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Association and Haplotype Analysis of the PON1, ITGB3 and CYP3A4 Genes, Strong Candidates for Familial Coronary Artery Disease Susceptibility

Ailesel Koroner Arter Hastalığına Yatkınlıkta Güçlü Adaylar PON1, ITGB3 ve CYP3A4 Genlerinin Hastalıkla İlişkisi ve Haplotip Analizi

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ABSTRACT

Objective: Genetic predisposition is very common among the patients with coronary artery disease (CAD), a complex and multifactorial disease. Our objective was to determine the possible association between the most remarkable functional variants in the paraoxonase 1(PON1), cytochrome P450 3A4 (CYP3A4), integrin subunit beta 3 (ITGB3) genes and familial CAD.

Materials and Methods: We included 117 patients diagnosed with familial CAD and 99 healthy subjects with no family history of CAD. PON1 Q192R, PON1 L55M, CYP3A4*1G and ITGB3 L33P single nucleotide polymorphisms were genotyped using the Sequenom MassARRAY system.

Results: Comparison of genotype and allele frequencies in inheritance models of polymorphisms between the patient and control groups did not reveal any significant findings related to CAD. Stratified analysis by gender did also not display any association both in females and males. There was no significant difference in the frequencies of the haplotypes of the PON1 Q192R and L55M polymorphisms between the groups.

Conclusions: Our findings confirmed previous studies that did not consider PON1, CYP3A4 and ITGB3 genes as risk loci. The fact that our study was conducted only in patients with familial CAD shows the originality and importance of our results.

Keywords: CYP3A4, familial coronary artery disease, PON1, ITGB3

ÖZ

Amaç: Kompleks ve multifaktöriyel bir hastalık olan koroner arter hastalığında (KAH) genetik yatkınlık çok yaygındır. Amacımız; paraoksonaz 1(PON1), sitokrom P450 3A4 (CYP3A4), integrin subunit beta 3 (ITGB3) genlerindeki en dikkat çekici fonksiyonel varyantlar ile ailesel KAH arasındaki olası ilişkiyi belirlemekti.

Materyal ve Metot: Çalışmamıza ailesel KAH tanısı almış 117 hasta ile ailesinde KAH öyküsü olmayan 99 sağlıklı bireyi dahil ettik. PON1 Q192R, PON1 L55M, CYP3A4*1G ve ITGB3 L33P tek nükleotid polimorfizmleri Sequenom MassARRAY sistemi kullanılarak genotiplendirildi.

Bulgular: Polimorfizmlerin kalıtım modellerindeki genotip ve allel frekanslarının hasta ve kontrol grupları arasında karşılaştırılması KAH ile ilişkili anlamlı bir bulgu ortaya çıkarmadı. Cinsiyete göre tabakalı analiz yöntemi de hem kadınlarda hem de erkeklerde herhangi bir ilişki göstermedi. PON1 Q192R ve L55M polimorfizmlerinin haplotip frekansları hasta ve kontrol grupları arasında analiz edildiğinde ise yine anlamlı bir fark yoktu.

Sonuç: Bulgularımız, PON1, CYP3A4 ve ITGB3 genlerini risk lokusları olarak kabul etmeyen önceki çalışmaları doğrulamış oldu. Çalışmamızın sadece ailesel KAH hastalarında yapılmış olması, sonuçlarımızın özgünlüğünü ve önemini göstermektedir.

Anahtar Kelimeler: Ailesel koroner arter hastalığı, CYP3A4, PON1, ITGB3

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INTRODUCTION

Myocardial infarction, the most serious complication of coronary artery disease (CAD), is the leading cause of death in the world.[1] With the use of microarray technology enabling the large scale analysis of functional variants, a growing number of genetic risk factors for CAD were identified.[2]

Paraoxonase 1 (PON1) has a key role in the prevention of the lipoprotein oxidation through the hydrolyzation of the lipid peroxides in oxidized LDL (oxLDL), plays an important role in the initiation and progression of atherosclerosis. Amino acid substitution of glutamine (Q) to arginine (A) at codon 192 (Q192R, c.575A>G, rs662) and the amino acid substitution of leucine (L) to methionine (M) at codon 55 (L55M, c.163T>A, rs854560) are the most common polymorphisms, which affect the PON1 enzyme level.[3]

Several cytochrome P450 (CYP) enzymes were found in the heart, endothelium and smooth muscle cells of the blood vessels and it was demonstrated that they participated in the catalyzation of the metabolites, which played a role in the protection of the cardiovascular health.[4] CYP3A protein group constitutes of the CYP3A4 and CYP3A5 enzymes. Minamiyama et al. discovered that endothelial cells expressed the CYP3A4 enzyme in the endocardium and coronary vessels.[5] In addition, He et al. with a high sample size study determined that the G to A substitution in intron 10 of the CYP3A4 (CYP3A4*1G, rs2242480) gene was related to the coronary artery disease.[6]

Glycoprotein IIb/IIIa (GpIIb/IIIa) complex serves as a receptor for the ligands like fibrinogen, von Willebrand factor and vitronectin, which enable the aggregation and binding of the platelets to the extracellular matrix found in the walls of the blood vessels. This aggregation leads to thrombus formation in atherosclerosis. It was suggested that a single polymorphism (Leu33Pro, c.176T>C, rs5918), which is emerging in the gene encoding the GpIIIa subunit of the receptor, increases the platelet adhesion and aggregation.[7]

Until today, several case-control studies based on the opinion that the variants in the genes encoding the proteins regarding the pathophysiology of CAD might be genetic risk factors, has resulted in conflicting findings. Therefore, we believed that it would be more proper to investigate the variants, which might be genetic risk factors for the multifactorial CAD, in the patients with a positive familial medical history. Our objective was to investigate the association of the functional PON1 Q192R, PON1 L55M, CYP3A4*1G and ITGB3 L33P gene polymorphisms with CAD in the patients with familial history.

MATERIALS AND METHODS

Ethical Statement: The study protocol was approved by the Eskişehir Osmangazi University Clinical Trials Ethics Committee (Date: 28/02/2011, decision no: 2011/17). All the participants were informed about the content of the study and written consent form was taken from all of them. The study protocol was designed in accordance with the Helsinki Ethical Principles and Declaration of Good Clinical Practices and carried out in accordance with these standards.

Subjects and Study Design*:* This study was conducted with the individuals who admitted to the Cardiology Department of the Training and Research Hospital in the Eskişehir Osmangazi University between February 2014 and January 2016. 177 patients (age interval: 18-80 years) diagnosed with familial CAD and had no familial relationship with each other and 99 healthy individuals were included in the study. CAD was defined as the presence of a 50% stenosis at least in one epicardial coronary artery. Stenosis was confirmed by angiography for all patients. Familial CAD was defined as evidence of coronary artery disease in a parent or sibling before 60 years of age.[8] Healthy volunteers were included in the control group, if they and their family had no cardiovascular disease, diabetes, hyperlipidemia and hypertension. Control subjects underwent a physical examination and clinical screening for the confirmation of their health. Exclusion criteria included acute coronary syndromes, congenital heart disease, renal dysfunction, neurological or hematological disorders, morbid obesity, alcohol and drug abuse and pregnancy or lactation.

Genotyping: DNA samples were isolated from 200 µl of peripheral blood using the PureLink Genomic DNA Mini Kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions. The amount and purity of the DNA samples was optimized using the Thermo Scientific NanoDrop™ 1000 (Thermo Fisher Scientific, Wilmington, DE, USA) spectrophotometer. Primer sequences for the amplification and extension PCR reactions are designed in the Sequenom Assay Designer 3.1 (Sequenom, San Diego, CA, USA) software. PON1 Q192R (rs662), PON1 L55M (rs854560), CYP3A4*1G (rs2242480) and ITGB3 L33P (rs5918) polymorphisms are then genotyped by single base extension reactions (iPLEX, Sequenom Inc., San Diego, CA, USA) using the MassARRAY system (Sequenom Inc., San Diego, CA, USA). MALDI-TOF mass spectrometry is used to analyze the amplicons in this system. The MassARRAY System with high levels of accuracy is widely used for fine mapping and validation of GWAS studies.

Statistical Analysis: Statistical analyses were performed using the IBM SPSS (Statistical Package for the Social Sciences) Statistics 21.O (IBM Corporation, NY, USA) software package. The difference in the average age of the CAD and control groups was analyzed using an independent-sample *t*-test. Pearson Chi-square analysis was used for the categorical variables between the groups. Hardy-Weinberg equilibrium (HWE) was assessed for each polymorphism using a chi-square analysis. It was performed a haplotype-based case-control analysis based on the genotype data of the PON1 Q192R and L55M polymorphisms using the SHEsis software (http://analysis.bio-x.cn/myAnalysis.php). Haplotypes with a frequency of <0.03 were excluded. The statistical significance level was accepted as being $p<0.05$.

RESULTS

When compared between the control and CAD group in terms of gender ratio, there was no statistically significant difference (p=0.263). The mean age was 55.6±9.03 years in the 117 CAD patients and 42.53±6.12 years in the control group. There was a significant difference between the mean ages of the groups (p<0.001). On the other hand, smoking, which is the most important risk factor for CAD was similarly distributed between the patient and control group (p=0.969).

The genotype and allele frequencies of the polymor-

Table 1. Genotype and allele frequencies of the PON1 Q192R (rs662, c.575A>G) polymorphism in patients with CAD and control subjects.

Genotype/Allele		CAD n (%)	Total Control n (%)	p value	CAD n (%)	Men Control n (%)	p value	CAD n (%)	Women Control n (%)	p value
	AA	49 (41.9)	49 (49.5)		28 (41.2)	24 (48)		21 (42.9)	25 (51)	
Genotyping	AG	57 (48.7)	36 (36.4)	0.756	32 (47.1)	15 (30)	0.800	25 (51)	21 (42.9)	0.503
	GG	11 (9.4)	14 (14.1)		8 (11.8)	11 (22)		3 (6.1)	3 (6.1)	
Recessive model	GG	11 (9.4)	14 (14.1)	0.592	8 (11.8)	11 (22)	0.381	3 (6.1)	3 (6.1)	0.840
	AA+AG	106 (90.6)	85 (85.9)		60 (88.3)	39 (78)		46 (93.9)	46 (93.9)	
Dominant model	AA	49 (41.9)	49 (49.5)	0.262	28 (41.2)	24 (48)	0.460	21 (42.9)	25 (51)	0.418
	AG+GG	68 (58.1)	50 (50.5)		40 (58.9)	26 (52)		28 (57.1)	24 (49)	
Additive model	AG	57 (48.7)	36 (36.4)	0.067	32 (47.1)	15 (30)	0.061	25 (51)	21 (42.9)	0.418
	AA+GG	60 (51.3)	63 (63.6)		36 (53)	35 (70)		24 (49)	28 (57.1)	
Allele	A	155 (66.2)	134 (67.7)	0.751	88 (64.7)	63 (63)	0.787	67 (68.3)	71 (72.4)	0.531
	G	79 (33.8)	64 (32.3)		48 (35.3)	37 (37)		31 (31.7)	27 (27.6)	

p<0.05 was considered a statistically significant difference. CAD, coronary artery disease; n, number of patients; rs, the accession number of the variant in the National Center for Biotechnology Information.

Table 2. Genotype and allele frequencies of the PON1 L55M (rs854560, c.163T>A) polymorphism in patients with CAD and control subjects.

Genotype/Allele		CAD n (%)	Control n (%)	p value	CAD n (%)	Men Control n (%)	p value	CAD n (%)	Women Control n (%)	p value
	TT	19 (16.2)	11 (11.1)		10 (14.7)	7 (14)		9 (18.4)	4 (8.2)	
Genotyping	TA	44 (37.6)	36 (36.4)	0.237	24 (35.3)	16 (32)	0.728	20 (40.8)	20 (40.8)	0.149
	AA	54 (46.2)	52 (52.5)		34 (50)	27 (54)		20 (40.8)	25 (51)	
Recessive model	AA	54 (46.2)	52 (52.5)	0.229	34 (50)	27 (54)	0.820	20 (40.8)	25 (51)	0.115
	TT+TA	63 (53.8)	47 (47.5)		34 (50)	23 (46)		29 (59.2)	24 (49)	
Dominant model	TT	19 (16.2)	11(11.1)	0.277	10 (14.7)	7 (14)	0.914	9 (18.4)	4 (8.2)	0.136
	TA+AA	98 (83.8)	88 (88.9)		58 (85.3)	43 (86)		40 (81.6)	45 (91.8)	
Additive model	TA	44 (37.6)	36 (36.4)	0.850	24 (35.3)	16 (32)	0.708	20 (40.8)	20 (40.8)	1.000
	TT+AA	73 (62.4)	63 (63.6)		44 (64.7)	34 (68)		29 (59.2)	29 (59.2)	
Allele	T	92 (37.7)	58 (29.3)	0.203	44 (32.4)	30 (30)	0.700	38 (38.8)	28 (28.6)	0.130
	A	152 (62.3)	140 (70.7)		92 (67.6)	70 (70)		60 (61.2)	70 (71.4)	

p<0.05 was considered a statistically significant difference. CAD, coronary artery disease; n, number of patients; rs, the accession number of the variant in the National Center for Biotechnology Information.

Table 3. Genotype and allele frequencies of the CYP3A4*1G (rs2242480, IVS10+12G>A) polymorphism in patients with CAD and control subjects.

Genotype/Allele		CAD n (%)	Total Control n (%)	p value	CAD n (%)	Men Control n (%)	p value	CAD n (%)	Women Control n (%)	p value
	GG	78 (66.7)	68 (68.7)		46 (67.6)	32 (64)		32 (65.3)	36 (73.5)	
Genotyping	GA	37 (31.6)	29 (29.3)	0.807	22 (32.4)	17 (34)	0.541	15 (30.6)	12 (24.5)	0.346
	AA	2 (1.7)	2 (2)		0 (0)	1 (2)		2 (4.1)	1 (2)	
Recessive model	AA	2 (1.7)	2 (2)	0.892	0 (0)	1 (2)	0.234	2 (4.1)	1 (2)	0.505
	GG+GA	115 (98.3)	97 (98)		68 (100)	49 (98)		47 (95.9)	48 (98)	
Dominant model	GG	78 (66.7)	68 (68.7)	0.751	46 (67.6)	32 (64)	0.679	32 (65.3)	36 (73.5)	0.380
	GA+AA	39 (33.3)	31 (31.3)		22 (32.4)	18 (36)		17 (34.7)	13 (26.5)	
Additive model	GA	37 (31.6)	29 (29.3)	0.710	22 (32.4)	17 (34)	0.850	15 (30.6)	12 (24.5)	0.497
	GG+AA	80 (68.4)	70 (70.7)		46 (67.6)	33 (66)		34 (69.4)	37 (75.5)	
Allele	G	193 (82.5)	165 (83.3)	0.814	114 (83.8)	81 (81)	0.571	79 (80.6)	84 (85.7)	0.339
	A	41(17.5)	33 (16.7)		22 (16.2)	19 (19)		19 (19.4)	14 (14.3)	

p<0.05 was considered a statistically significant difference. CAD, coronary artery disease; n, number of patients; rs, the accession number of the variant in the National Center for Biotechnology Information.

Table 4. Genotype and allele frequencies of the ITGB3 Leu33Pro (rs5918, c.176T>C) polymorphism in patients with CAD and control subjects.

Genotype/Allele		CAD n (%)	Total Control n (%)	p value	CAD n (%)	Men Control n (%)	p value	CAD n (%)	Women Control n (%)	p value
	TT	87 (77.7)	72 (72.7)		55 (80.9)	37 (74)		36 (73.5)	35 (71.4)	
Genotyping	TC	23 (20.5)	25 (25.3)	0.437	12 (17.6)	13 (26)	0.511	12 (24.5)	12 (24.5)	0.701
	CC	2 (1.8)	2 (2)		1 (1.5)	0 (0)		1 (2)	2 (4.1)	
Recessive model	CC	2 (1.8)	2 (2)	0.851	1 (1.5)	0 (0)	0.413	1 (2)	2 (4.1)	0.556
	TT+TC	110 (98.2)	97 (98)		67 (98.5)	50 (100)		48 (98)	47 (95.9)	
Dominant model	TT	87 (77.7)	72 (72.7)	0.404	55 (80.9)	37 (74)	0.372	36 (73.5)	35 (71.4)	0.821
	TC+CC	25 (22.3)	27 (27.3)		13 (19.1)	13 (26)		13 (26.5)	14 (28.6)	
Additive model	TC	23 (20.5)	25 (25.3)	0.414	12 (17.6)	13 (26)	0.272	12 (24.5)	12 (24.5)	1.000
	TT+CC	89 (79.5)	74 (74.7)		56 (82.4)	37 (74)		37 (75.5)	37 (75.5)	
Allele	T	197 (87.9)	169 (85.4)	0.433	122 (89.7)	87 (87)	0.518	84 (85.7)	82 (83.7)	0.691
	C	27 (12.1)	29 (14.6)		14 (10.3)	13 (13)		14 (14.3)	16 (16.3)	

p<0.05 was considered a statistically significant difference. CAD, coronary artery disease; n, number of patients; rs, the accession number of the variant in the National Center for Biotechnology Information.

phisms and their genotype distributions in the recessive, dominant and additive models were analyzed both in overall and when grouped by gender. The genotype frequencies of the PON1 Q192R, PON1 L55M, CYP3A4*1G and ITGB3 L33P polymorphisms did not show any significant difference between the CAD and control group in respect of the volunteers in total, females and males (Table 1, Table 2, Table 3 and Table 4, respectively). Additionally, there was also no statistical difference in the genotype distribution of the recessive, dominant and additive models of the polymorphism between the CAD and control group. Similarly, allele frequencies of the polymorphisms did not show a statistical difference between the groups.

Table 5. Haplotype analysis in patients with CAD and control subjects for the PON1 Q192R (rs662) and L55M (rs854560) polymorphisms.

Haplotypes*	Frequency in Control Group	Frequency in CAD Group	p value	OR (95% Confidence Interval)
QL	0.293	0.338	0.274	1.256 (0.834-1.892)
QM	0.384	0.320	0.196	0.770 (0.517-1.145)
RM	0.323	0.330	0.818	1.048 (0.700-1.571)

*Order of the polymorphisms is PON1 Q192R, PON1 L55M, Haplotypes with frequencies 0.03 were analyzed using the SHEsis software. p<0.05 was considered a statistically significant difference. CAD, coronary artery disease; rs, the accession number of the variant in the National Center for Biotechnology Information; OR, odds ratio.

Case-control analysis depending on the haplotypes was carried out for the PON1 Q192R and L55M polymorphisms. Haplotypes were generated with the usage of all combinations, which could be formed for the polymorphism alleles. Only frequencies of the RL haplotype were <0.03 in our study group, so this haplotype was excluded from the statistical assessment. The comparison of the haplotype frequencies between the CAD and control group did not reveal any significant difference (Table 5).

DISCUSSION AND CONCLUSION

Atherosclerosis underlying CAD is a complex and chronic pathophysiological process involves endothelial dysfunction, vascular remodeling, plaque formation, inflammation, leukocyte adhesion, platelet aggregation and thrombus formation.[9] Hypertension, hypercholesterolemia, diabetes, obesity and smoking are conventional risk factors, which might have the potential to contribute to these pathophysiological stages.[6] There are several case-control studies focused on the variants in the genes, which encode the proteins known for their role at the onset and progress of atherosclerosis.[1,10] However, these studies were designed without respecting the homogenization of the groups regarding the several environmental risk factors and the CAD family history of the subjects. A molecular genetics research, especially in the patients with a family history of CAD, will enable a reliable and correct determination of the genetic risk factors. Therefore, we conducted this study only on CAD patients with family history and healthy individuals without a family history of CAD. Besides, the healthy volunteers did not have hypertension, hypercholesterolemia, diabetes or obesity. The genetic variants contributing to the onset and progress of these risk factors were indirectly our target in the study.

PON1 Q192R and L55M are functional polymorphisms, which were commonly encountered in populations and investigated as the genetic predisposing factor in many diseases, especially in CAD. Association studies of these polymorphisms with CAD revealed several conflicting findings.[11] The strongest association was found in the PON1 Q192R polymorphism. The preventive role of the allele 192Q and the damaging effect of the allele 192R were demonstrated.[12-14] However, there are a remarkable number of studies in the literature, which did not confirm this finding.[15,16] Similarly, there are studies showing the protective effect of the allele PON1 55M against CAD,[17] but also studies resulted with the contrary findings.[15,18] The first researchers to evaluate the relationship between PON1 Q192R polymorphism and CAD in the Turkish population were Aynacıoğlu et al. Although Aynacıoğlu et al. suggested that there were no significant association,[19] Özkök et al. showed that PON1 Q192R and L55M polymorphism was correlated to CAD.[20] In a different study with Turkish subjects, it was claimed that PON1 L55M plays an important role in the progression of CAD.[21] We intended to contribute to this conflicting topic with our proposal of a new point of view in respect of a study focused on patients with a familial CAD history. We did not find any significant association between the PON1 Q192R and L55M polymorphisms and CAD regarding both the genotype and allele frequencies and dominant, recessive and additive models. The haplotype analysis of these polymorphisms revealed the same result. In a recently published study supporting our findings, no association was found between PON1 Q192R and L55M polymorphisms and coronary artery disease. Paszek et al. achieved this result in a study they conducted in 367 patients and 660 healthy individuals.[22] If the important role of the PON1 activity in the prevention of the lipoprotein oxidation is taken into the consideration, these results in our study bring the studies to mind, which demonstrated that feeding habits might affect this activity. A newly published meta-analysis study also revealed an interesting result, reporting that only the PON1 Q192R polymorphism is associated with CAD. When they performed the same analysis for the PON1 L55M polymorphism, they could not obtain a significant finding.[23]

There are only very few findings in the literature, which demonstrated the association between CYP3A4 and CAD. However, the important role of the CYP enzymes in the protection of the cardiovas-

cular health and in the disorders was well documented.[9] The importance of CYP3A4*1G polymorphism in vitamin D metabolism, which is an important protective agent in the treatment of cardiovascular diseases, has been demonstrated by studies.[24] In addition, a recently published microarray analysis study demonstrated that downregulation of the CYP3A4 gene, which is central to fatty acid metabolism, is associated with the pathogenesis of CAD.[25] He et al. suggested that CYP3A4*1G polymorphism increases the risk of CAD in their cohort study with 322 CAD patients and 306 healthy subjects.[6] Consideration of the sample size and the evaluation of the effect of CYP3A4*1G polymorphism on the enzyme function in their study, proves to be a remarkable finding. We intended to clarify this finding in a different patient population namely in the patients with a family history of CAD. Detailed genotype and allele frequency comparisons in our study revealed that the CYP3A4*1G polymorphism did not have any association with CAD.

Although, some remarkable preliminary studies were published displaying the relation between the ITGB3 Leu33Pro polymorphism and CAD, the subsequent studies did not confirm these findings.[7,26] In a study comparing angiographic findings with polymorphism genotypes, it was suggested that ITGB3 Leu33Pro polymorphism is not a risk factor for coronary atherosclerosis.[27] However, in a large-scale meta-analysis study that included 57 studies, it was suggested that it may be a significant risk factor for the development of acute coronary events in young people.[28] Our analysis of genotype and allele frequencies in overall and inheritance models did not reveal any significant difference between the ITGB3 Leu33Pro polymorphism and CAD. In a recently published study supporting our findings, ITGB3 Leu33Pro polymorphism was compared in Sudanese patients with atherosclerotic plaque and healthy individuals, and no significant results were obtained.[29] A newly published study found similar results to our findings when they analyzed the association of the same polymorphism in Iranian coronary artery patients.[30]

Although there are findings in the literature, which show that PON1 Q192R, PON1 L55M, CYP3A4*1G and ITGB3 L33P polymorphisms might be risk factors for CAD, our detailed statistical analysis did not display any significant association. Thus, our findings confirmed the GWAS studies, in which large-scale genome screening was carried out with the microarray technology and did not show PON1, CYP3A4 and ITGB3 genes as risk loci. The inclusion of only the patients with CAD history and control subject with no family history of CAD in our study is important in respect of our study's reliability. The present study was limited by the relatively small sample size and incompatible mean ages of the control and patient groups.

Ethics Committee Approval: The study protocol was approved by the Eskişehir Osmangazi University Clinical Trials Ethics Committee (Date: 28/02/2011, decision no: 2011/17). . All the participants were informed about the content of the study and written consent form was taken from all of them. The study protocol was designed in accordance with the Helsinki Ethical Principles and Declaration of Good Clinical Practices and carried out in accordance with these standards.

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

Author Contributions: Concept – FS, İD; Supervision–NA, HVG, İD, AB; Materials – FS, İD, CÖ; Data Collection and/or Processing – FS, AB, TU, MÖ, CÖ; Analysis and/or Interpretation – NA, HVG, İD, MÖ; Writing – FS, İD.

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