The mutations in ISDR of NS5A gene are not associated with response to interferon treatment in Turkish patients with chronic hepatitis C virus genotype lb infection

Hepatic C genotip 1b ile infekte Türk hastaların interferon tedavisine verdiği cevap NS5A geninin ISDR bölgesindeki mutasyonlarla ilişkili değildir

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Background/aims: *A significant association between variations in amino acid sequences resides between 2209-2248 nucleotides of HCV non-structural 5A (NS5A) gene, and response to interferon treatment has been proposed. The aim of this study was to determine whether the amino acid sequence changes in ISDR could be correlated to response to alpha interferon treatment in Turkish patients infected with HCV genotypes lb and la.* ***Methods:*** *Thirty-nine patients with chronic C virus infection (35 and 4 patients with genotype lb and la, respectively), receiving 3x3-5 MU of interferon α-2b for six months were included in the study. Following PCR amplification of the region from pre-treatment serum samples, the products were directly sequenced. The amino acid sequence of NS5A was compared with the published sequence for HCV-J (AA 2209-2248). Mutant type was defined as three or more amino acid mutations, and intermediate type as 1-3 amino acids in this region. Otherwise, they were defined as the wild type (no amino acid mutations). HCV RNA serum viremia levels were analyzed by branched DNA assay.* ***Results:*** *Eighteen patients were responders (R; 46%), whereas 21 patients were non-responders (NR; 54%). Amino acid changes in both R and NR groups did not show significant difference. Intermediate or wild type strains were detected in both groups.* ***Conclusions:*** *In this study, we could not determine a significant association between number of amino acid changes in $NS5A_{2209-2248}$ and response to interferon treatment. In the majority of the patients, it seems that amino acid sequences in this region are well conserved.*

Key words: Hepatic B, NS5A, interferon, treatment, Turkey, genotype 1b

Amaç: HCV-NS5A geni (2209-2248 nt) üzerindeki aa değişimleri ile interferon tedavisine yanıt arasında bir ilişki olduğu rapor edilmiştir. Bu çalışmamızın amacı HCV genotip lb ile enfekte Türk hastalarının IFN tedavisine verdikleri yanıtla ISDR bölgesindeki aa değişimleri arasında bir ilişki olup olmadığını incelemektedir. Yöntem: Çalışma grubumuzda 6 ay boyunca 3x3-5 MIU interferon α 2b tedavisi alan 39 kronik C hastası (35:1b, 4:1a) bulunmaktadır. Tedavi öncesi serum örneklerinden yapılan PZR amplifikasyonları sonrası sekanslar gerçekleştirildi. Mutant tiplerde 4 aadan faza mutasyonlar tespit edilirken, orta tiplerde 1-3 aa mutasyon bulunmaktadır. Yaban tipte ise hiçbir aa değişimi yoktur. HCV-RNA serum virüs düzeyleri bDNA yöntemi ile analiz edildi. ***Bulgular:*** *Onsekiz hasta tedaviye yanıt verirken (%46), 21 hastada yanıt alınamamıştır(%54). Aminoasit değişimleri bu iki hasta grubunda önemli bir farklılık göstermemiştir. İki grupta da orta ve yaban tip mutasyon türleri gözlenmiştir. Sonuç: Bu çalışmada IFN'a cevap ile $NS5A_{2209-2248}$ bögesindeki aa değişimleri arasında bir ilişki bulunamamıştır. Hastaların büyük bir kısmında aa dizileri korunmuştuk göstermektedir.*

Anahtar Kelimeler: Hepatit C, NS5A, interferon, tedavi, Türkiye, genotip 1b

INTRODUCTION

Hepatitis C virus (HCV) is a major cause of chronic viral hepatitis, cirrhosis and hepatocellular carcinoma (1). Interferon (IFN) was the only available treatment until a nucleoside analogue ribavirine was recently approved for the treatment of chronic hepatitis C virus infection as well (2-6). Several viral factors, including high pretreatment virus load, genotype lb, advanced histological

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changes and a high degree of amino acid substitutions of hypervariable regions have been suggested to predict a poor response to antiviral IFN therapy (7-9).

In addition, the HCV nonstructural 5A (NS5A) protein has been implicated in HCV antiviral resistance in many studies. Amino acid substitutions in the NS5A 2209-2248 region, termed interferon sensitivity-determining region (ISDR), were found to be related to response to IFN therapy in Japanese patients (10-11). It was further clarified that the double-stranded-RNA-activated protein kinase (PKR), which causes phosphorylation of a eucaryotic translation initiation factor, is involved in the mediation of IFN non-response. The mutations within the PKR-binding domain, which comprise the NS5A 2209-2248 region, may abolish the binding ability of ISDR to PKR (12-16). Supporting this, the number of mutations in the ISDR was found to be higher in patients with sustained response than in patients with transient or no response in studies from Japan (10,11,17-19). On the contrary, several other studies from the United States and Europe could not confirm a correlation between ISDR sequence and response to IFN (20-24).

A similar discrepancy between Japanese studies and the studies from Western Europe and the united stated also exists in the results of therapeutic trials of IFN. The mutant type ISDR sequence has been reported to be less common in European than in Japanese patients. A possible explanation suggested for the contradictory results in different geographical regions is that the mutant ISDR sequence may be too low in frequency in the European population for meaningful analysis (25). It is thus imperative to extend studies on the NS5A region in the context of its effect on IFN response to different geographical regions. We therefore decided to analyze the relationship between the amino acid (aa) mutations in the NS5A 2209-2248 region and response to IFN-α in Turkish patients with chronic hepatitis C.

MATERIALS AND METHODS

Patients

Serum samples of 39 native patients (22 female, 17 male; mean age, 50±10 years) with histopathologically proven chronic C hepatitis who received 3-5 MIU/TIW (total dose 216 MIU) IFN-α-2b for six months were enrolled in this study. The patients were all positive for anti-HCV on a third generation microparticle enzyme immunoassay but negative for HBsAg, anti-HBc and Anti-HIV (Abbott Laboratories, North Chicago, Illinois, US). Pre-treatment sera obtained from the patients were stored at -80°C. Patients having bDNA levels lower and higher than 0.2 mEq/ml at the end of therapy were considered responder (R) and nonresponder (NR) patients, respectively. The study protocol conformed to the ethical guidelines of the 1995 Declaration of Helsinki and was approved by the ethics committee of the University of Ankara Medical School.

RNA Extraction and RT PCR

RNA was extracted from serum samples by the acid-guanidinium-phenol-chloroform method (26). Obtained RNA was stored at -80°C until further use. The RNA pellet was reverse transcribed to complementary DNA (cDNA) using random hexanucleotide mix, dNTP and 10 units of AMV RT in a final volume of 20 uL. This mixture was incubated in a thermal cycler at 42°C for 1 hour.

Amplification of HCV $NS5A_{2209-2248}$

The NS5A sequence of HCV was amplified by reverse transcription polymerase chain reaction (RT-PCR). The first round of PCR was performed using external sense (5' CAGTGCTCACTTCCATGCTCA 3') and antisense primers (5' ACGGATATTTCCCTCTCA TCC 3'). After an initial denaturation step at 950C for 5 minutes, 30 cycles at 950C for 30 sec, at 550C for 40 sec and at 720C for 60 sec were performed. In the second round of the nested PCR, 35 cycles using internal sense (5' ACCCCTCCCACAT TACAGCAG 3') and antisense primers (5' CCGAAAGCGGATCGAAAGAGTCCA3') were performed as described previously (20). The second PCR products were analyzed on a 2% agarose gel stained with ethidium bromide, and the expected length (241 base pair) was confirmed.

Amplification of HCV 5' Non-Coding Region:

Five microliters of the cDNA mixture were added to PCR mixture containing 5 pmol of primers derived from the 5' non-coding region of the virus. The first round of PCR was performed using external sense and antisense primers (5' ATACTCGAAGGTG CAGTCTACGAGACCT 3'; 5' CTGTGAGGAACTACTGTCTT 3'). A thermal cycler program with 30 cycles, following initial denaturing at 94°C for 5 minutes, denaturing at 94°oC for 1 minute, annealing at 60°C for 1 minute, extension at 72°C for 2 minutes, and followed by 7 minutes final ex-

tension at 72°C, was performed. In the second round of the nested PCR, the same cycle program was performed using internal sense and antisense primers (5'CTGTGAGGAACTTCTG TCTT 3' ;5' TTCACGCAGAACGTCTAG 3'). The second PCR products were analyzed on a 2% agarose gel stained with ethidium bromide, and the expected 256 base pair length was confirmed (27).

HCV Genotyping:

Amplified RT-PCR products from the 5' non-coding region were used for restriction fragment length pattern analysis. Secondary PCR products were double digested with the restriction enzyme pairs Haelll and Rsal, and Mval and Hinfl. The contents of each digest were 20 uL PCR products, 2.5 (uL of 10X enzyme buffer and 10 units of each enzyme. Reactions were incubated at 37°C for 4-16 hours. Subtypes la/b were distinguished by incubating the mixture at 60°C overnight using the restriction enzyme BstUI. Digestion products were visualized under UV light after electrophoresis through an ethidium bromide stained 6% polyacrylamide gel in IX Tris-borate-EDTA buffer at 200 V for 4 hours (27).

Quantitative Detection of Serum HCV RNA

The levels of HCV RNA were determined by bDNA assay (Quantiplex, Bayer, US). The cut-off value of the assay was 0.2 HCV RNA mEq/ml.

Direct Sequencing of PCR Product

The amplified PCR products were sequenced by cycle sequencing method (fmol; Promega, US) and bands were visualized by silver staining. The resulting aa sequences were compared with the $NS5A_{2209-2248}$ sequence identified in NS5A-CR for genotype 1b isolates and in HCV-K1-R2 for la isolates (NIH.GOV.NEW GENBANK, DDBJ/EMBL /GENBANK).

Statistical Analysis:

$\%^2$ and Fisher's exact test were used where appropriate. A p value of 0.05 or less was considered to represent statistical significance.

RESULTS

Thirty-five patients had genotype lb and four patients had genotype la infection. There were no statistically significant differences between responders and non-responders with regard to age, sex, basal bDNA levels, basal ALT levels, total aa changes, and wild/intermediate aa change rates ($p>0.05$) (Table 1).

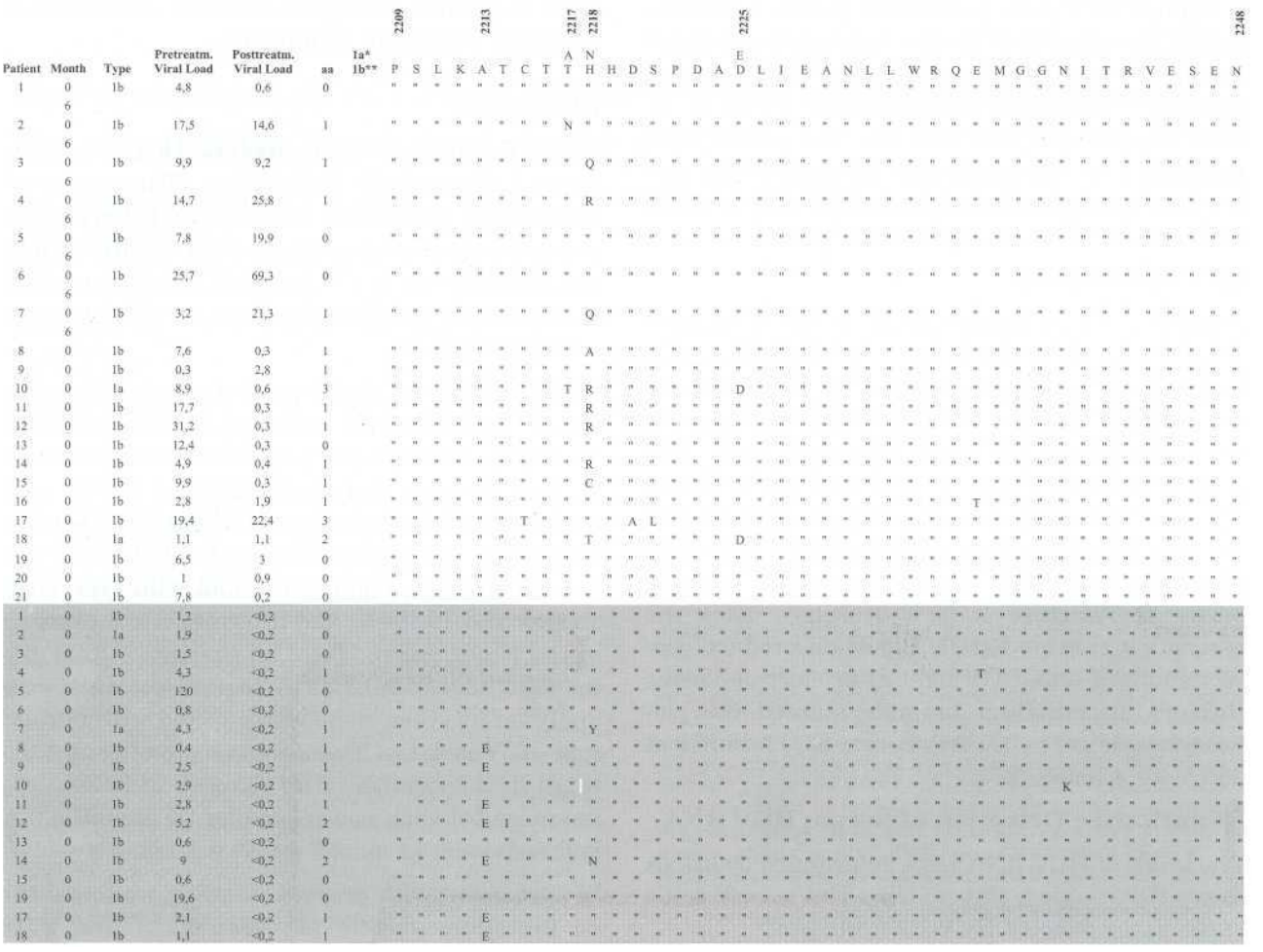
Eighteen (46%) of the 39 patients were responders and 21 (54%) patients were non-responders. Fourteen (67%) of the 21 NR patients had intermediate (1-3) aa changes and seven (33%) of the 21 patients had wild type sequences (Figure 1). Mean aa changes in NR patients were 0.9 ± 0.2 aa. In responder patients, eight (44%) had wild type virus strains and 10 (56%) had intermediate mutations in the NS5A region. Total aa changes in this group were only 0.7±0.2 aa. When all patients were analyzed together, none was infected with mutant type HCV isolates. There was no significant difference in the number of aa changes in $NS5A_{2209-2248}$ sequence between non-responder (D aa: 0.9 ± 0.2) and responder (A aa: 0.7 ± 0.2) patients.

In agreement with previous studies, we found the aa mutations mostly at codon 2213 and 2218 (H_{2218}->R) (10). However, we detected a specific aa substitution pattern in R patients at codon 2213. Of the 18 NR patients, seven had an alanine to glutamic acid change at codon 2213, whereas this variation was not observed in any of the R patients. In addition, the mutation at codon 2218 was determined more in NR patients at codon 2218 (10/21; 48%) than in R patients (2/18; 11%).

The sequence of the ISDR in HCV genotype lb or genotype la infection might change during IFN therapy. To examine this issue, we analyzed pre-treatment and post-treatment ISDR sequences in

Table 1. Demographic and clinical characteristics of patients with and without response to interferon treatment (R: responders to interferon treatment; NR: non-responders to interferon treatment; NS: not significant; aa: amino acid)

	R (n: 18) 46 %	NR (n: 21) 54 %	P value
Age (Years; mean ± SD)	47 ±8	52 ± 12	NS
Sex (Male/Female)	11/7	11/10	NS
Genotype (lb/la)	16/2	19/2	NS
Pre-treatment ALT (IU/L)	103± 50	120± 69	NS
Pre-treatment viral load (mEq/ ml)	9.5± 27.1	10.2± 8.2	NS
Post-treatment viral load (mEq/ml)	<0.2	9.7 ± 16.6	>0.01
Wild/intermediate A aa	8/10	0.7± 0.2	7/14
Total A aa	0.9 ± 0.2	NS	NS
Total IFN dosage (MIU)	216	216	NS



Patient	Month	Type	Pretreatm. Viral Load	Posttreatm. Viral Load	aa	Ia* Ib**	2209 P	S	L	K	2213 A	T	C	T	2217 A T	2218 N H	H	D	S	P	D	A	2225 E D	L	I	E	A	N	L	L	W	R	Q	E	M	G	G	N	I	T	R	V	E	S	E	2248 N
1	0	1b	4,8	0,6	0		"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
	6																																															
2	0	1b	17,5	14,6	1		"	"	"	"	"	"	"	"	N	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
	6																																															
3	0	1b	9,9	9,2	1		"	"	"	"	"	"	"	"	"	Q	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
	6																																															
4	0	1b	14,7	25,8	1		"	"	"	"	"	"	"	"	"	R	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
	6																																															
5	0	1b	7,8	19,9	0		"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
	6																																															
6	0	1b	25,7	69,3	0		"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
	6																																															
7	0	1b	3,2	21,3	1		"	"	"	"	"	"	"	"	"	Q	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
	6																																															
8	0	1b	7,6	0,3	1		"	"	"	"	"	"	"	"	"	A	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
9	0	1b	0,3	2,8	1		"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
10	0	1a	8,9	0,6	3		"	"	"	"	"	"	"	"	T	R	"	"	"	"	"	"	D	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
11	0	1b	17,7	0,3	1		"	"	"	"	"	"	"	"	"	R	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
12	0	1b	31,2	0,3	1		"	"	"	"	"	"	"	"	"	R	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
13	0	1b	12,4	0,3	0		"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
14	0	1b	4,9	0,4	1		"	"	"	"	"	"	"	"	"	R	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
15	0	1b	9,9	0,3	1		"	"	"	"	"	"	"	"	"	C	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
16	0	1b	2,8	1,9	1		"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	T	"	"	"	"	"	"	"	"	"	"	"	"	"
17	0	1b	19,4	22,4	3		"	"	"	"	"	"	T	"	"	"	"	"	A	L	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
18	0	1a	1,1	1,1	2		"	"	"	"	"	"	"	"	"	T	"	"	"	"	"	"	D	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
19	0	1b	6,5	3	0		"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
20	0	1b	1	0,9	0		"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
21	0	1b	7,8	0,2	0		"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
1	0	1b	1,2	<0,2	0		"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
2	0	1a	1,9	<0,2	0		"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
3	0	1b	1,5	<0,2	0		"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
4	0	1b	4,3	<0,2	1		"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	T	"	"	"	"	"	"	"	"	"	"	"	"	"
5	0	1b	120	<0,2	0		"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
6	0	1b	0,8	<0,2	0		"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
7	0	1a	4,3	<0,2	1		"	"	"	"	"	"	"	"	"	Y	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
8	0	1b	0,4	<0,2	1		"	"	"	"	E	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
9	0	1b	2,5	<0,2	1		"	"	"	"	E	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
10	0	1b	2,9	<0,2	1		"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	K	"	"	"	"	"	"	"
11	0	1b	2,8	<0,2	1		"	"	"	"	E	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
12	0	1b	5,2	<0,2	2		"	"	"	"	E	I	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
13	0	1b	0,6	<0,2	0		"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
14	0	1b	9	<0,2	2		"	"	"	"	E	"	"	"	"	N	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
15	0	1b	0,6	<0,2	0		"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
19	0	1b	19,6	<0,2	0		"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
17	0	1b	2,1	<0,2	1		"	"	"	"	E	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"
18	0	1b	1,1	<0,2	1		"	"	"	"	E	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"	"

Figure 1. Pre-treatment amino acid sequences of NS5A2209-2248 of all patients. Dark shaded area shows the data of responder patients. Reference sequences are NS5A-CR for genotype lb isolates and HCV-K1-R2 for la isolates (GENBANK)

seven NR patients. All of these patients were infected with genotype lb. No aa change was observed for five of the seven analyzed. Amino acid changes were observed in only two patients (Patients 5, 6). The aa changes were mainly at codon 2218 (H_{2218}->R). In this group pre-treatment total aa changes were 0.57±0.53, and post-treatment total aa changes were only 1.6± 0.9. Pre-treatment viral load (11.9±7.9 mEq/ml) increased to 23.3 ± 24.1 mEq/ml at the end of treatment. Hence, no correlation was found between decreased HCV RNA levels and H_{2218}->R substitution in these two patients (9, 10).

DISCUSSION

A positive correlation between mutations in the region of the NS5A gene of HCV genotype lb ($NS5A_{22092248}$) and sensitivity to IFN-α was reported in several studies from Japan. Accordingly, genotype lb patients with 4-11 aa changes in the $NS5A_{2209\text{-}2248}$ sequence were more likely to have a complete response to IFN, compared to patients with no aa changes (wild type) (10, 11). To investigate the validity of this hypothesis in Turkish patients, we examined the impact of mutations in the ISDR of the NS5A region with respect to the outcome of IFN treatment in patients with chronic hepatitis C. However, in line with other studies from Europe, we found no difference in the number of aa mutations in ISDR between patients who responded to an IFN treatment regime and those who did not.

In our R patient group (18/39), no mutant type $NS5A_{2209\text{-}2248}$ sequence was detected. Seven of the 18 R patients (39%) infected with wild type virus had complete response at the end of the therapy. In addition, patients infected with intermediate type

sequence (11/18) also had complete response (63.2%). In the NR patient group (21/39), there were only eight patients (8/21) infected with the wild type virus and the rate of intermediate type virus (13/21) was nearly two times higher than the wild type virus. Thus, we found no correlation between the sequence of the ISDR and sensitivity to IFN. Our results differ from those which have reported that mutant type sequences in the NS5A gene correlated with sustained response to IFN (10,11,17-19), but are in line with those in European groups that failed to demonstrate such a correlation. Most of the findings indicate that the ISDR NS5A regions of European HCV isolates do not have a common ISDR to confirm Japanese groups results (20-24,28). European investigators have shown that the sequences of the ISDR, the PKR-binding domain are unlikely to have predictive value for IFN treatment success in patients infected with HCV genotype 1. However, the finding of greater variability among treatment responders in the carboxy-end of NS5A suggests that the V3 region (codons 2356-2358) may be relevant, but merits further investigations (29). Recently, Sarazzin et al. developed a new mathematical model to investigate NS5A sequences of HCV 1 infected patients and also showed and confirmed Murphy et al.'s results that mutations correlating with treatment response were found to be the sequence from aa 2350-2370(30). Moreover, the conformational analysis of NS5A by secondary structure prediction allowed the differentiation of the most sensitive strains from resistant ones. It can be concluded that the regions other than ISDR involved in resistance to interferon may have been via the interaction between NS5A and PKR(31).

In contrast to the hypothesis that H2218->R mutation leads to an increase in response rate (10), we found all 6 H2218->R mutations in NR rather than R patients. There was no H2218->R mutation in any R patient. On the other hand, the aa sequence of $NS5A_{2209-2248}$ showed a unique feature of one particular aa change at codon 2213 in some IFN responders and this pattern has not been described so far. Alanine was replaced by glutamic acid at this position in seven of 18 R patients, but in none of the 21 NR patients. The lack of such an aa change in all NR patients raises the intriguing possibility that in a long mutation number in the ISDR of the HCV, a particular type of aa change may affect IFN response. This data certainly needs verification, and ISDR data bases and aa substitution models can be further developed (32). Because mutation patterns in the ISDR affect the virological response to IFN and reflect different influences on the function of the NS5A protein (33), it is necessary to compose mutation patterns in the ISDR in different geographical regions, and consider their effect on IFN response.

Our results demonstrated that no significant association exists between the number of aa changes in ISDR and response to IFN-α treatment. Amino acid sequences in ISDR are well conserved in the majority of patients infected with genotype lb and la.

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