



Interleukin 28B rs12979860 CT, rs12980275 GA, rs8099917 GT and TT genotypes are the Predictors of Rapid Viral Response in Hepatitis C Virus-Infected Patients

Hepatit C Virüs Enfeksiyonu Olan Hastalarda İnterlökin 28B rs12979860 CT, rs12980275 GA, rs8099917 GT ve TT Genotipleri Hızlı Viral Yanıtın Göstergesidir

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ABSTRACT

Objective: In this study, the effects of genotypic differences on the clinical course of the disease, response to treatment and fibrosis were investigated in patients with hepatitis C virus (HCV) infection.

Materials and Methods: Ninety-nine chronic HCV-infected patients and 95 controls were enrolled. The patients received pegylated interferon (PegIFN) + ribavirin (RBV) for 48 weeks and followed up for the next 48 weeks. Aspartate aminotransferase/platelet ratio index was used to determine the stage of liver fibrosis. DNA specimens were extracted from peripheral blood mononuclear cells and the interleukin (IL) 28B gene rs12979860, rs12980275, and rs8099917 were genotyped by the immune polymerase chain reaction-restriction fragment length polymorphism method. Results were analysed using the SPSS 16.0 and OpenEpi 2.2 softwares.

Results: All patients had HCV genotype 1. Among the 99 HCV+ patients, in 26.3% spontaneous viral clearance, in 42.8% rapid viral response, 92% early viral response and in 72.6% sustained viral response was observed. The allele frequencies of IL28B single nucleotide polymorphisms (SNP), rs12979860, rs12980275, and rs8099917 were not identical in all samples (p<0.005). SNP rs12979860 CT genotype (p=0.010); rs12980275 GA genotype (p=0.010); and rs8099917 GT and TT genotypes (p=0.019 and 0.020, respectively) were strongly associated with rapid viral response in the overall sample.

Conclusion: The determination of IL28B polymorphisms may be useful to individualize treatment options when using PEG/RBV-based therapies for chronic HCV infection but genetic characteristics of populations of the countries must be known.

Keywords: Interleukin 28B, hepatitis C virus, single nucleotide polymorphisms, polymorphism, genotype

ÖZ

Amaç: Bu çalışmamızda hepatit C virüs (HCV) enfeksiyonu olan hastalarda genotipik farklılıkların hastalığın klinik gidişi, tedavi yanıtları ve fibrosis üzerine etkileri araştırılmıştır.

Gereç ve Yöntemler: Çalışmaya 99 kronik aktif HCV enfeksiyonu olan hasta ve 95 sağlıklı kontrol dahil edildi. Hastaların tümü 48 hafta boyunca pegile interferon (PegIFN) + ribavirin (RBV) tedavisi aldı ve tedavi sonu 48 hafta takip edildi. Karaciğer fibrosis evresi için aspartat aminotransferaz/platelet skoru kullanıldı. DNA örnekleri deneklerin periferik kan mononükleer hücrelerinden elde edildi ve immün polimeraz zincir reaksiyonu-restriksiyon parça uzunluk polimorfizmi yöntemiyle interlökin (IL) 28B rs12979860, rs12980275 ve rs8099917 genotiplendirmeleri yapıldı. Sonuçlar SPSS 16,0 ve OpenEpi 2,2 yazılımı ile analiz edildi.

Bulgular: Tüm hastalar HCV genotip 1 hastası idi. Çalışmaya alınan deneklerde IL28B tek nükleotid polimorfizmi (TNP) (rs12979860, rs12980275 ve rs8099917) dağılımı farklılık gösteriyordu (p<0,005). TNP rs12979860 CT genotipi (p=0,010); rs12980275 GA genotipi (p=0,010); ve rs8099917 GT ve TT genotipleri (p=0,019 ve 0,020 sırasıyla) ve hızlı viral yanıt arasında kuvvetli ilişkili bulundu.

Sonuç: Kronik HCV enfeksiyonu olan hastaların PegIFN+RBV ile tedavilerinin bireysel olarak belirlenmesinde IL28B polimorfizmlerinin bilinmesi faydalı olabilir. Fakat her ülkenin kendi genotipik karakteristiklerini bilmesi gereklidir.

Anahtar Kelimeler: İnterlökin 28B, hepatit C virüs, tek nükleotid polimorfizmi, polimorfizm, genotip

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Introduction

Hepatitis C virus (HCV) is the etiological factor for hepatitis C, which is one of the most important pathogenic factors of chronic liver diseases, cirrhosis and, even hepatocellular carcinoma. When infected with HCV, only a small proportion of patients clear the virus spontaneously and the majority develops chronic hepatitis C (CHC) (1). There are viral and host factors that are important in the development of chronic infection. Baseline viral load, rapid virologic response (RVR) and host characteristics (e.g. alcohol consumption, steatosis, liver fibrosis, metabolic syndrome, ethnicity, and host genetic polymorphisms) are the examples that have impact on chronicity (2).

Hepatocytes are the target cell of the virus. After infection, the innate immune system reacts to the virus and after 4 to 8 weeks, CD8+ T cells recognize viral peptides bound to human leukocyte antigen class 1 molecules on virus-infected hepatocytes. This initiates signaling pathways that lead to the synthesis of interferon (IFN) and a variety of other cytokines. IFN- λ 3 (λ 3) belongs to the type 3 IFN family (IFN- λ). IFN- λ is rapidly induced during HCV infection and has antiviral activity against HCV (3,4). The virus is eliminated during the acute phase of the infection by T cell-mediated antiviral mechanisms. The rate of spontaneous viral clearance in acute HCV infection is approximately 26% (range: 15%-40%) (5,6,7). In the remaining patients who do not defeat the virus at first glance, HCV persists for decades unless treated. Until recently, the effective treatment of chronic HCV infection includes pegylated interferon (PegIFN) and ribavirin (RBV) regimen (1). IFN, especially IFN- λ 3 interacts with its acceptor, a heterodimer [IFN- λ R1 x interleukin (IL)-10R2]. Even the most perfect therapeutic molecules (PegIFN+RBV) do not guarantee 100% efficacy and sustained virologic response (SVR) remains 40% (2,8).

There are variations that contribute to therapeutic success of HCV infection. Genotype of HCV is the most important parameter that has impact on treatment response. Genotype 1 is regarded as "difficult-to-treat" (2). According the HCV genotypes involved, SVR rates of genotypes 2, 3, 5 and 6 is 70%-90%, but it is less than 50% for genotypes 1 and 4 (1,9,10). Two postdoctoral thesis including 500 and 115 patients (11,12) conducted in Turkey revealed that HCV genotype 1b was the most common (81.7-90%), followed by genotype 1a (5.2-7.2%).

Besides HCV genotype, host genetic background could impact HCV infection, viral clearance, and treatment. Although studies demonstrated associations between cytokine gene polymorphisms and outcome of HCV infection, no general consensus has been reached, possibly due to differences between ethnic groups. Four recent studies (13,14,15,16) demonstrated that predictive role of single nucleotide polymorphisms (SNPs) of the IL28B locus was more likely to be associated with spontaneous viral clearance and treatment effectiveness of HCV in genotype 1 patients who were cured by PegIFN combined with RBV: IL28B rs12979860 C (good-response allele) versus T (poor-response allele) and rs809917 T (good-response allele) versus G (poor-response allele) showed the strongest association with SVR.

SNPs of the IL28B gene has been extensively described in the literature but allele frequencies, in particular rs809917, differs somewhat between world-wide populations (17,18,19,20). Therefore, the predictive power of SNPs may vary between

different cohorts. For example rs809917 was only a weak predictor of SVR in African-American patients (13). The aim of this study was to examine the prevalence and clinical significance of the outlined SNPs in a population from Turkey, a region with a high prevalence of HCV infection and a high prevalence of genotype 1b.

Materials and Methods

A total of 99 HCV-infected patients (26 spontaneous clearance and 73 chronic HCV genotype 1b patients) and 95 healthy control subjects were included in the study by Gaziosmanpaşa University Faculty of Medicine, Department of Infectious Diseases and Clinical Microbiology. CHC patients, who had received weekly injections of PegIFN department of Infectious Diseases and Clinical Microbiology. CHC patients, who had received weegIFN department of Infectious Diseases and Clinical Microbiology. CHC paegIFN department of Infectious Diseases and Clinical Microbio.5 μ g/kg body weight. Ribavirin was orally administered daily in two divided doses (1.000 mg for \leq 75 kg, 1.200 mg for $>$ 75 kg) (21).

Genomic DNA was extracted from blood samples using an Invitrogen Genomic DNA Isolation Mini Kit K1820-02 (Invitrogen Life Technologies, Carlsbad, CA, USA). Polymerase chain reaction (PCR) of rs12979860, rs12980275, and rs809917 polymorphisms of IL28B gene were performed in a total volume of 25 μ L, using 100 ng of genomic DNA with 20 pmol primers each (for rs12979860 F:5'-AGG GCC CCT AAC CTC TGC ACA GTC T-3', R: 5'-GCT GAG GGA CCG CTA CGT AAG TCA CC-3'; for rs12980275 F:5'-GAG AGC AAG AGG AGG GAA GGA A-3', R: 5'-GTG TGC CAT TAG CCA GTC AGA T-3'; and for rs809917 F:5'-TTC ACC ATC CTC CTC TCA TCC CTC AT -3', R: 5'-TCC TAA ATT GAC GGG CCA TCT GTT TC-3'), 0.2 mM each dNTP, 1X buffer, 2 mM MgCl₂ and 1 U Taq DNA polymerase (Invitrogen Life Technologies, Carlsbad, CA, USA). Cycling was performed in a Techne TC-4000 Thermal Cycler (Bibby Scientific Limited, Staffordshire, UK) as follows: amplification consisted of a 2-minute denaturation step at 94 °C; 40 cycles for 60 seconds at 94 °C, 40 seconds at 58 °C, 60 seconds at 72 °C and final extension of 7 minutes at 72 °C followed by cooling to 4 °C.

Genotype analysis of three IL28B gene loci (rs12979860, rs12980275 and rs809917) was conducted using restriction fragment length polymorphism for all three polymorphic loci. PCR products were digested with specific restriction enzymes: BstU I for rs12979860, Bsl I for rs12980275, and Mae III for rs809917. The digested PCR products were resolved by electrophoresis on 2.5% agarose gels containing 0.5 μ g/mL ethidium bromide. Restriction fragments were visualized with the use of a Vilber-Lourmat Gel Quantification and Documentation System QUANTUM-ST4 (Vilber Lourmat BP 66 Torcy, France).

Statistical Analysis

Statistical analysis was performed by SPSS 16.0 Software (SPSS Inc., Chicago, IL, USA). The distribution of IL28B gene polymorphisms between HCV patients and healthy controls and their deviations from Hardy-Weinberg equilibrium were compared by using the Fisher's exact chi-square test. A p value of less than 0.05 was considered statistically significant.

Odds ratios (ORs) and 95% confidence intervals (CIs) were used to determine the association of IL28B allelic and genotypic variants, compound genotypes and haplotypes with the occurrence of HCV disease were also calculated by Win PEPI version 11.39 software.

Results

Seventy-four patients completed treatment with PegIFN- α plus RBV (two patients could not receive the treatment because of the side effects). There was no significant difference between the patient and the control group in terms of age, gender, and viral genotype ($p>0.05$). Genotype and allele frequencies are given in Table 1. SNP *rs12979860* C allele (OR, 0.56; 95% CI, 0.37-0.83; $p<0.005$) and CC genotype (OR, 0.42; 95% CI, 0.23-0.77; $p<0.006$); *rs12980275* A allele (OR, 0.57; 95% CI, 0.38-0.87; $p<0.009$) and GG genotype (OR, 3.96; 95% CI, 1.41-11.12; $p<0.007$); *rs8099917*

T allele (OR, 0.56; 95% CI, 0.36-0.88; $p<0.014$) and TT genotype (OR, 0.50; 95% CI, 0.28-0.87; $p<0.022$) were strongly associated with the disease development compare to controls.

To evaluate the clinical applicability of individual SNPs, we calculated the predictive ORs for each SNP between rapid RVR, early virologic response, and SVR (Table 2, 3, 4). There were 25 patients who had spontaneous viral clearance. Rapid viral response was seen in 27 patients who had SNP *rs12979860* CT genotype ($p=0.010$), *rs12980275* GA genotype ($p=0.010$), and both *rs8099917* GT and TT genotypes ($p=0.019$, $p=0.020$, respectively)

Table 1. Genotype and allele frequencies of interleukin 28B gene loci among patients and control

Loci	Genotypes	Patients N (F)	Controls N (F)	p (OR, 95% CI)	Allels	Patients N (F)	Controls N (F)	p (OR, 95% CI)
rs12979860	CC	24 (0.2424)	42 (0.4330)	0.006(0.42, 0.23-0.77)	C	105 (0.5303)	130 (0.6701)	0.005 (0.56, 0.37-0.83)
	CT	57 (0.5758)	46 (0.4742)	0.198(1.50, 0.86-2.64)	T	105 (0.5303)	130(0.6701)	
	TT	18 (0.1818)	9 (0.0928)	0.097(2.17, 0.93-5.09)				
p for HWE		0.1212	0.4746					
rs12980275	AA	26 (0.2680)	37 (0.4022)	0.064 (0.54, 0.30-1.00)	A	105 (0.5412)	124 (0.6739)	0.009 (0.57, 0.38-0.87)
	GA	53 (0.5464)	50 (0.5435)	1.000 (1.01, 0.57-1.79)	G	89 (0.4588)	60 (0.3261)	
	GG	18 (0.1856)	5 (0.0543)	0.007 (3.96, 1.41-11.12)				
p for HWE		0.3234	0.0233					
rs8099917	TT	40 (0.4040)	56 (0.5773)	0.022 (0.50, 0.28-0.87)	T	129 (0.6515)	149 (0.7680)	0.014 (0.56, 0.36-0.88)
	GT	49 (0.4949)	37 (0.3814)	0.116 (1.59, 0.90-2.80)	G	129 (0.6515)	149 (0.7680)	
	GG	10 (0.1010)	4 (0.0412)	0.164 (2.61, 0.80-8.58)				
p for HWE		0.3706	0.4872					
N: Number, F: Frequency, OR: Odds ratio, CI: Confidence interval, HWE: Hardy Weinberg equilibrium								

Table 2. Genotype and allele frequencies of interleukin 28B- *rs12979860* for disease symptoms

CC	Genotypes		Alleles		
	CT	TT	C	T	
Patients	CHC (n=73)	14 (0.1918)	45 (0.6164)	14 (0.1918)	73 (0.5000)
	Carriers (n=25)	10 (0.4000)	11 (0.4400)	4 (0.1600)	31 (0.6200)
p	0.057	0.161	1.000	0.189	
OR, 95% CI	0.36, 0.13-0.94	2.05, 0.83-5.05	1.25, 0.38-4.12	0.61, 0.32-1018	
RVR	Yes (27)	9 (0.3333)	10 (0.3704)	8 (0.2963)	28 (0.5185)
	No (36)	4 (0.1111)	26 (0.7222)	6 (0.1667)	34 (0.4722)
p	0.060	0.010	0.240	0.719	
OR, 95% CI	3.75, 1.03-13.67	0.23, 0.08-0.65	2.11, 0.64-6.89	1.20, 0.60-2.43	
EVR	Yes (58)	13 (0.2241)	32 (0.5517)	13 (0.2241)	58 (0.5000)
	No (5)	0	4 (0.8000)	1 (0.2000)	4 (0.4000)
p	0.574	0.381	1.000	0.744	
OR, 95% CI	3.26, 0.22-49.08	0.31, 0.04-2.33	1.16, 0.15-9.01	1.50, 0.43-5.26	
SVR	Yes (45)	9 (0.2000)	26 (0.5778)	10 (0.2222)	44 (0.4889)
	No (17)	4 (0.2353)	9 (0.5294)	4 (0.2353)	17 (0.5000)
p	0.739	0.780	1.000	1.000	
OR, 95% CI	0.81, 0.22-3.00	1.22, 0.41-3.63	0.93, 0.26-3.37	0.96, 0.44-2.09	
OR: Odds ratio, CI: Confidence interval, CHC: Chronic hepatitis C, RVR: Rapid virological response, EVR: Early virological response, SVR: Sustained virological response					

predicted the most positive response to treatment outcome in the overall study population.

We did not find any difference between aspartate aminotransferase/platelet ratio index (APRI) and genotype frequencies.

Discussion

HCV infection is a major health problem worldwide. The virus is the main cause of chronic hepatitis and liver cirrhosis. Studies on entire viral genomes split HCV into seven major genotypes (22).

Table 3. Genotype and allele frequencies of interleukin 28B- *rs12980275* for disease symptoms

GG		Genotypes			Alleles	
		GA	AA	G	A	
Patients	CHC (n=72)	13 (0.1806)	42 (0.5833)	17 (0.2361)	68 (0.4722)	76 (0.5278)
	Carriers (n=24)	5 (0.2083)	10 (0.4167)	9 (0.3750)	20 (0.4167)	28 (0.5833)
p		0.768	0.166	0.196	0.616	
OR, 95% CI		0.84, 0.27-2.60	1.96, 0.78-4.92	0.52, 0.19-1.36	1.25, 0.65-2.41	
RVR	Yes (27)	8 (0.2963)	9 (0.3333)	10 (0.3704)	26 (0.4727)	29 (0.5273)
	No (35)	5 (0.1429)	24 (0.6857)	6 (0.1714)	34 (0.4857)	36 (0.5143)
p		0.209	0.010	0.089	1.000	
OR, 95% CI		2.53, 0.73-8.70	0.23, 0.08-0.66	2.84, 0.89-9.04	0.95, 0.47-1.91	
EVR	Yes (57)	12 (0.2105)	29 (0.5088)	16 (0.2807)	53 (0.4649)	61 (0.5351)
	No (5)	1 (0.2000)	4 (0.8000)	0	6 (0.6000)	4 (0.4000)
p		1.000	0.360	0.315	0.516	
OR, 95% CI		1.07, 0.4-8.36	0.26, 0.03-1.96	4.37, 0.29-65.25	0.58, 0.17-2.03	
SVR	Yes (44)	9 (0.2045)	24 (0.5455)	11 (0.2500)	42 (0.5385)	36 (0.4615)
	No (17)	4 (0.2353)	8 (0.4706)	5 (0.2941)	16 (0.4706)	18 (0.5294)
p		1.000	0.776	0.752	0.543	
OR, 95% CI		0.84, 0.23-3.09	1.35, 0.45-4.03	0.80, 0.24-2.70	1.31, 0.59-2.91	

OR: Odds ratio, CI: Confidence interval, CHC: Chronic hepatitis C, RVR: Rapid virological response, EVR: Early virological response, SVR: Sustained virological response

Table 4. Genotype and allele frequencies of interleukin 28B- *rs8099917* for disease symptoms

GG		Genotypes			Alleles	
		GT	TT	G	T	
Patients	CHC (n=73)	7 (0.0959)	42 (0.5753)	24 (0.3288)	56 (0.3836)	90 (0.6164)
	Carriers (n=25)	3 (0.1200)	7 (0.2800)	15 (0.6000)	13 (0.2600)	37 (0.7400)
p		0.712	0.019	0.020	0.126	
OR, 95% CI		0.78, 0.19-3.19	3.48, 1.32-9.21	0.33, 0.13-0.82	1.77, 0.87-3.60	
RVR	Yes (27)	3 (0.1111)	11 (0.4400)	13 (0.4815)	17 (0.3148)	37 (0.6852)
	No (36)	4 (0.1111)	23 (0.6389)	9 (0.2500)	31 (0.4306)	41 (0.5694)
p		1.000	0.190	0.067	0.200	
OR, 95% CI		1.00, 0.21-4.76	0.44, 0.16-1.24	2.79, 0.98-7.96	0.61, 0.29-1.27	
EVR	Yes (58)	7 (0.1207)	29 (0.5000)	22 (0.3793)	43 (0.3707)	73 (0.6293)
	No (5)	0	5 (1.0000)	0	5 (0.5000)	5 (0.5000)
p		1.000	0.056	0.153	0.503	
OR, 95% CI		1.60, 0.10-25.05	0.09, 0.01-1.34	6.78, 0.46-100.26	0.59, 0.17-2.02	
SVR	Yes (45)	5 (0.1111)	24 (0.5333)	16 (0.3556)	34 (0.3778)	56 (0.6222)
	No (17)	2 (0.1176)	10 (0.5882)	5 (0.2941)	14 (0.4118)	20 (0.5882)
p		1.000	0.780	0.768	0.837	
OR, 95% CI		0.94, 0.17-5.14	0.80, 0.27-2.41	1.32, 0.41-4.30	0.87, 0.39-1.92	

OR: Odds ratio, CI: Confidence interval, CHC: Chronic hepatitis C, RVR: Rapid virological response, EVR: Early virological response, SVR: Sustained virological response

The HCV genotype 1 is the most prevalent genotype worldwide (46% of all HCV cases), followed by genotype 3 (30%) but the distribution of these genotypes are different between countries (23).

Human hepatocytes are the primary target cell for HCV infection. The first line of immune defense comprises activation of innate immunity following HCV recognition. Local production of IFNs disrupts HCV genome replication and spreading in the liver parenchyma (24). The rate of the treatment of chronic HCV infection (SVR) varies under the influence of ethnicity. For example, it was found that patients of European ancestry were cured more successfully than patients of African ancestry (25).

Besides ethnicity, genetic polymorphism of certain genes influences treatment response. A cohort study with 1000 patients infected with HCV genotype 1 revealed that carrying the IL28B *rs12979860* CC genotype was associated with two-fold chance of SVR compared to TT genotype (13). Its effect has been shown in HCV+HIV co-infected patients as well (25,26). This CC genotype was also reported to be associated with a higher rate of spontaneous clearance in European and Asian populations (20,27). On the other hand, these significant SNPs observed in Europe and Asia were not strongly associated with Japanese population (17). Moreover, it was found that genomic ancestry did not interfere with therapy response among HCV genotype 1 patients with C/C genotype in a Brazilian study (28).

The frequency of homozygote genotype (*rs12979860* CC) is different among countries (29). It is found in 24.2% of the patient group and 43.3% of the control group in our study. In a German study, the *rs12979860* CC was 33.9% in genotype 1 and 49% in the control group, which is pretty much, same as in our study (30). When we compared the genotype frequencies between the each group, *rs12979860* CC, *rs12980275* GG, and *rs8099917* GT genotypes and *rs12979860* C, *rs12980275* A, and *rs8099917* T alleles were found to be higher in the patients but *rs12979860* CT (57.6%), *rs12980275* GA genotype (54.6%); and *rs8099917* GT genotypes were the most common genotypes and all were associated with RVR and the RVR was found to be the best indicator for treatment outcome (31).

The main focus of the present study was the importance of the SNP of the IL28B gene. However, not only the *rs12979860* CC variability may influence the treatment response but also the *rs12980275* and *rs8099917*. We wanted to bring out the importance of differences between variabilities among countries so that, focusing only the *rs12979860* CC genotype should not accurately identify those patients who would respond to the therapy and who would not need longer treatment period. Although our study revealed results consistent with that in many studies, the frequency of the *rs12979860* CC genotype has been found lower in HCV genotype 1 vs. genotype 2/3 patients in a German study (30). It was the same in a Spanish cohort: the CC genotype was overrepresented among patients infected with viral genotypes non-1 (66.7% versus 39.1% in patients) (32). In Taiwan, not the *rs12979860* but the *rs8099917* TT genotype had benefit from a shorter duration of combination therapy in HCV-1 patients (33). In Uzbekistan, SNP *rs8099917* was found the most predictive of outcome for Central Asians (18) and in Chile, all the three genotypes (the IL28B *rs12979860* CC, *rs12980275* AA and

rs8099917 TT) have been found frequent in patients with SVR compared to null responders (38%, 44% and 50% vs. 2%, 8.2% and 8.2%, respectively) (34). Two recent studies have failed to show such an association: status of IL-28B polymorphism neither affected nor had an impact on virologic response in France and Japan (35,36).

In this study, we also evaluated fibrosis. Although we did not search HAI and Ishak fibrosis scoring, based on APRI, there was no association observed in terms of fibrosis and IL28B polymorphism in this study. A study observed an association between IL28B and fibrosis progression in CHC patients with IL28B CC genotype had significantly higher portal inflammation (2.4 versus 2.2) and ALT levels (37).

Study Limitations

The study was conducted before the start of the use of new treatments.

Conclusion

The determination of IL28B polymorphisms may be useful to individualize treatment options when using Peg/RBV-based therapies for CHC but countries must know their population's genetic characteristics.

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Ethics

Ethics Committee Approval: The study were approved by the Gaziosmanpaşa University of Local Ethics Committee, Informed Consent: Consent form was filled out by all participants.

Peer-review: Externally and Internally peer-reviewed.

Authorship Contributions

Medical Practices: Aydın Rüstemoğlu, Özgür Günel, Concept: Aydın Rüstemoğlu, Özgür Günel, Didem Yalçın, Design: Özgür Günel, Didem Yalçın, Betül Çelik, Data Collection or Processing: Şener Barut, Ömer Ateş, Analysis or Interpretation: Didem Yalçın, Betül Çelik, Literature Search: Aydın Rüstemoğlu, Şener Barut, Ömer Ateş, Writing: Özgür Günel, Didem Yalçın, Betül Çelik.

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