up to 90% for the surgical treatment of SUI confirmed by long-term follow-up data [3, 7]. The TVT procedure is associated with some complications, and BP is one of the most reported problems [7, 8]. The incidence of BP after TVT insertion is between 3.6 to 4.5% according to the literature [6]. The rate of BP in our case series, which was 3.9%, is consistent with the reports.

Modifications in TVT techniques to eliminate BP have been developed. However, BP is still inevitable for all modified techniques with varying rates [15, 16]. For all techniques, slinging the urethra requires a close operational space to the bladder, and the placement of the trocars is performed by blind movements out of the operator's sight. Therefore, elimination of this complication is difficult. A randomized clinical trial demonstrated that the route of retropubic trocar insertion, whether top to bottom or bottom to top, did not affect the BP rates [17], although some moderate quality evidence claimed the opposite [6]. The efforts to switch the insertion of tape to the groin (trans-obturator) instead of the abdomen (retropubic) decreased the complication rates; however, the BP rates, despite being lower, remained significant [8, 18]. An incidence of BP as high as 24% was also reported [17]. Reversing the trocar route (outside-in or in-outside) did not change the complication rates [19]. Moreover, the BP rates may be underreported due to unrecognized cases [7, 20]. Thus, in TVT and in trans-obturator tape (TOT) operations or even in the latest mini sling operations, routine cystoscopy is recommended for early detection of BPs [10, 12].

Recommendations for routine cystoscopy mainly depend on early recognition of BPs intraoperatively. Cetinel et al. [21] emphasized that cases of missed diagnosis would later require open or endoscopic surgery for removal of the tape inside the bladder. Otherwise, reinsertion of the tape intraoperatively and a few days of urethral catheterization postoperatively would be an adequate and timely solution.

However, cystoscopic evaluation may not detect all BPs [12, 21-22]. Cases with normal cystoscopic evaluation may still have perforated bladders diagnosed mostly by the related postoperative symptomatology. The misdiagnosis may be due to inadequate filling of the bladder during cystoscopy and/or using 30° lenses instead of 70°, which may result in not seeing the perforation site that mostly occurs anteriorly. Shobeiri et al. [23] suggested that the improper placement of a trocar through the submucosa with no visible tape at cystoscopy may later turn to an occult bladder injury when the trocars are extracted due to trauma from the rough edge of the tape. In our study, we checked the fluid leakage after the extraction of the trocar, a technique that may exclude this probable injury and may eliminate all the imperfections of cystoscopy. Another study reported an undiagnosed occult perforation even using a 70°cystoscope, which was only detected by flexible cystoscopy soon after the operation [24]. Cetinel et al. [21] reported two patients who had BP with normal cystoscopy and the perforation was determined only by fluid leakage at the abdominal incision site after the trocars were removed. Buchsbaum et al. [25] reported a case of occult BP that could not be identified by cystoscopy, but was suspected after clear fluid leakage at the abdominal incision site. In another case report, missed perforation was diagnosed by vulvar edema presented four hours after the operation and confirmed by repeated cystoscopy [26]. All these case reports indicate the possible effectiveness of the method presented in this study, and to the best of our knowledge, there is no study in the literature that has investigated bladder filling technique systematically in a case series.

No patients from our study group presented with occult perforation during the follow-up period. Although we believe that our method could detect it intra-operatively, large scale, prospectively randomized studies are required to test the accuracy of the bladder filling test for occult BPs.

Along with the known risk of complications, cystoscopy increases costs and prolongs the surgical time. In addition, it requires access to a camera stack, light source, and cystoscope. Additional time due to cystoscopy was only mentioned in the literature at comparative studies between TVT and TOT reporting that surgical time for TOT operations was shorter. This outcome was postulated to be due to the surgical time saved from not performing routine cystoscopy in TOT operations [27, 28]. The mean cystoscopy time was 8.4 (1.14) minutes for our study group with a range of 5-13 minutes and mean total operation time was 34.8 (5.3) minutes. Cystoscopy took approximately 20% of mean total operation time, which supports the fact that the procedure significantly prolongs the operation when compared to the bladder filling test that usually takes 1-2 minutes. Therefore, the method we present in this study may help to shorten the surgical time and eliminate the requirement of cystoscopy instrumentation in most of the TVT procedures.

The cases with BP had longer operation, cystoscopy, and hospitalization times. However, statistical power was not sufficient to draw a firm conclusion due to the small number of perforated cases (n = 11).

Success and complication rates of this study were consistent with the current literature [29]. Although the outcomes of three months follow-up are not sufficient for a complete comparison, this follow-up period fills the requirements for the objective of this study.

This study has some limitations. Although specificity and sensitivity of the bladder filling test for diagnosing BP in TVT procedures were 100% in our study, large scale studies must be performed to validate these results for safely excluding cystoscopy in cases without leakage after the bladder filling test. No patients presented with occult BP in our study group; therefore, it was not possible to test the diagnostic performance of the bladder filling test for occult BPs.

Conclusion

Presence or absence of fluid leakage through paraurethral or abdominal incision sites following the bladder filling test accurately determined the existence or absence of BPs during TVT operations. Large-scale studies are required to validate these results, which may end in limiting the routine cystoscopy only to cases with leakage after the bladder filling test, thus, decreasing the total surgical time and the cost.

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Dietary polyphenols in the treatment of inflammatory bowel diseases

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Abstract

Ulcerative colitis and Crohn's disease, caused by chronic inflammation in the digestive tract, are inflammatory bowel diseases and have similar symptoms. Abnormal immune responses play a pretty important role in the pathogenesis of the disease. Proinflammatory mediators trigger inflammation, stimulate cell signaling molecules, and induce disease onset. Corticosteroids, anti-tumor necrosis factor-α antibodies, and immunosuppressants are some drugs used to treat the disease. However, these drugs have some side effects. In addition, surgical methods might be used in the treatment, but these methods may have some complications. Due to the negative impact on treatment options, alternative methods for reliable, inexpensive, and effective treatment are being sought. Secondary plant compounds with an aromatic or phenolic ring structure, so-called polyphenols or phenolic compounds, may modulate cellular signaling pathways and reduce intestinal inflammation due to their antioxidant and anti-inflammatory effects. Polyphenols may be evaluated as alternative methods for inflammatory bowel disease based on these properties. This review aims to investigate the effect of some polyphenols on inflammatory bowel disease.

Keywords: Inflammatory bowel diseases, Ulcerative colitis, Crohn's disease, Polyphenols

Introduction

Inflammatory bowel disease (IBD) is a condition characterized by chronic inflammation in the gastrointestinal tract. It usually displays involvement in the bowel [1]. The disease includes phases of relapse and remission. It is also considered a progressive disease in recent years [2].

IBD is mainly classified as ulcerative colitis (UC) or Crohn's disease (CD) based on clinical and pathological features [1]. CD and UC cause indigestion and inflammation in the gastrointestinal tract [3]. CD may occur anywhere from the mouth to the anus and is characterized by transmural inflammation [4]. It is usually seen in the terminal ileum, cecum, perianal region, and colon, but intermittent lesions may be seen in any part of the intestine [5]. On the other hand, UC originates in the rectum and involves the entire colon. It also causes superficial damage to the intestinal wall by leading to mucosal inflammation [6].

The incidence of IBD has been increasing day by day in recent years [7]. While the incidence of IBD in developed Western countries has been high in the past, it has recently increased in developing countries such as Asia and South America [7–9]. This increase is considered to be due to changes in lifestyle and eating habits due to industrialization and Westernization [10].

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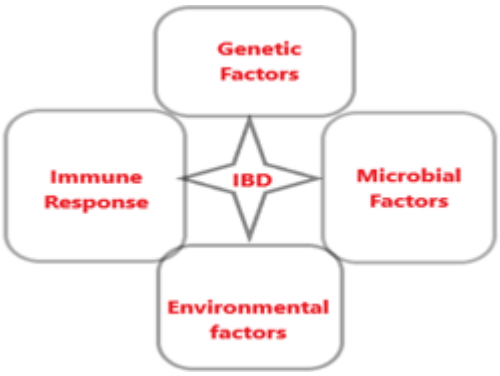
Etiology and symptoms of IBD

The disease is associated with immune system dysregulation, altered gut microflora, disrupted digestive mucosal barrier, altered oxidative stress, and increased permeability [11]. Figure 1 shows these factors that affect the etiology of IBD [8].

Figure 1: Factors affecting the etiology of IBD



Dysfunction of the mucosal barrier induces intestinal permeability and causes luminal contents to leak into underlying tissues [12]. In addition, there is an increase in free radicals, proteolytic enzymes, and cytokines in IBD. This condition causes inflammation, abdominal pain, bloody stools, weight loss, diarrhea, and ulcers [5]. Meanwhile, the adaptive immune response triggers chronic inflammation in the colon, leading to the formation of IBD lesions [12].

Unlike other inflammatory diseases, IBD may not be quickly suppressed because the immune system is stimulated and part of the intestine is destroyed. This condition may cause pain, diarrhea, fever, and other symptoms [13]. Bloody diarrhea is a common symptom in most patients with UC, and the severity of the bleeding depends on its location in the colon. Weight loss is common in CD patients. Weight loss in these patients is thought to be due to chronic diarrhea, malabsorption, and fear of food [14]. In addition, the inflammatory effects of the disease may pass through the intestinal lumen and affect the extraintestinal organs, forming abscesses, fissures, and fistulas. Extraintestinal symptoms are common, especially in the eyes, hematologic system, joints, and skin [15]. Moreover, these symptoms may vary from person to person and can reduce normal daily activities, absenteeism, eating disorders, and psychological effects [16].

Treatment of IBD

Clinical, endoscopic, histologic, and radiologic tests diagnose IBD [17]. After diagnosis, the disease's severity and location are critical in determining treatment strategies and evaluating possible side effects. The main goal of diagnosis and treatment of the disease is to reduce the symptoms and improve the patient's health, eliminate the symptoms of the disease or keep the disease in a sound stage and avoid surgical treatment [13]. Remission, known as mucosal healing and normalization of blood biomarkers, is critical for the maintenance of therapy [18].

Treatment options for the disease include nutrition, medication, or surgical interventions [16]. Recently, drugs such as immunosuppressants, anti-tumor necrosis factor (TNF)-α antibodies, and corticosteroids have been used as drug therapy. These drugs are associated with an increased risk of opportunistic infections and malignancies [11]. It is also one of the treatment options in surgical methods, but it can cause some complications [19]. In addition to the negative aspects of treatments, the cost of health care services in treatment, and social costs such as lost work and daily activities impose a significant economic burden. This economic burden ranges from $8.1 billion to $14.9 billion in the United States [20].

Because of these adverse effects in treatment, alternative methods are being sought for reliable, inexpensive, and effective treatment [11]. Recently, some nutraceutical compounds, such as bioactive peptides and phytochemicals, have been investigated as alternative methods to treat IBD [21]. Therefore, this review aims to examine the effects of some polyphenol components on IBD.

Polyphenols

Polyphenols, known as secondary plant metabolites, contain more than 7000 compounds [22, 23]. These compounds are classified according to the bonds that connect the rings and the number of rings [22]. They are grouped into flavonoids such as isoflavonoids, anthocyanins, and non-flavonoids such as stilbenes, phenolic acids, tannins, and coumarins [23]. Polyphenols with these components are found in different amounts in different foods [22]. For example, flavonols are commonly found in fruit, apples, and red onions, while flavanols are mainly in tea, cocoa, and chocolate. Isoflavones are also in soybeans, while flavones are in thyme and rosemary. Furthermore, grapes and wine are rich in resveratrol [24].

The relationship between polyphenols and IBD

Polyphenols modulate cellular signaling pathways and have antioxidant and anti-inflammatory effects [22]. Due to these effects, polyphenols are thought to be effective in treating IBD [25]. These compounds may regulate the intestinal immune response and the production of molecular mediators involved in inflammation [26]. These components limit the production of cytokines such as interleukin-8, interleukin-1β, and TNF-a and increase the activities of intracellular antioxidants such as superoxide dismutase and glutathione peroxidase. Moreover, these components act as antioxidants and scavenge free radicals. In addition, intestinal inflammation is reduced by interrupting redox signaling pathways [24, 27]. It has been reported that flavonoids protect the cell against oxidative stress and the epithelial mucosal layer of the intestine in vivo and in vitro [28]. Curcumin, a component of Indian curry spices, has been reported to inhibit the proinflammatory transcription factor Nuclear Factor Kappa B due to its anti-inflammatory effects. Curcumin has also improved symptoms in patients with reduced corticosteroid therapy [29]. Another study investigated the protective effects of Curcumin (50 mg/kg/day) and resveratrol (80 mg/kg/day) and the underlying mechanisms in colitis-induced mice. As a result of the study, it was reported that mice treated with Curcumin or resveratrol decreased weight loss, disease severity, and proinflammatory cytokine production and prolonged life span compared to the colitis group. In addition, Curcumin and resveratrol were found to suppress inflammation in the gut, reduce autophagy, and have a protective effect against colitis by regulating sirtuin 1/mTOR signaling [30]. In another study, the therapeutic effects of pretreatment with 10 mg/kg/day of resveratrol were investigated in colitis-induced rats. It was found that resveratrol treatment increased glutathione peroxidase and catalase activities, while the microscopic score and

malondialdehyde levels were decreased. As a result of the study, it was emphasized that resveratrol had a beneficial effect on colitis in rats [31]. Similarly, in another study, 56 patients with ulcerative colitis received 500 mg of resveratrol daily for six weeks. It was found that resveratrol treatment in these patients increased total antioxidant capacity, serum superoxide dismutase, and quality of life while decreasing serum malondialdehyde levels and disease activity [32].

In the study examining the effect of grape seed polyphenol on colitis, 500mg/kg and 750mg/kg grape seed polyphenol were given orally to colitis-induced mice, respectively. As a result of the study, it was determined that grape seed polyphenol reduced diarrhea, mucosal damage, weight loss, bloody stool, inflammatory infiltration, and mRNA expression of interleukin-6, interleukin-1β, and TNF-a and signal converter and transcription 3 phosphorylation activator [33].

Luteolin is a flavonoid with anti-inflammatory properties. In a study aimed to qualify the anti-inflammatory property of luteolin against intestinal inflammation and its effect on the underlying molecular mechanisms, it was observed that luteolin negatively regulates inflammatory pathways by decreasing the expression of interleukin-8, cyclooxygenase-2, nitric oxide, and inducible nitric oxide synthase. It was also concluded that luteolin may be effective against intestinal inflammation and could be considered a therapeutic option for IBD [34]. A study investigating the protective effect of lycopene against colitis found that treatment with 10 mg/kg of lycopene increased superoxide dismutase levels and total antioxidant levels in colitis-induced rats. As a result, it was found that treatment with lycopene improved biochemical and pathological outcomes in rats with colitis [35]. In another study, the effects of tomatoes called Bronz, enriched with three different classes of polyphenols, on the microbiota, inflammatory responses, and chronic IBD symptoms were investigated, and as a result, Bronz tomatoes were found to reduce IBD symptoms significantly [26].

Another animal study examining the effects of gallotannin-rich mango and ellagitannin-rich pomegranate found that mango and pomegranate drinks reduced intestinal inflammation, mucosal damage, and proinflammatory cytokines [36]. In a different study on pomegranate polyphenols, the effects of purified punicalagin and pomegranate juice on nuclear factor kappa B signaling pathways and its expression in colitis were studied in rats with experimental colitis. It was concluded that the severity of the disease decreased, and the levels of TNF-a, interleukin-18, and interleukin-1 B mRNA decreased. It was concluded that pomegranate juice and its main ingredient, punicalagin, may be used to control inflammatory diseases such as IBD and to inhibit nuclear factor kappa B directly [37].

In another study investigating the anti-inflammatory properties of flavonoid compounds, eupatilin and quercetin were administered to rats 48 hours before colitis was induced. Rats receiving flavonoid extracts had fewer mucosal lesions, nitric oxide production, and TNF-α levels. Higher glutathione levels have also been reported. Finally, it was observed that these compounds improved the inflammatory response and colon damage in colitis by reducing oxidative stress and neutrophil activation [38]. Dönder et al. [4] studied the effects of quercetin on bacterial translocation in IBD in an experimental colitis model and reported that quercetin has a substantial therapeutic effect. In addition, histopathologic improvement, inflammation reduction, and bacterial translocation were observed in the treatment group.

In the study, which investigated the effect of epigallocatechin gallate (EGCG) in green tea, rats were given 20 mg/kg and 50 mg/kg EGCG orally daily. Different doses of EGCG therapy were found to reduce weight loss and clinical manifestations of the disease. It also prevented colon shortening, decreased intestinal permeability, reduced colon inflammation, and provided histopathological improvements[39]. Zhao et al. concluded that different doses of magnolol (5 mg/kg, 10 mg/kg, and 15 mg/kg) reduced myeloperoxidase activity, colon lesions, proinflammatory cytokines, and disease activity index in colitis-induced mice [40]. In the study evaluating the protective effect of different doses of gallic acid treatment (25 mg/kg, 50 mg/kg, 75 mg/kg, and 100 mg/kg), it was concluded that treatment with 100 mg/kg gallic acid reduced disease activity index, macroscopic and microscopic colon damage, and myeloperoxidase activity. This effect is believed to be due to the gallic acid's anti-inflammatory and antioxidant properties [41].

Conclusion

Dietary polyphenols have several health benefits, such as protecting against chronic diseases and supporting healthy aging. The main problems of therapeutic approaches to the disorder are limited benefits, side effects, and poor response in patients taking anti-inflammatory drugs. Polyphenols modulate cellular signaling pathways and exert antioxidant and anti-inflammatory effects. Polyphenols are believed to have beneficial effects on reducing the severity of IBD and slowing its progression. For these reasons, dietary polyphenols are now complementary to treating IBD.

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Isolated cardiac hydatid cyst of the right ventricle

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Abstract

A hydatid cyst is a parasitic disease that most commonly affects the liver and lungs: it rarely affects the heart: right ventricular involvement is even less common. A 33-year-old male patient with a cardiac cystic mass, detected during echocardiography, was evaluated. Early surgery was the best treatment option. A hydatid cyst is located in the right ventricular wall and detected during surgery. The cyst was drained and the defect in the right ventricle was quilted. Postoperative follow-ups occurred, and he was discharged with albendazole in good health.

Keywords: Hydatid cyst, Asymptomatic, Right ventricle

Introduction

Hydatid disease is a parasitic disease caused by echinococcus granulosus. It is endemic in subtropical and tropical regions and occurs in approximately 2-6% of the population [1]. It can be seen in any part of the body, but the pericardium and heart are rarely involved. Cardiac involvement occurs in 0.5–2% of cases [2], with right ventricular involvement about 10% of them [3-5]. The main treatment is surgery; its rate of recurrence of is unknown, but is extremely rare. Serological tests are generally ineffective in showing the possibility of recurrence.

We present a very rare case of asymptomatic isolated cardiac hydatid cyst in the right ventricular wall, which was successfully treated with surgery.

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Informed Consent
The authors stated that the written consent was obtained from the patient presented with images in the study.

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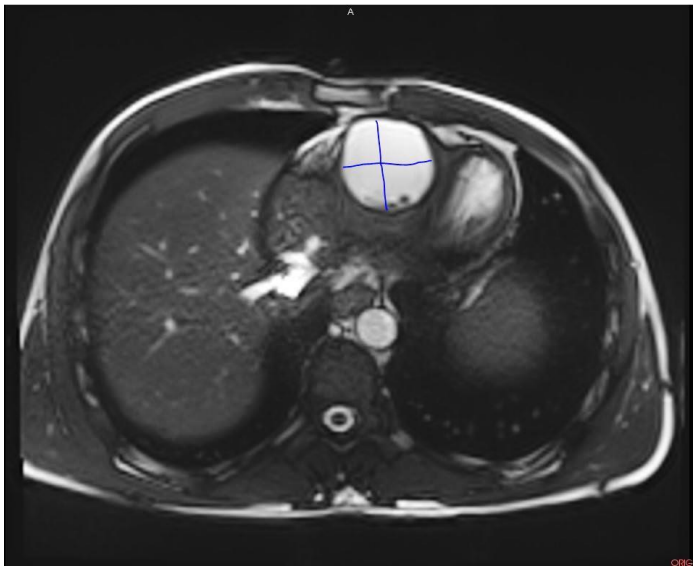
Case presentation

We evaluated a 33-year-old male patient, with a mass in the heart detected during echocardiography. No pathology was found on physical examination. His body temperature was 36.6 degrees C, pulse 81, and blood pressure 130/80. ECG abnormality exhibited T wave negativity in D_{2-3} leads and a biphasic T wave in V_{2-3} leads. Transthoracic echocardiography showed a 48x42 mm cyst mass in the right ventricle. Blood tests were completely normal. MRI showed a lesion compatible with hydatid cyst originating from the free wall of the right ventricle, filling the lumen of the right ventricular apex, creating mild pressure to the interventricular septum. The patient was scanned for other lesions, which were not found (Figure 1).

Figure 1: Abdominal computed tomography examination of the case.



After obtaining written informed consent from the patient, he was taken to the operating room. Surgery started in the supine position under general anesthesia. Median sternotomy was performed and the pericardium was opened, respectively. There was a 6x6 cm cystic lesion at the edge of the acute margin in the right ventricular wall. Heparinization aorto-bicaval cannulation was performed and then extracorporeal circulation was started, with the x-clamp placed on the ascending aorta to achieve arrest with antegrade isothermal blood cardioplegia. Moreover, 3% NaCl with gauze was placed around the cyst, with 40 cc of cystic content aspirated, while the same amount of 3% NaCl was used in the cavity. After ten minutes, the cyst was opened, with a germinative membrane removed (Figure 2, 3). The cavity was irrigated with 1000 ml of 3% NaCl (Figure 4). Subsequently, the cystic cavity was plicated (capitonnage procedure). The outer layer of the ventricle was sutured with six 2/0 TiCron sutures. After de-airing cardiac chambers, the X-clamp was removed. Sinus rhythm was achieved, with the surgery completed.

The postoperative course was uneventful, with the patient discharged on the fifth postoperative day with albendazole, as per the recommendation of the infectious diseases department. The pathological examination confirmed cardiac ecchinococcosis.

Figure 2: Operative view of the right ventricular cyst.

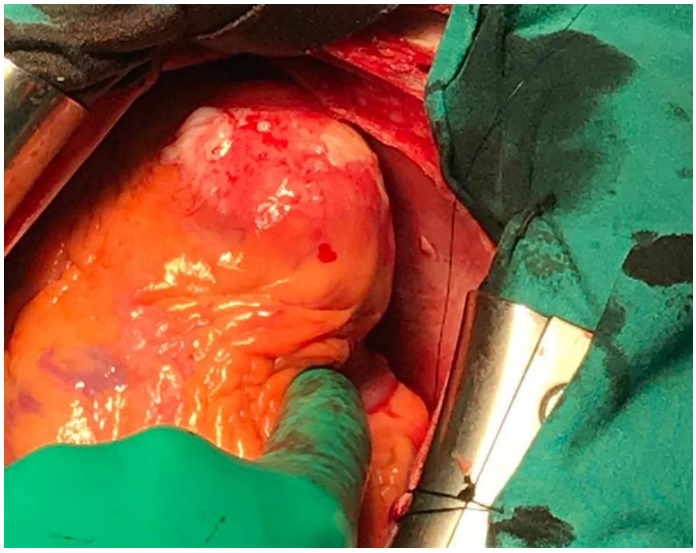


Figure 3: Operative view of the right ventricular cyst.

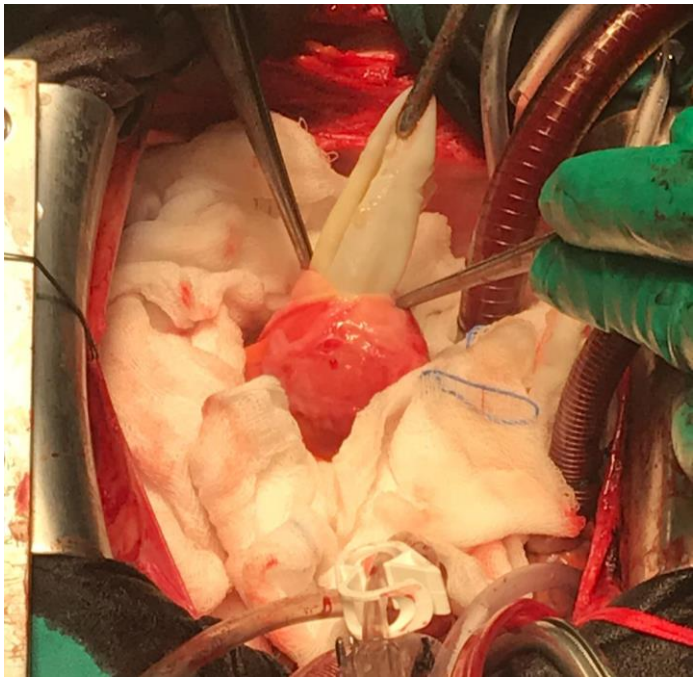
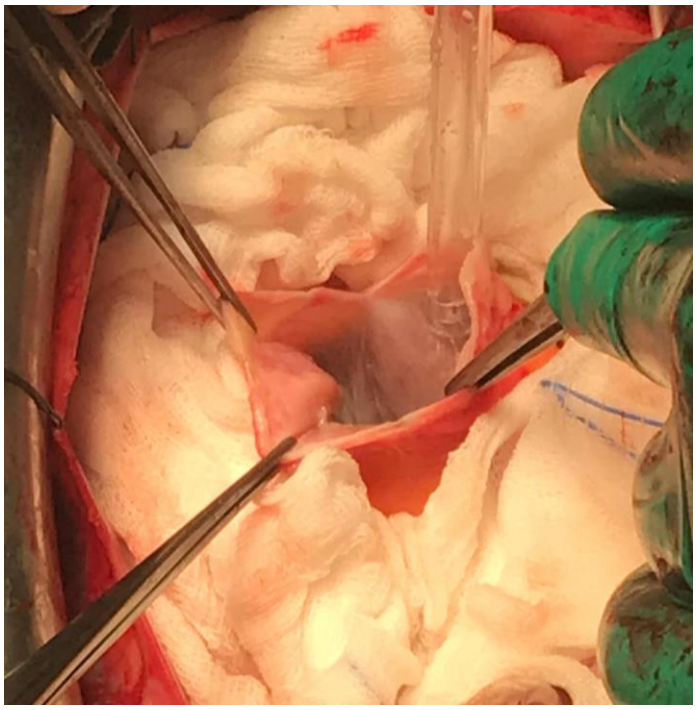


Figure 4: Operative view of the right ventricular cyst.



Discussion

As stated, a cardiac hydatid cyst is a rare disease and right ventricular involvement is infrequently detected [2-5]. Areas of cardiac involvement include the left ventricle 60%, right ventricle 10%, pericardium 7%, pulmonary artery 6%, and the left atrial appendage 6% [3]. Right-sided cysts tend to expand intracavity and subendocardial area, with right ventricular cysts rupturing more often [6]. The type of operation may vary depending on the case. Cysts unrelated to the heart chambers can be operated on without using extracorporeal circulation [7, 8]. In our case, extracorporeal bypass was preferred, as the cyst was associated with the right ventricle. As such, we determined that it would be easier to control local dissemination.

The operation was performed in the supine position under general anesthesia. We preferred a median sternotomy, given our experience, which also provides better access to all cardiac structures. Although several agents have been used intraoperatively for protoscolicidal effect, such as iodine solution, cetrimide, ethanol, hypertonic saline, chlorhexidine, and methylene blue, the gold standard agent has not been identified [1, 7, 9, 10]. There are publications that do not mention scolicidal impact during the operation [11]. According to the recommendation of the thoracic surgery clinic, which has more experience in hydatid cysts, with the risk that it might have reached the ventricle - we preferred 3% hypertonic saline solution. The area around the cyst required 3% NaCl impregnated gauze for Vakilian et al. [11], removing the cyst directly. We aspirated and irrigated it with hypertonic saline. The cyst was opened, with the germination membrane carefully and completely excised, in an extremely important step: this leads to anaphylaxis, pulmonary embolism, sudden death, relapse, and secondary seeding of daughter cysts into other areas of the body [1, 6]. The right ventricular chamber was reached when all cystic structures were excised, but some surgeons do not close the cyst cavity. For example, Gupta and Priyadarshini left the cavity open after removing the cyst in the left ventricular wall [7]. We performed a capitonnage procedure with six 2/0 TiCron sutures, as the cavity is connected to the ventricular chamber. Ipek et al. [12] used teflon sutures in this procedure, as they have generally been used in other publications. We decided to use 2/0 TiCron sutures for the heart, a strong and continuously mobile organ: this suture offers high tensile strength, ease of tissue passage, permanent support, and surface lubricity. The case was terminated routinely in the sinus rhythm.

Conclusion

Early and accurate diagnosis is crucial in cardiac hydatid disease. In addition, treatment is surgical and must not be delayed. As in our case, cardiac echinococcosis should be considered as a differential diagnosis, especially in asymptomatic cases from endemic regions. Surgical results are generally satisfactory, and most patients completely recover.

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Recurrent cardiac rhabdomyosarcoma with multiple metastases: A case report

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Abstract

Rhabdomyosarcoma, which accounts for 20% of all malignant tumors of the heart, is an aggressive tumor originating in the ventricular wall. These tumors are the second most common malignant primary tumor of the heart after angiosarcoma. Despite treatment options, such as surgical resection, radiotherapy, and chemotherapy, recurrence is common and mortality is high. Among these patients, survival with surgical resection is around six months to one year. In this case report, we discuss a patient who presented with recurrent rhabdomyosarcoma with distant metastasis after surgical intervention. A 56-year-old male patient who underwent left atrial mass excision and mitral valve replacement ten months prior was admitted with recurrent metastatic rhabdomyosarcoma.

Keywords: Rhabdomyosarcoma, Cardiac, Recurrent tumor

Introduction

Primary cardiac tumors are rare and have been reported at between 0.001% and 0.28% in autopsy series [1]. Of these primary tumors, 25% are malignant [2,3]. Rhabdomyosarcoma accounts for 20% of these malignant tumors [4,5]. In fact, rhabdomyosarcoma is very rare in adults compared to children [6]. Rhabdomyosarcoma, which often originates in the ventricular wall, spreads aggressively and has a high mortality rate [5]. In this case report, we will present a patient who was operated on for an intracardiac tumor with recurrent metastatic rhabdomyosarcoma detected ten months later.

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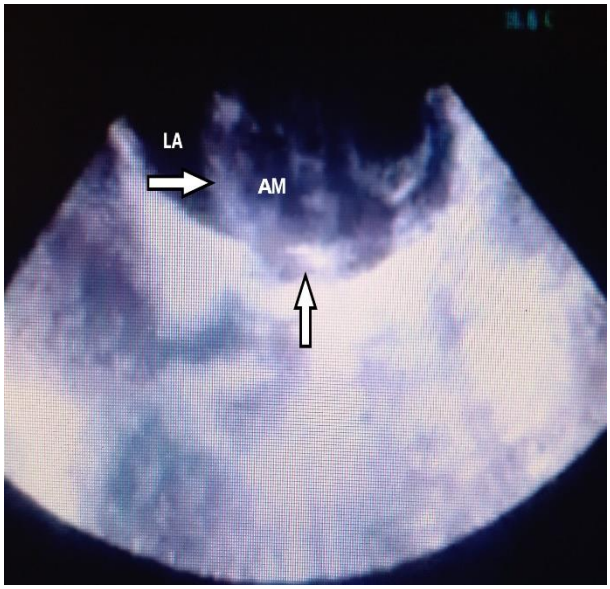
Case presentation

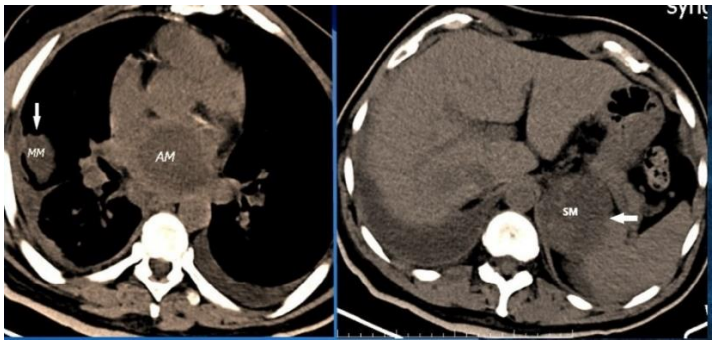
A 56-year-old male patient who had undergone left atrial mass excision and mitral valve replacement ten months previously at another center presented to the cardiology outpatient clinic with dyspnea, malaise, and fatigue. The international normalized ratio (INR) test showed an effective INR value. Other laboratory results revealed no pathology except a hemoglobin value of 10.6 gr/dl and a C-reactive protein (CRP) value of 163 mg/lt. The patient's transthoracic echocardiography (TTE) revealed a mitral valve gradient of 25/19 mmHg and a suspicious mass surrounding the left atrium. The patient was initially diagnosed as having possible left atrial mass and mechanical valve dysfunction. He was scheduled for transesophageal echocardiography (TEE) for a definitive diagnosis. The TEE showed a mass completely covering the left atrium and a raised gradient across the mechanical valve (Figure 1).

Figure 1: Transesophageal echocardiography revealed a mass filling the left atrium (LA: left atrium, AM: atrial mass)



Given that the patient had symptoms of decompensated heart failure, emergency surgical preparation was made. Preoperative computed tomography (CT) was performed for possible metastases. The CT examination showed multiple mediastinal lymph nodes, with the largest one measuring 13 mm, a mass appearance of 7 x 4 cm in the lower lobe of the right lung, a right paracardiac mass of 42 x 30 mm and a mass of 7 cm in the left adrenal gland, as well as a hypodense lesion with a diameter of 58 mm in the left atrium (Figure 2). The patient's data from the hospital where he had been previously been operated on showed that the postoperative histopathological result of the intracardiac mass excised was rhabdomyosarcoma. An interview with the patient and his relatives revealed that he was called to the hospital for radiotherapy and chemotherapy planning after the diagnosis, but the treatment was not continued, because he did not want to receive chemotherapy. After a council discussion with the cardiology, cardiovascular surgery, and oncology departments, the patient was considered to have a possible metastatic rhabdomyosarcoma and it was decided that this patient's condition was inoperable. The patient, who was followed up in the intensive care unit, died on the fifth day after diagnosis due to multiorgan failure secondary to cardiac failure.

Figure 2: Computed tomography showing recurrence of the mass lesion in the left atrium, right lung, and surrenal gland (MM: metastatic mass in the lung, AM: left atrial mass, SM: surrenal metastatic mass)



Discussion

Rhabdomyosarcoma, which was first described by Raycoff in 1937, is a malignant tumor arising from embryonic mesenchymal cells that can differentiate into skeletal muscle [1]. The literature shows that cardiac sites involved include the left atrium (55%), left ventricle (15.7%), right ventricle (15.7%), and right atrium (13%) [3].

This condition can be diagnosed by TTE, TEE, CT and magnetic resonance imaging [5]. The prognosis is often poor, with 75% of patients having distant metastases at the time of diagnosis. The spread of metastases occurs via lymphatic and hematogenous routes. Symptoms develop due to intracardiac obstruction by the mass. Patients often present with heart failure, dyspnea, mitral/tricuspid stenosis, arrhythmia, inferior/superior vena cava obstruction, restrictive cardiomyopathy, tamponade, and sudden death [1]. They may present with tumor-induced cerebral, pulmonary and peripheral embolism. Among these patients, survival with surgical resection is around six months to one year [3]. Although complete surgical resection is the most important prognostic factor, systemic chemotherapy and local radiotherapy for residual tumors are both important for possible survival [1]. However, there is no consensus on radiotherapy and adjuvant chemotherapy due to the poor prognosis [5]. Heart transplantation has also been attempted in cases where these treatments were ineffective and metastasis was excluded [7]. In their series of four patients, Uberfuhr et al. [7] reported a mean survival of 18 months, while Li et al. [8] reported a mean survival of 16 months after transplantation in a literature analysis of 46 patients.

In our case, a review of the patient's past surgical notes showed that the tumor was located in the left atrium and had invaded the mitral valve. During the surgical intervention, the tumor was removed, the mitral valve was completely resected and mechanical valve replacement was performed to avoid residual tumor tissue. Although the surgical intervention was successful, we do not know whether there was a residual focus or microscopic metastasis during the surgery. Failure to administer postoperative chemotherapy and radiotherapy may have caused his early recurrence within ten months. Notably, surgical intervention would have been a controversial decision in terms of prolonging survival if no metastasis had been detected in the patient.

Conclusion

Intracardiac rhabdomyosarcoma is a malignancy with an aggressive course and high mortality despite all available treatment modalities, such as surgical resection, chemotherapy, and radiotherapy. Continuation of chemotherapy and radiotherapy after surgical intervention is important in terms of prolonging the survival of such patients.

References

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