How does scavenger receptor B1 polymorphism (rs4238001) affect the progression of Hepatitis C patients with persistent viral responses?

Osman Ozdogan[1], Zuhal Altintas[2]

[1]Mersin University, Faculty of Medicine, Department of Gastroenterology, Mersin, Turkey
[2]Mersin University, Faculty of Medicine, Department of Medicine Genetics, Mersin, Turkey

Abstract

Aim: Scavenger receptor B1 (SR-B1) is an important glycoprotein that plays an important role in the entry of hepatitis C virus (HCV) to hepatocyte. SR-B1 also acts as a modulator in many situations, such as both the natural and adaptive immune system. The effects of HCV on entry to hepatocyte and response to treatment in SR-B1 mutations were investigated in many studies performed before. We aimed to investigate the effects of SR-B1 polymorphism on liver in patients with noncirrhotic HCV who received pegylated-interferon + ribavirin treatment and had persistent viral response (SVR).

Material and Methods: We included naive, non-cirrhosis patients diagnosed with HCV in our clinic between 2008 and 2009. We monitored approximately 53 patients form whom we obtained SVR after treatment and followed up regularly for at least twice a year for ten years. We evaluated ultrasonography, laboratory parameters and calculated non-invasive fibrosis scores (APRI and FIB-4).

Results: A total of 91% of patients were genotype 1b and 9% were genotype 1a. 2 patients developed cirrhosis in 4th and 6th year of their follow-up. One of these patients had Wild type SR-B1 rs4238001 (Gly2Ser) (GG) genotype and the other had heterozygous SR-B1 (GA) genotype. We detected no cirrhosis in none of the other 51 patients. We found improvement in APRI and FIB4 scores during follow-up in patients with both wild type and heterozygous SR-B1 genotype. It was more pronounced in the heterozygous SR-B1 group (pretreatment and 10th year values, respectively 0.82±0.65, 0.38±0.24 for APRI, 1.79±1.12, 1.31±0.65 for FIB-4) (p< 0.05).

Conclusion: The improvement of noninvasive fibrosis scores of SR-B1 mutations in non-cirrhotic HCV patients with SVR was more prominent in the group with heterozygous mutations. Although no difference was detected between SR-B1 mutations in terms of risk of cirrhosis progression and HCC development, larger studies are needed in this direction.

Keywords: Hepatitis C virus (HCV); scavenger receptor; sustained virologic response (SVR)

INTRODUCTION

Scavenger receptors (SR) form a large family of glycoproteins with many different types of structures and involved in a wide range of biological functions. In a consensus report, SRs are typically defined as structures that function by signaling mechanisms that bind multiple ligands and often lead to degradation through endocytosis, phagocytosis, adhesion or destruction of harmful substances (1). Many SRs have been identified until this time and these are divided into eight subgroups (A-H). One of these is the SR-B1. SR-B1s, also called as CD36 and LIMPII analogous 1, CD36 antigen-like 1, SCARB1, are most commonly expressed by hepatocytes and steroidogenic cells. They are also found in a wide variety of tissues and cell types, such as arterial wall and macrophage, adipocyte, adrenal, ovarian and testicular Leydig cells (2,3). The main function of SR-B1s is to mediate the passage of lipids such as cholesteryl esters (CE) from HDL to circulating lipoproteins into cells, unlike the classical endocytic LDL receptor pathway. It also mediates the selective uptake of other lipid components of receptor-bound HDL particles, including triglycerides (TGs), phospholipids, and α-tocopherol, and facilitates free cholesterol flow from peripheral cells to HDL (4). Structurally, SR-B1 forms a hydrophobic tunnel in the plasma membrane that facilitates the selective cellular flow of certain molecules such as lipid molecules, vitamins, viruses and apoptotic cells (5). SR-B1 plays a role in the entry of Hepatitis C Virus (HCV) into hepatocytes with receptors such as claudin-1, occludin, CD81. This is a complicated pathway. In genetically humanized mice for HCV infection, it has been observed that HCV infection is

reduced in whom with SR-BI deficiency (6). SR-B1s are also involved in metabolic processes such as endothelial nitric oxide synthase activation, lymphocyte hemostasis, inflammation and regulation of autoimmunity, and sepsis (3,7).

In HCV infection, all-cause mortality, cirrhosis, and risk of hepatocellular carcinoma (HCC) are reduced in HCV patients who provide a persistent virological response (SVR) with interferon-based antiviral therapy (8,9). SVR was defined as absence of viremia 24 weeks after discontinuation of all antiviral drugs. The primary goal in HCV treatment is virologic response. It has been shown that progression of fibrosis decreases and sometimes regresses in HCV patients with SVR (10,11). Some patients with advanced liver disease should be followed as HCC and cirrhosis may develop despite SVR. In some studies, it was emphasized that cirrhosis or HCC may develop in the follow-up of nonsirotic HCV patients with SVR, although at a low rate (12,13).

Many factors have been discussed as predictors of fibrosis regression after antiviral treatment, and there is still no consistent method alternative to biopsy. Biopsy is an invasive procedure with serious complications. Several blood tests have been proposed as an alternative to liver biopsy to identify fibrosis or cirrhosis. Aspartate transaminase to Platelet Ratio Index (APRI), FIB-4 are a few of them. In a systematic review performed, the sensitivity and specificity of these values for showing significant fibrosis (Ishak stage ≥3) and cirrhosis for APRI> 0.55 (81%, 55%, respectively),> 1.55 (37%, 95% respectively), FIB -4 was found to be> 1.45 (64%, 68%, respectively) and > 3.25 (50%, 79%, respectively) (14). In some studies, it has been stated that patients can be followed with noninvasive tests (15,16). In some publications, it was stated that noninvasive tests cannot distinguish between the middle stage of fibrosis and their performance is not high (17, 18).

In previous studies investigating the relationship between HCV and SR-B1, it was focused that more on the entry of HCV into hepatocytes and investigated how HCV was affected by changes in SR-B1 level or gene polymorphisms. We investigated whether SR-B1 alleles had different effects on liver progression in non-cirrhotic HCV patients with SVR of whom we have evaluated by biopsy and treated. We used non-invasive fibrosis scores in the follow-up.

MATERIAL and METHODS

Approval of Local Ethics Committee
Mersin University Local ethics committee approved the pre-treatment stage (ethics committee no: B.30.2.MEÜ.0.01.00.00/1871). Written and verbal informed consent was obtained from the patients.

Study participants and design
HCV patients older than 18 years who did not have previous cirrhosis, had not previously received treatment, had not viral infection such as hepatitis B and HIV and accompanying primary biliary cholangitis, primary sclerosing cholangitis, chronic liver disease such as hemochromatosis and who applied to our clinic between 2008 and 2009 were included in the study. Patients who could not provide a permanent viral response (SVR), died due to extrahepatic reasons during follow-up and who did not come regularly were excluded from the study. These patients underwent biopsy and then received 48 weeks of pegylated interferon alpha (2a or 2b) + ribavirin (1000-1200mg) treatment. SR-B1 alleles were examined in the patients before treatment. Patients with negative HCV RNA levels were included in the study. Patients were evaluated for ultrasonography and laboratory tests, HCV RNA and alfafetoproetin levels at least twice a year. Patients suspected to have cirrhosis and HCC underwent MRI and endoscopy was performed for portal hypertension; patients who were followed for 10 years of regular follow-up were included in the study.

In our study, we analyzed 29 HCV patients who did not respond to treatment to determine whether SR-B1 rs4238001 (Gly2Ser) polymorphism plays a role in determining the response to treatment and compared with patients with SVR.

Blood samples were taken after fasting for at least 8 hours. Hemogram, biochemical and other tests were evaluated in the laboratory of our hospital. Homeostatic Model Assessment for Insulin Resistance (HOMA) was calculated as = fasting insulin (microU/L) x fasting glucose (mg(dL)/405. APRI and FIB-4 were calculated by the following formulas.

AST to Platelet Ratio Index (APRI)= ((AST level(IU/L) / AST(upper Limit of normal)(IU/L)) / Platelet Count (109/L)) X 100

FIB-4= (AST level(IU/L) x Age (years)) / (Platelet Count (109/L) X √ALT(IU/L))

HCV-RNA viral level was performed by "real time" polymerase chain reaction (RT-PCR) technique with COBAS TaqMan 48 (Roche Diagnostic, USA). HCV genotype determination was performed with the AMPLIQUALITY HCV-TS (AB Analitica, Italy) kit called "line probe assay" (LiPA).

Liver biopsies were performed in our clinic under the guidance of ultrasonography (16-gauge Hepafix needle) needle. Patients for whom at least 8 portal areas obtained were included in the study. Ishak scoring system [fibrosis stage (range, 0-6) and histology activity index (HAI) (range, 0-18)] were used for histopathological evaluation.

SR-B1 polymorphism

We used PCR-RFLP methods for the determination of SC-B1 Exon1, +4 G/A, rs4238001 gene polymorphisms in HCV patients and non-responders HCV. The blood samples were collected from HCV patients and control. The blood samples were placed into tubes containing 0.5M EDTA and were stored at -20°C until DNA extraction. DNA isolation The genomic DNA was extracted from whole

peripheral blood sample using the Miller et al., 1988 extraction protocol (19).

PCR (Polymerase Chain Reaction) was performed to determine the amplification of the 263 bp gene region in the SC-B1 gene Exon1, +4 G / A, rs4238001 (Gly2Ser) polymorphism. The sequences of oligonucleotide primers used to determine the polymorphism of +4 G / A, rs4238001 (Gly2Ser) are the previously described sequences (20).

Genotyping determination
DNA was extracted from peripheral blood leukocytes; PCR was performed in a 25-μL volume with 100 ng DNA, 100 μm dNTPs, 20 pmol of each primer, 1.5 mM MgCl2, 1 x PCR buffer with (NH4)2SO4 (Fermentas, Vilnius, Lithuania) and 2 U Taq DNA polymerase (Fermentas, Vilnius, Lithuania). Amplification was performed on an automated Thermal Cycler (Techne Flexigene, Cambridge, UK). These genetic polymorphisms were determined by fragment separation at 120 V for 40–50 minutes on a 3.5% agarose gel containing 0.5 mg/mL ethidium bromide. A 100-bp DNA Ladder (Fermentas Vilnius, Lithuania) was used as a size standard for each gel lane. The gel was visualised under UV light using a gel electrophoresis visualising system (Vilber Lourmat). PCR RFLP conditions for the SR-B1 (rs4238001), polymorphisms of the SR-B1 gene are shown in Table 1.

Statistical analysis
Mean and standard deviation are given as descriptive statistics for continuous structure parameters and number and percentage values are given for parameters in categorical structure. Chi-square analysis was used to test the relationships between categorical variables. Student t test was used to check whether there was a difference between the mean biomarker, APRI and FIB-4 scores of SR-B1GG and SR-B1 GA genotype groups. Repeated measurement variance analysis was used to check whether the mean values of APRI and FIB-4 scores differed between baseline and sequential measurements in each group. In addition, Error Bar graph was used for visual presentation.

RESULTS
53 Hepatitis C patients for whom SVR was obtained were included in the study. 28 (52.8%) of our patients aged between 23 and 66 years were female. All of our patients were genotype 1 and 91% of them were genotype 1b. 31 patients received pegylated interferon alfa 2a+ ribavirin and 22 patients received pegylated interferon alfa 2b + ribavirin treatment. There were stage 4 fibrosis in one patient and stage 3 fibrosis in 5 patients for whom we performed pre-treatment biopsy (according to ISHAK scoring system). While one patient had high hepatostetatosis, 8 had moderate hepatosteatosis (Table 2).

Table 1. PCR-RFLP conditions of DR4 gene polymorphisms				
SR-B1 gene	Primers	Anneling temperature	Restriction enzymes	PCR products
rs4238001 (Gly2Ser)	5'-CCGGCGATGGGG CATAAAACCACT-3' 5'-CGCCCAGCACAGCGC ACAGTAGC-3'	64°C	AluI	Allele G: 263bp Allele A:192bp, 71bp

Table 2. Rates of fibrosis and hepatostetatosis according to liver biopsy			
Fibrosis (stage)	Number of patients	Hepatosteatosis	Number of patients
0	10 (18.9%)	no (0%)	17 (32.2%)
1	23 (43.4%)	mild (1-33) %	27 (50.9%)
2	14 (26.4%)	moderate (34-66) %	8 (16%)
3	5 (9.4%)	high (67-100) %	1 (1.9%)
4	1 (1.9%)		

First, we investigated whether SR-B1 genotypes had an effect on treatment response rate. Therefore, we detected SR-B1 genotype status in 29 nonsirotic patients without SVR and compared them with patients with SVR. The proportion of patients with GA genotype was higher in the SVR group (p=0.033) (Table 3).

A total of 39 patients had SR-B1 GG genotype and 14 patients had SR-B1 GA genotype. There was no patient with SR-B1 AA genotype. When we evaluated patients with GG genotype and patients with GA genotype, pre-treatment age, sex, body mass index (BMI), fibrosis (stage), HCV RNA level, aspartate transaminase (AST), alanine

transaminase (ALT), and gamma glutamyl transpeptidase (GGT); there was no difference between fasting blood glucose (FBG), HOMA, lipid parameters and noninvasive fibrosis scores (p> 0.005) (Table 4).

Table 3. Comparison of SVR group and non-SVR group			
	group with SVR (n=53)	Group with Non-SVR (n=29)	P value
Age (years)	46.6 ± 9.6	53.9 ± 4.3	**0.0003**
Gender (female)	28 (52.8%)	19 (65.5%)	0.267
SR-B1 GG genotype	39 (73.6%)	27 (93.1%)	**0.033**
SR-B1 GA genotype	14 (26.4%)	2 (6.9%)	**0.033**
BMI	26.71 ± 4.33	26.31 ± 3.73	0.458
HCV RNA (10^3)IU/mL	1441 ± 3034	1595 ± 2481	0.329
HCT (%)	39.7 ± 4.8	40.2 ± 4.9	0.414
Plt (x 10^3/μL)	212 ± 60	188 ± 63	0.053
ALT (U/L)	68 ± 49	83 ± 53	0.103
AST (U/L)	55 ± 33	66 ± 42	0.089
GGT (U/L)	47 ± 53	58 ± 38	0.169
HOMA	3.53 ± 3.33	4.29 ± 3.54	0.117
APRI-0	0.814 ± 0.657	1.344 ± 1.529	0.282
FIB4-0	1.787 ± 1.122	2.401 ± 1.546	0.498

ALP. Alkaline Phosphatase; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; BMI: Body Mass Index; GGT: Gamma Glutamyl Transpeptidase; HCT: Hematocrit; Plt: Platelet; wbc: leukocyte

Table 4. Demographic and laboratory patient data (prior to treatment)			
	SR-B1 GG genotype (n=39)	SR-B1 GA genotype (n=14)	P value
Age (years)	45.6 ± 9.4	49.4 ± 10.1	0.208
Gender (female)	20 (51.3%)	8 (57.1%)	0.706
Fibrosis (stage)			
0	9 (23.1%)	1 (7.1%)	0.363
1	14 (35.9%)	9 (64.3%)	0.127
2	12 (30.7%)	2 (14.3%)	0.398
3	3 (7.7%)	2 (14.3%)	0.848
4	1 (2.6%)	0	-
BMI	26.46 ± 4.56	27.40 ± 3.68	0.489
HCV RNA (10^3) IU/mL	1256 ± 2514	2056 ± 4216	0.492
HCT (%)	39.5 ± 5.1	40.5 ± 3.9	0.506
Plt (x 10^3/μL)	217 ± 60	202 ± 64	0.446
ALT (U/L)	64 ± 46	80 ± 59	0.313
AST (U/L)	52 ± 31	63 ± 38	0.263
GGT (U/L)	51 ± 60	37 ± 24	0.525
FBG	100 ± 28	91 ± 15	0.115
Insulin	13.6 ± 7.5	13.8 ± 9.4	0.614
HOMA	3.64 ± 3.58	3.22 ± 2.63	0.402
TC	165 ± 46	170 ± 36	0.721
Triglyceride	124 ± 45	133 ± 61	0.643
HDL-C	47 ± 14	46 ± 10	0.754
LDL-C	93 ± 40	97 ± 32	0.759
APRI-0	0.727 ± 0.511	1.064 ± 0.935	0.246
FIB4-0	1.666 ± 1.079	2.124 ± 1.211	0.183

ALP. Alkaline Phosphatase; ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; BMI: Body Mass Index; FBG: Fasting Blood Glucose; GGT: Gamma Glutamyl Transpeptidase; HCT: Hematocrit; HDL: High-Hensity lipoprotein; LDL: low-Density lipoprotein; Plt: Platelet; TC: Total Cholesterol; wbc: leukocyte

While none of our patients developed hepatocellular carcinoma (HCC), cirrhosis developed in 2 patients. The first of these patients was a 63-year-old female with pre-treatment biopsy with stage 3 fibrosis and moderate hepatostetosis. The patient with SR-B1 GA genotype developed cirrhosis in 6th years of treatment and is now being followed up as compensated liver cirrhosis. The second patient was 58-year-old female with pre-treatment stage 4 fibrosis and moderate hepatosteatosis. This patient with SR-B1 GG developed cirrhosis at the 4th year of treatment. This patient is currently being followed up for compensated liver cirrhosis.

In the study, where we followed each patient for a total of 10 years, we calculated and evaluated the APRI and FIB-4 of our patients every year. We found that these noninvasive fibrosis scores improved statistically in both SR-B1 GG genotype group and SR-B1 GA genotype group (p <0.05) (Table 5, Figures 1, 2). The decrease in the SR-B1 GA genotype group was more evident than in the SR-B1 GG genotype group (P< 0.05).

Table 5. Change of noninvasive fibrosis scores over the years							
APRI	SR-B1 GG genotype (n=39)	SR-B1 GA genotype (n=14)	p value	FIB-4	SR-B1 GG genotype (n=39)	SR-B1 GA genotype (n=14)	p value
APRI-0	0.73±0.51	1.06±0.93	0.246	FIB4-0	1.67±1.08	2.13±1.21	0.183
APRI-1	0.63±0.44	0.81±0.45	**0.048**	FIB4-1	1.51±0.85	1.95±0.97	0.088
APRI-2	0.55±0.35	0.73±0.48	0.276	FIB4-2	1.47±0.71	1.80±0.78	0.141
APRI-3	0.48±0.27	0.63±0.38	0.241	FIB4-3	1.38±0.69	1.53±0.74	0.190
APRI-4	0.46±0.23	0.58±0.39	0.479	FIB4-4	1.39±0.68	1.44±0.72	0.414
APRI-5	0.45±0.26	0.52±0.26	0.299	FIB4-5	1.39±0.69	1.47±0.82	0.586
APRI-6	0.43±0.26	0.43±0.24	0.896	FIB4-6	1.37±0.67	1.41±0.68	0.614
APRI-7	0.38±0.20	0.39±0.23	0.747	FIB4-7	1.31±0.65	1.37±0.64	0.380
APRI-8	0.36±0.19	0.45±0.22	0.090	FIB4-8	1.28±0.62	1.31±0.69	0.968
APRI-9	0.37±0.24	0.41±0.21	0.455	FIB4-9	1.34±0.67	1.25±0.62	0.694
P value	**0.030**	**<0.001**			**0.027**	**0.029**	
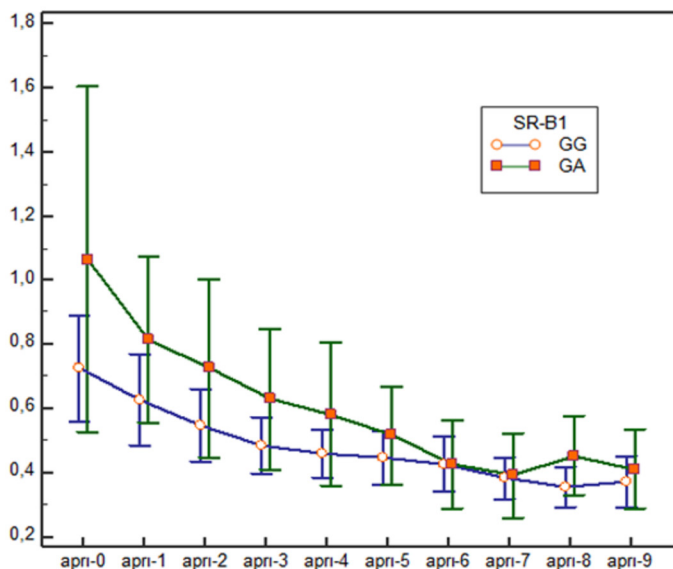
Figure 1. Evaluation of APRI scores by years
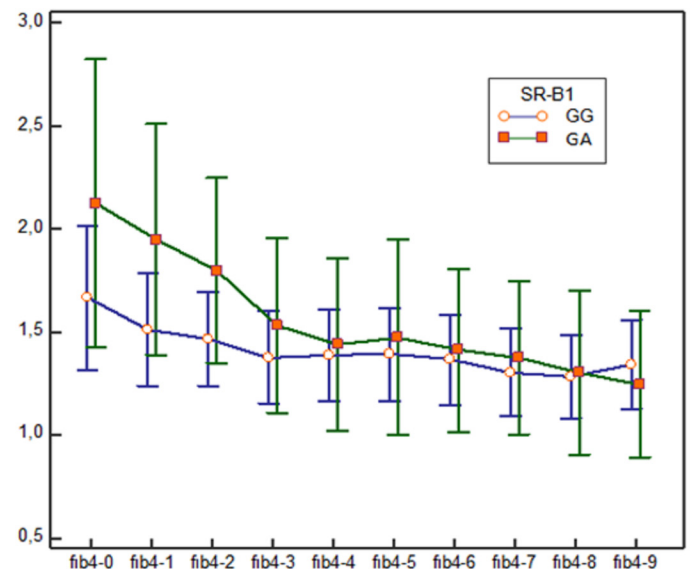
Figure 2. Evaluation of FIB-4 scores by years

DISCUSSION

SR-B1s are particularly one of the receptors involved in the entry of HCV into hepatocytes. SR-B1 is also known to be involved in many processes such as lymphocyte hemostasis, inflammation regulation, nonopsonic phagocytosis of pathogens and apoptotic cells (3). It has been shown in different studies that those who have single nucleotide polymorphisms (SNP) in different genes in SR-B1 may play a role in reducing cell entry of HCV (21, 22). In the study conducted by Hsu CS et al. (23), it wa shown that SCARB1 rs10846744 GG genotype may be a negative therapeutic factor for HCV patients taking pegylated interferon plus ribavirin treatment. In addition, previous studies performed in mice have shown that antibodies against SR-BI can prevent and/or reduce the spread and entry of HCV infection into hepatoma (24, 25). In the first part of our study, we compared 53 patients who received SVR with interferon treatment and 29 patients who did not respond to HCV treatment. Here, we found that unresponsive patients had higher levels of wild-type SR-B1 GG genotype. SC-B1 gene Exon 1.4 G / A rs4238001 (Gly2Ser) GG genotype was interpreted as probable HCV facilitating hepatocyte entry and reducing response to treatment.

One of the most important causes of liver cirrhosis and HCC is Hepatitis C. When hepatitis C is eradicated, the risk of developing cirrhosis and HCC is significantly reduced. In previous studies performed, it has been shown in previous publications that the risk of HCC development may continue even if the risk of HCC is decreased in HCV-induced cirrhosis patients with SVR (8, 9, 26). However, there are controversial results about whether cirrhosis progression and HCC develops in the follow-up of non-cirrhotic patients with SVR. Some studies investigating the follow-up of non-cirrhotic patients with SVR with pegylated interferon + ribavirin treatment have suggested that cirrhosis progression and HCC may develop (12, 13, 27- 29). In these studies, it was seen that the probability of developing cirrhosis varies between 1-10%. While no HCC was detected in any of our 10-year follow-up patients, but progressive cirrhosis was monitored in only 2 (3.7%) of them. In these two patients, fibrosis stage was significant (stage 3 and 4; according to ISHAK scoring system) and moderate hepatostetaosis was present in the pre-treatment biopsy. The reason for progression of cirrhosis in these two patients was interpreted as the possibility that fibrosis values were significant before treatment and that steatohepatitis may have developed over time. It has been emphasized in non-hepatitis C causes in patients with fibrosis progression, HCC and cirrhosis developing non cirrhotic patients with SVR (12,29). We could not determine whether SR-B1 genotypes would have an effect on progression to cirrhosis due to the small number of patients in this study. In the literature, we could not find a study examining the effects of SR-B1 polymorphism on cirrhosis progression in hepatitis C patients.

In our 10-years follow-up study that we evaluated 53 patients, we found significant improvement in noninvasive-fibrosis scores (APRI and FIB-4) and this improvement was more evident in patients with SR-B1 rs4238001 (Gly2Ser) GA genotype. In a study from Taiwan (12), the researchers followed a mean of 93 months for 31 patients with SVR with a METAVIR score of 2.35 (25.9% of patients had advanced fibrosis (F4, F3: 19.4, 6.5%)). Fibrosis regression was observed in 19% of patients, stable condition was seen in 45% of patients, and progression of liver disease was evaluated in 36% of patients with liver biopsy. 71% improvement in inflammation was found here. In another study (30) involving 150 patients with SVR who underwent a 5-year follow-up, biopsy was performed before and at the end of 5 years and they found fibrosis scores or improvement in inflammation in more than 90%. In our study, we used noninvasive fibrosis scores and these results showed similar studies that fibrosis also improved. In our study, we found a more significant improvement in fibrosis score in patients with SR- B1 rs4238001 (Gly2Ser) GA genotype. We could not find any study in the literature that examined the relationship between scavenger receptor fibrosis similar to our study. We think that large volume studies are needed to be performed in this direction.

One of the important limitations of our study is the low number of patients included in the study. In addition, the absence of cirrhotic patients in the study, non-follow-up of non-SVR patients, the lack of evaluation of patients with direct-acting oral antiviral agents are our shortcomings.

CONCLUSION

As a result; we followed non-cirrhotic patients receiving pegileinterferon + ribavirin and developing SVR fo 10 years. We evaluated cirrhosis progression, HCC development, changes in noninvasive fibrosis scores (APRI and FIB-4) in these patients and their relationship with SR-B1 rs4238001 (Gly2Ser) polymorphism. We found a significant improvement in noninvasive fibrosis scores and this was evident in patients with SR-B1 rs4238001 (Gly2Ser) GA genotype. We detected 3.7% cirrhosis progression, but we could not detect its association with the SR-B1 rs4238001 (Gly2Ser) polymorphism. Further studies are needed to determine whether scavenger receptors may be a useful value in the follow-up of HCV patients with SVR.

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