ORIGINAL RESEARCH

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The Relationship Between the Number of Miscarriages and Diagnostic Parameters in Couples with Recurrent Pregnancy Loss: A Retrospective Cohort Study

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ABSTRACT Objective: The aim of this study was to compare the ovarian reserve tests and abnormal test results in couples with 2 to more than 2 recurrent pregnancy losses in our population. **Material and Methods:** Seventy-five couples aged 20-44 years, who had two or more consecutive abortions at 20 weeks or less from the last menstrual period, were included in the study. The women were divided into two groups as those who had two miscarriages (Group 1, n=34) and those who had three or more miscarriages (Group 2, n=41). Women in both groups were compared in terms of serum hormone levels, biochemical parameters, karyotype analysis, thrombophilic, and uterine factors. The spermiogram values and karyotype analyzes of the male partners in both groups were compared. **Results:** The women in Group 2 were older than those in Group 1, and live birth history and Factor V Leiden heterozygosity were found to be statistically significantly higher. The follicle stimulating hormone values in the secondary recurrent pregnancy loss patients were found to be statistically significantly higher than in the primary recurrent pregnancy loss patients. **Conclusion:** Maternal age and Factor V Leiden heterozygosity may be associated with the number of miscarriages in couples with recurrent pregnancy loss.

Keywords: Factor V Leiden; recurrent miscarriage; thrombophilia

Recurrent pregnancy loss (RPL) is a problem that affects approximately 1-5% of couples who want to conceive. The loss of any desired pregnancy, regardless of which trimester, is very distressing for couples. Among the causes of RPL, uterine anomalies, fetal chromosomal anomalies, hereditary, and acquired thrombophilias, immunological factors, infectious diseases, endocrine disorders, and endometrial receptivity disorders are often blamed.[1] Unfortunately, there are many conflicting studies on this subject and despite investigations, often the cause cannot be determined.[2] The existence of many problems such as whether pregnancy loss is primary or secondary, whether the upper limit of pregnancy is 20 weeks or 24 weeks from the last menstrual period, whether biochemical pregnancies are included in the entity, the effect of ovarian reserve, female age, infertility, and recurrent implantation failure makes it difficult to standardize the groups in the studies. There is not even a consensus on when to start research. The Royal College of Obstetricians and Gynecologists recommends evaluation after three or more consecutive pregnancy losses, while the American Society for Reproductive Medicine (ASRM) Practice Committee recommends investigating etiology after two or more pregnancy losses that need not be consecutive.[3,4] Although the guidelines recommend a minimal approach to research because there is no clear evidence-based treatment and diagnostic testing is expensive, the psychological effects of the problem on couples leave the clinician helpless. It is very important for couples to find out if there is a

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modifiable cause before planning the next pregnancy.[5] For this reason, special approaches are recommended for couples. It is decided when to start the research, previous pregnancy losses, the age of the woman and other maternal conditions.[6] In studies, the number of previous miscarriages and advanced maternal age associated with ovarian reserve appear to be the most important prognostic factors.[7,8]

In order to contribute to the lack of knowledge on this subject, in this study, it was aimed to examine the association between the number of miscarriages, live birth history, ovarian reserve and the parameters blamed in the etiology in a group of couples with RPL in our population.

MATERIAL AND METHODS

This retrospective cohort study was conducted in a tertiary university hospital. After ethical approval from the institutional University of Health Sciences, Kanuni Sultan Süleyman Training and Research Hospital Ethics Committee (no: 2022.04.82) on 08.04.2022, 132 patient files that applied with the complaint of RPL between January 2011 and December 2019 were reviewed. Fifty-seven couples were excluded from the study due to the lack of data. Seventy-five couples were included in the study. All couples have their informed consent before the evaluation of their files. The study was conducted in accordance with the principles of the Declaration of Helsinki. Women aged 20-44 years, from the same partners, consecutive, 20 weeks or less according to the last menstrual period, 2 or more, ultrasound-proven abortions were included in the RPL category. Women were divided into two groups as those with 2 miscarriages (Group 1, n=34) and those with 3 or more (Group 2, n=41). Women with no previous living children were considered as primary RPL, and those with a history of at least one live birth were considered as secondary RPL.

All couples were evaluated according to ASRM's recommendations.[9] Following a complete systemic evaluation, all women underwent gynecological examination and were evaluated for possible uterine anomalies by transvaginal ultrasonography, hysterosalpingography, or office hysteroscopy. On the 2nd or 3rd day of menstruation, venous blood sampling was performed after an overnight fasting from 8:00 am to 10:00 am. Serum follicle stimulating hormone (FSH), estradiol (E_2), thyroid stimulating hormone (TSH), free T_3 (fT_3), free T_4 (fT_4), antithyroid antibodies [antithyroid peroxidase (anti-TPO), antithyroglobulin (anti-TG)], antimullerian hormone (AMH), lupus anticoagulant (LA), antiphospholipid antibodies [anticardiolipin immunoglobulin (Ig) M and Ig G, anti B_2 glycoprotein Ig M and Ig G], protein C, protein S, antithrombin 3 (AT III), Factor V G1691A [Factor V Leiden (FVL], prothrombin gene mutation G20210A (Factor II), methylenetetrahydrofolate reductase (MTHFR) A1298C, MTHFR C677T, activated protein C (APC) resistance, antinuclear antibody (ANA), Factor XII, homocystein, plasminogen activator inhibitor-1 (PAI-1) 4G/5G levels and Factor XIII V34L were assayed.

All hormones and antibodies were measured using the UniCel DxI 800 immunochemistry analyzer (Beckman Coulter Inc., USA) according to manufacturer's assay instructions and requirements in the biochemistry laboratory of our hospital. Access FSH, TSH, anti-TPO, and anti-TG values were assayed following a two-site immunoenzymatic (sandwich) method. Access E_2, sT_3, and sT_4 values were assayed with the competitive immunoenzymatic method. AMH, antiphospholipid antibodies, and ANA concentrations were assayed following a highly specific enzyme-linked immunosorbent method. AT III, protein C, protein S, and homocystein were analyzed by chromogenic method. APC resistance and Factor XII were measured by coagulometric method. LA was determined by the modified Dilute Russell's Viper Venom method. The coefficients of variation intraassay and interassay tests of these hormones are as follows (mean±standard deviation): 7.40±3.43 mIU/mL for FSH, 45.2±18.52 ng/mL for E_2, 3.94±3.25 ng/mL for AMH. Thyroid disorders were defined as abnormal T_4 level with serum levels of TSH <0.45 mIU/L or TSH>4.5 mIU/L. When antiphospholipid antibodies were positive twice with an interval of at least 12 weeks, if clinical criteria including thrombosis or pregnancy morbidity were present, it was accepted that antiphospholipid syndrome (aPLs), which is the cause of acquired thrombophilia, was present (Sapporo classification criteria).[10] Less

than 70% functional protein C activity and functional protein S activity were considered abnormal. After DNA extraction, genotyping of Factor II G20210A, FVL, MTHFR C677T, and MTHFR A1298C, PAI-1 4G/5G, Factor XIII V34L polymorphisms was performed by real-time polymerase chain reaction. Heterozygous or homozygous mutations were defined as abnormal. Karyotyping was performed on both spouses. The spermiogram test of the male partner was evaluated. Women with active urogenital infections were excluded from the study.

Mean standard deviation, median, minimum and maximum values were given in descriptive statistics for continuous data, and number and percentage values were given in discrete data. The Shapiro-Wilk test was used to examine the compatibility of continuous data to the normal distribution. In the comparison of continuous variables, the t-test was used for data showing normal distribution, and the Mann-Whitney U test was used for data that did not comply with the normal distribution. Chi-square test was used for comparisons between groups of categorical variables (cross tables). The IBM SPSS Statistics 20 program (Chicago, IL, USA) was used for evaluations and a p value <0.05 was accepted to be statistically significant.

RESULTS

The mean age of 75 women with RPL included in the study was 30 (20-44). Thirty-four (45.3%) women with two miscarriages were accepted as Group 1, and forty-one women (54.7%) with three or more miscarriages (3-6) were accepted as Group 2. In Group 2, there were 5 women with 4 miscarriages (12.1%), 7 women with 5 miscarriages (17.07%), and 1 woman with 6 miscarriages (2.43%). Group 1 included 2 women with multiple fibroids (5.88%) and 1 women with diabetes mellitus (2.94%). Group 2 included 2 women with diabetes mellitus (4.87%) and 1 woman with beta thalassemia (2.43%). None of the patients had a history of thromboembolic events. Comparisons of the live birth history, hormonal values and male partners' spermiogram parameters of the women in the groups are shown in Table 1. The comparison of biochemical parameters, uterine factors of women, genetic factors of both women, and their male partners is shown in Table 2. The comparison of age, hormone levels, FVL, and spermiogram parameters of male partners of primary RPL patients and secondary RPL patients are given in Table 3. Comparison of AMH (when threshold value is 1.5 ng/mL), FSH (when threshold value is 10 mIU/mL), and miscarrieges numbers are given in Table 4.

DISCUSSION

In this study, women with more miscarriages were older, live birth history and FVL heterozygosity rates were found to be statistically significantly higher. However, there was no statistically significant difference between the groups' AMH, FSH, E_2, TSH, fT_3, fT_4, anti-TPO, anti-TG, LA, antiphospholipid antibodies, protein C, protein S, AT III, prothrombin gene mutation G20210A, MTHFR A1298C, MTHFR C677T, APC resistance, ANA, homocysteine, PAI-1 4G/5G, Factor XII, Factor XIII V34L values and the spermiogram parameters of spouses.

TABLE 1: Comparison of age, AMH, FSH, E2 values of women and spermiogram parameters of male partners between groups.

	Group 1 (n=34) $\bar{X}$±SD Median (minimum-maximum)	Group 2 (n=41) $\bar{X}$±SD Median (minimum-maximum)	p value
Age (year)	30.56±5.22	30.76±6.09	**<0.001***
Live birth history	1 (0-3)	1 (0-4)	**0.011****
AMH (ng/mL)	3.25 (0.89-12.20)	3.30 (0.07-21.90)	0.987**
FSH (mIU/mL)	7.05 (4.5-12.39)	7.30 (1.60-32.40)	0.919**
E_2 (ng/mL)	45.10 (13.30-86.90)	40.10 (12.70-101.39)	0.698**
Spermiogram Total motile sperm count (million)	54.06±37.54 41.74 (18.80-180.20)	8.78±44.19 48.30 (3.70-199)	0.573**

*Independent samples t-test; **Mann-Whitney U test, p<0.05; AMH: Antimullerian hormone; FSH: Follicule stimulating hormone; E_2: Estradiol; SD: Standart deviation.

TABLE 2: Characteristics of 75 patients with recurrent pregnancy loss.

	n=75 (%)	Group 1 (n=34)	Group 2 (n=41)	p value
Thyroid diseases				
Normal	64 (85.4)	29 (85.3)	35 (85.4)	
Hypothyroidism	1 (1.3)	0	1 (2.4)	
Hyperthyroidism	1 (1.3)	0	1 (2.4)	1.000**
Subclinical hypothyroidism	7 (9.3)	4 (11.8)	3 (7.3)	
Subclinical hyperthyroidism	2 (2.7)	1 (2.9)	1 (2.4)	
Antithyroid peroxidase				
Normal	56 (74.7)	28 (82.4)	28 (68.3)	0.163*
High	19 (25.3)	6 (17.6)	13 (31.7)	
Anti thyroglobulin				
Normal	63 (84)	29 (85.3)	34 (82.9)	0.781*
High	12 (16)	5 (14.7)	7 (17.1)	
Lupus anticoagulant				
Negative	74 (98.7)	33 (97.1)	41 (100)	0.453**
Positive	1 (1.3)	1 (2.9)	0	
Anticardiolipin antibody Ig M and Ig G				
Negative	75 (100)	34 (100)	41 (100)	
Anti B2 glycoprotein antibody Ig M				
Negative	75 (100)	34 (100)	41 (100)	
Anti B2 glycoprotein antibody Ig G				
Negative	74 (98.7)	34 (100)	40 (97.6)	1.000**
Positive	1 (1.3)	0	1 (2.4)	
Protein C				
Low	2 (2.7)	1 (2.9)	1 (2.4)	0.725**
Normal	72 (96)	32 (94.1)	40 (97.6)	
High	1 (1.3)	1 (2.9)	0	
Protein S				
Low	7 (9.3)	2 (5.9)	5 (12.2)	0.386**
Normal	66 (88)	32 (94.1)	34 (82.9)	
High	2 (2.7)	0	2 (4.9)	
Antithrombin 3				
Normal	71 (94.7)	31 (91.2)	40 (97.6)	0.323**
Low	4 (5.3)	3 (8.8)	1 (2.4)	
Factor V Leiden				
Normal	69 (92)	34 (100)	35 (85.4)	0.029**
Heterozygous	6 (8)	0	6 (14.6)	
Prothrombin gene mutation G20210A				
Normal	72 (96)	33 (97.1)	39 (95.1)	1.000**
Heterozygous	3 (4)	1 (2.9)	2 (4.9)	
MTHFR A1298C				
Heterozygous	27 (36)	12 (35.3)	15 (36.6)	
Homozygous	5 (6.7)	2 (5.9)	3 (7.3)	1.000**
Normal	43 (57.3)	20 (58.8)	23 (56.1)	
MTHFR C677T				
Heterozygous	14 (18.7)	8 (23.5)	6 (14.6)	
Homozygous	6 (8)	1 (2.9)	5 (12.2)	0.264**
Normal	55 (73.3)	25 (73.5)	30 (73.2)	
Homocystein				
Low	1 (1.3)	1 (2.9)	0	
Normal	69 (92)	31 (91.9)	38 (92.7)	0.812**
High	5 (6.7)	2 (5.2)	3 (7.3)	

TABLE 2: Characteristics of 75 patients with recurrent pregnancy loss *(continue)*.

	n=75 (%)	Group 1 (n=34)	Group 2 (n=41)	p value
Activated protein C resistance				
Low	5 (6.7)	0	5 (12.2)	0.060**
Normal	70 (93.3)	34 (100)	36 (87.8)	
Factor 12				
Low	1 (1.3)	0	1 (2.4)	1.000**
Normal	74 (98.7)	34 (100)	40 (97.6)	
Anti nuclear antibody				
Negative	75 (100)	34 (100)	41 (100)	
PAI-1 4G/5G				
Heterozygous	12 (16)	3 (8.8)	9 (22)	
Homozygous	5 (6.7)	1 (2.9)	4 (9.8)	0.140**
Normal	58 (77.3)	30 (88.2)	28 (68.3)	
Factor 13 V34L				
Heterozygous	4 (5.3)	2 (5.9)	2 (4.9)	
Normal	71 (94.7)	32 (94.1)	39 (95.1)	
Female karyotype				
46, XX	71 (94.7)	33 (97.1)	38 (92.7)	
46, XX, inv(9)p12q13	1 (1.3)		1 (2.4)	
46, XX, t(3;8)(p13;p23.1)	1 (1.3)		1 (2.4)	
47, XXX(3)/46, XX(47)	1 (1.3)		1 (2.4)	
46, XX, t(1;5)(p31;p15.3)	1 (1.3)	1 (2.9)		
Male karyotype				1.000**
46, XY	71 (94.7)	33 (97.1)	38 (92.7)	
46, XY, 16qh+	1 (1.3)	1 (2.9)		
46, XY,qh	2 (2.7)		2 (4.9)	
46, XY,t(5;18)q23;q23)	1 (1.3)		1 (2.4)	
Hysterosalpingography/hysteroscopy findings				
Normal	67 (89.3)	30 (88.2)	37 (90.2)	
Arcuat uterus	2 (2.7)	1 (2.9)	1 (2.4)	
Endometrial polyp	2 (2.7)	2 (5.9)		
Uterine septum	2 (2.7)	1 (2.9)	1 (2.4)	
Uterine septum+hydrosalpenx	1 (1.3)		1 (2.4)	
T-shape uterus+endometrial polyp	1 (1.3)		1 (2.4)	

*Pearson chi-square tests; **Fisher's exact test, p<0.05; MTHFR: Methylene tetrahydrofolate reductase; PAI-1: Plasminogen activator inhibitor; Ig: immunoglobulin.

Decreased ovarian reserve with advancing age in women increases the possibility of aneuploidy and miscarriage.[11] While the probability of miscarriage is 20% at the age of 35, this rate increases to 40% at the age of 40.[12] In support of this finding, in this study, the age of the women who made three or more miscarriages was found to be older than the women who made two miscarriages. However, women in Group 2 were found to have higher previous live birth rates than women who had 2 miscarriages. Although this seems like a contradiction, the fact that the woman had a successful live birth at a young age supports the finding that pregnancies result in miscarriage as she gets older. This is why we may have found the FSH values of women with a previous live birth history (secondary RPL) to be high. However, when the AMH threshold value was 1.5 ng/mL, no difference was found between those with low and high AMH values in terms of miscarriage numbers. Likewise, no difference was found between the miscarriage numbers of those with FSH values less than 10 mIU/mL and those with a FSH value greater than 10 mIU/mL. Probably because the number of patients in this study was small and relatively young, a significant differ-

TABLE 3: Comparison of age, AMH, FSH and E2 values of primary RPL patients and secondary RPL patients.

	Primary RPL (n=27) X̄±SD Median (minimum-maximum)	Secondary RPL (n=48) X̄±SD Median (minimum-maximum)	p value
Age (Year)	30.15±5.66	30.96±5.72	0.557*
AMH (ng/mL)	2.60 (0.29-12.20)	3.40 (0.07-21.90)	0.825**
FSH (mIU/mL)	6.45 (1.60-9.90)	7.50 (4.05-32.40)	**0.039****
E_2 (ng/mL)	44.90 (12.70-86.90)	41.85 (13.30-101.39)	0.573**
Spermiogram			
Concentration (million/mL)	42.33±26.72	35.96±21.18	0.472**
	36 (14-134)	33.5 (3-102)	
Sperm motility Grade A (%)	42.63±8.91	45.50±14.36	0.537**
	42 (27-63)	45 (17-74)	
Sperm motility Grade B (%)	9.93±2.46	10.81±3.46	0.405**
	10 (4-16)	10 (4-24)	
Total motile sperm count (million)	57.31±41.66	56.26±41.22	0.573**
	48.30 (14-187.5)	46.60 (3.70-199)	
Morphology (%)	3 (0-7)	3 (0-9)	0.507**
	n (%)	**n (%)**	
Thyroid function			
Normal	23 (85.2)	41 (85.4)	1.000***
Abnormal	4 (14.8)	7 (14.6)	
Anti-TPO			
Normal	22 (81.5)	34 (70.8)	0.309****
Elevated	5 (18.5)	14 (29.2)	
Anti-TG			
Normal	22 (81.5)	41 (85.4)	0.746***
Elevated	5 (18.5)	7 (14.6)	
Factor V Leiden			
Normal	25 (92.6)	44 (91.7)	1.000***
Heterozygous	2 (7.4)	4 (8.3)	

*Independent Samples t-test; **Mann-Whitney U test; ***Fisher's exact test; ****Pearson chi-square tests, p<0.05; SD: Standart deviation;
AMH: Antimullerian hormone; FSH: Follicule stimulating hormone; E_2: Estradiol; RPL: Reccurrent pregnancy loss; Anti-TPO: Antithyroid peroxidase; Anti-TG: Antithyroglobulin.

ence could not be found between the AMH and FSH threshold values. Whereas, in a study by Wald et al. in 264 women with RPL, they reported that patients with unexplained RPL had more decreased ovarian reserve than patients with an explainable cause (48% vs. 29%, p=0.005).[13] However, the American Congress of Obstetricians and Gynecologists, the European Society of Human Reproduction and Embryology, and the ASRM do not currently recommend ovarian reserve testing in the routine evaluation of RPL.[4,6,8] We prefer to perform ovarian reserve tests in selected patients in order to offer couples new treatment methods such as assisted reproductive techniques and preimplantation genetic diagnosis in terms of planning the next pregnancies.

TABLE 4: Comparison of AMH levels (when threshold value is taken as 1.5 ng/mL), FSH levels, and miscarrieges numbers.

	Number of miscarriages Median (minimum-maximum)	p value
AMH (ng/mL)		
≤1.5 (n=16)	3 (2-6)	0.753*
>1.5 (n=59)	3 (2-5)	
FSH (mIU/mL)		
<10 (n=72)	3 (2-6)	0.990*
>10 (n=3)	3 (2-3)	

*Mann-Whitney U test, p<0.05; AMH: Antimullerian hormone; FSH: Follicule stimulating hormone.

Thrombophilias are hypothesized to play a role in the etiology of RPL.[14] Pregnancy is a prothrombotic condition, and the risk of complications is even

higher if these women also have a thrombophilia. FVL as a cause of thrombophilia is reported as a common inherited risk factor for RPL, with the incidence range of 8-32% in patients and 4-10% in controls.[15-17] However, there are many studies showing that there is no relationship between all hereditary causes of thrombophilia (FVL, prothrombin gene mutation G20210A, protein C deficiency, protein S deficiency, and AT III deficiency, PAI-1 4G/5G, MTHFR C677T and MTHFR A1298C mutations) and RPL.[18-20] In this study, among all thrombophilic factors, only FVL heterozygosity was found to be significantly higher in women who had a high number of miscarriages. Acquired thrombophilias (aPLs) are more clearly associated with RPL.[21] It is reported that approximately 5-20% of women with recurrent miscarriage have aPL.[22,23] No patient was diagnosed with aPLs in this study. It is possible that this diagnosis could not be made because the patient population in the study was small. Because the prevalence of these markers varies according to ethnic and geographical regions.[24]

Genetic disorders in the sperm, oocyte, or embryo are also among the causes of RPL. It is estimated that 12% of couples with RPL, including those with healthy babies, have chromosomal abnormalities from structural rearrangements in both parents.[25] Studies comparing parental structural chromosomal abnormalities in women with two and three or more pregnancy losses have shown no difference between the two groups.[26,27] Therefore, it is recommended that routine karyotyping not be performed of all couples with RPL, but rather after an individual risk assessment.[6] Uterine causes of miscarriages can be divided into congenital (uterine septum) and acquired (uterine fibromas, endometrial polyps, intrauterine adhesions) disorders.[28] In a large systematic review on uterine anomalies in women with RPL, it was reported that there was no statistically significant difference between the prevalence of uterine anomalies in women with two pregnancy losses (15.4%) and women with three or more pregnancy losses (10.9%).[29] In our study, no statistical difference was found between the two groups in terms of uterine anomalies, structural and numerical chromosomal anomalies in couples. However, this is probably due to the small number of patients in the groups.

In a meta-analysis comparing women with two pregnancy losses and three or more pregnancy losses, no statistical difference was found between the rates of uterine anomalies, hereditary thrombophilia (prothrombin gene mutation, FVL, protein S deficiency, protein C deficiency, MTHFR C677T), aPLs, parental structural chromosome abnormalities, and thyroid diseases.[30] However, in our study, the prevalence of FVL heterozygosity was higher in women who had three miscarriages. No patient was diagnosed as homozygous for FVL in this study. This result may be different due to ethnic population difference. In only one of the few studies conducted in the Turkish population, a positive correlation was found between RPL and FVL and FII gene mutations.[31] In other studies, no association was found between thrombophilic factors, RPL, and miscarriage numbers.[32-35]

STUDY LIMITATIONS

The lack of a control group consisting of completely fertile couples and the small number of patients are the limitations of the study. Because in clinical practice, in line with the guidelines, we only perform the most comprehensive analyzes on selected RPL patients. In addition, the average age of the patients is in their 30s, which prevents us from generalizing the findings to all RPL patients.

CONCLUSION

In conclusion, maternal age and FVL heterozygosity may be associated with miscarriage number in couples with RPL. If the number of patients is increased, perhaps the difference will not be meaningful. Nevertheless, the aim of screening should be individualized management plan, supportive care, risk estimation for anticoagulant therapy, improvement of pregnancy morbidity, reducing anxiety in couples and to advise future options through ovarian reserve testing. Planning larger-scale prospective randomized controlled trials will help increase knowledge of whether the findings are specific to our population.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that pro-

vides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Evrim Ebru Kovalak;* ***Design:*** *Evrim Ebru Kovalak;* ***Control/Supervision:*** *Evrim Ebru Kovalak, Erdal Kaya;* ***Data Collection and/or Processing:*** *Özlem Karabay Akgül, Nurşen Kurtoğlu Aksoy;* ***Analysis and/or Interpretation:*** *Nurşen Kurtoğlu Aksoy;* ***Literature Review:*** *Neşe Hayırlıoğlu;* ***Writing the Article:*** *Evrim Ebru Kovalak;* ***Critical Review:*** *Erdal Kaya;* ***References and Fundings:*** *Özlem Karabay Akgül;* ***Materials:*** *Neşe Hayırlıoğlu.*

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