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Protease Inhibitors Drug Resistance Mutations in Turkish Patients with Chronic Hepatitis C

Elif Sargin Altunok [a], Murat Sayan [b,c], Sila Akhan [d], Bilgehan Aygen [e], Orhan Yildiz [e], Suda Tekin Koruk [f], Resit Mistik [g], Nese Demirturk [h], Onur Ural [i], Şükran Kose [j], Aynur Aynioglu [k], Fatime Korkmaz [l], Gülden Ersoz [m], Nazan Tuna [n], Celal Ayaz [o], Faruk Karakecili [p], Derya Keten [q], Dilara Inan [r], Saadet Yazici [s], Safiye Koculu [t], Taner Yildirmak [u]

[a] *Infectious Diseases and Clinical Microbiology, Bitlis Public Hospital, Bitlis 13000, Turkey*
[b] *Clinical Laboratory, Kocaeli University Faculty of Medicine, Kocaeli 41380, Turkey*
[c] *Near East University, Research Center of Experimental Health Sciences, Nicosia, Northern Cyprus*
[d] *Infectious Diseases And Clinical Microbiology, Kocaeli University Faculty of Medicine, Kocaeli 41380, Turkey*
[e] *Infectious Diseases And Clinical Microbiology, Erciyes University Faculty of Medicine, Kayseri 38030, Turkey*
[f] *Infectious Diseases and Clinical Microbiology, Koc University Faculty of Medicine, İstanbul 34010, Turkey*
[g] *Infectious Diseases and Clinical Microbiology, Uludag University Faculty of Medicine, Bursa, Turkey*
[h] *Infectious Diseases and Clinical Microbiology, Afyon Kocatepe University Faculty of Medicine, Afyonkarahisar, Turkey*
[i] *Infectious Diseases and Clinical Microbiology, Selçuk University Faculty of Medicine, Konya, Turkey*
[j] *Infectious Diseases and Clinical Microbiology, Tepecik Training and Research Hospital, İzmir, Turkey*
[k] *Infectious Diseases and Clinical Microbiology, Zonguldak Ataturk Public Hospital, Zonguldak, Turkey*
[l] *Infectious Diseases and Clinical Microbiology, Konya Training and Research Hospital, Konya, Turkey*
[m] *Infectious Diseases And Clinical Microbiology, Mersin University Faculty of Medicine, Mersin, Turkey*
[n] *Infectious Diseases and Clinical Microbiology, Sakarya University Faculty of Medicine, Sakarya, Turkey*
[o] *Infectious Diseases and Clinical Microbiology, Dicle University Faculty of Medicine, Diyarbakir, Turkey*
[p] *Infectious Diseases and Clinical Microbiology, Erzincan University Faculty of Medicine, Erzincan, Turkey*
[q] *Infectious Diseases and Clinical Microbiology, Necip Fazil City Hospital, Kahramanmaraş, Turkey*
[r] *Infectious Diseases and Clinical Microbiology, Akdeniz University Faculty of Medicine, Antalya, Turkey*
[s] *Infectious Diseases and Clinical Microbiology, Göztepe Training and Research Hospital, İstanbul, Turkey*
[t] *Infectious Diseases and Clinical Microbiology, Giresun Public Hospital, Giresun, Turkey*
[u] *Infectious Diseases and Clinical Microbiology, Okmeydanı Training and Research Hospital, İstanbul, Turkey*

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ABSTRACT

Background: Drug resistance development is an expected problem during treatment with protease inhibitors (PIs), this is largely due to the fact that Pls are low-genetic barrier drugs. Resistance-associated variants (RAVs) however may also occur naturally, and prior to treatment with Pls, the clinical impact of this basal resistance remains unknown. In Turkey, there is yet to be an investigation into the hepatitis C (HCV) drug associated resistance to oral antivirals.

Materials and methods: 178 antiviral-naïve patients infected with HCV genotype 1 were selected from 27 clinical centers of various geographical regions in Turkey and included in the current study. The basal NS3 Pls resistance mutations of these patients were analyzed.

Results: In 33 (18.5%) of the patients included in the study, at least one mutation pattern that can cause drug resistance was identified. The most frequently detected mutation pattern was T54S while R109K was the second most frequently detected. Following a more general examination of the patients studied, telaprevir (TVR) resistance in 27 patients (15.2%), boceprevir (BOC) resistance in 26 (14.6%) patients, simeprevir (SMV) resistance in 11 (6.2%) patients and faldaprevir resistance in 13 (7.3%) patients were detected. Our investigation also revealed that rebound developed in the presence of a Q80K mutation and amongst two V55A mutations following treatment with TVR, while no response to treatment was detected in a patient with a R55K mutation.

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Conclusion: We are of the opinion that drug resistance analyses can be beneficial and necessary in revealing which variants are responsible for pre-treatment natural resistance and which mutations are responsible for the viral breakthrough that may develop during the treatment.

1. Background

Hepatitis C virus (HCV), has a 9.6 kb-long genome and is a single-stranded RNA virus.[1,2] There are seven different HCV genotypes and more than 90 subtypes.[3] The most frequently encountered HCV genotypes worldwide are genotypes 1, 2 and 3. Similarly in Turkey, HCV genotype 1 infection is the most commonly detected HCV infection type.[4,5] HCV genotyping is critical for selecting the treatment type, predicting the possible response to the treatment and also in deciding the duration of the treatment.[6,7]

In chronic hepatitis C (CHC) infections, and in situations where the virus is not integrated into the host cell genome, the targets that the treatment interacts with is often regarded as a 'cure' unlike that in hepatitis B and Human Immunodeficiency Virus infection treatments.[8] Until now, the available standard treatment administered using pegylated interferon (PEG-IFN) and ribavirin (RBV) has had limited effects with significant associated side effects.[9,10] However, CHC treatment options have been gradually increasing along with the use of various direct-acting antiviral agents (DAAs). NS3/4A serine protease, NS5B polymerase and NS5A protein (the non-structural proteins necessary for virus replication), are three important main drug targets for DAAs.[11,12] The first generation NS3/4A PIs telaprevir (TVR) and boceprevir (BOC) both inhibit the NS3/4A serine protease enzyme required for HCV replication.[13,14] In 2011, TVR and BOC were approved to be used in combination with PEG-IFN and RBV for treatment of HCV genotype 1 infection. Although sustained virological response (SVR) rates have increased with these new regimes, treatment compliance has considerably decreased due to the increase in drug load and side effects. On the other hand, when SMV, a second generation PI is used in combination with PEG-IFN/RBV similar SVR rates can be obtained.

HCV has a high replication capacity ($10^{10\text{-}12}$ virion/day) and its polymerase activity does not have error-correcting ability.[2,8,15] Formation of variants due to mutations occurring during viral replication may lead to a decrease in the sensitivity to antiviral agents.[16–18] Treatment with TVR and BOC, may result in resistance development due to the fact that they are low genetic barrier antiviral drugs.[19] Resistant variants may also occur naturally and may be found prior to the commencement of treatment.[8,13,14,20,21] However, the clinical impact of basal resistance still remains unclear.

According to The World Health Organization's 2013 HCV disease burden report, Turkey has been categorized together with countries which form part of North Africa and the Middle East. The HCV prevalence amongst countries in these regions has been reported as 3.2 – 4.1%.[22] However, in recent studies conducted in Turkey anti-HCV positivity rates vary between 0.1-1%.[23,24] In Turkey, where there is mid-level HCV prevalence, PIs, TVR and BOC, have been in use since 2013. The use of SMV in Turkey however is yet to occur. Therefore, the data-based in Turkey with regards to antiviral use and the mutations that may occur as a consequence of their use is yet to be available.

The purpose of the current study was to determine PI resistance at the molecular level in CHC patients in Turkey infected with genotype 1, and to identify clinical reflections of drug resistance mutations.

2. Materials and methods

2.1. Patient population

The present study was conducted between May 2012 – March 2015, and included 178 HCV genotype 1 infected patients, who were diagnosed from across 27 infectious disease departments from 21 cities within Turkey.

Clinic and laboratory characteristics of the patients are shown in Table 1. The study was approved by the local ethics committee (Clinical Research Ethics Committee of Kocaeli University, KOU KAEK 2012/12 and written consent was obtained from each patient.

All of the patients were categorized as hepatitis C chronic carriers according to the European Association for the Study of the Liver Clinical Practice Guidelines.[25] Blood samples with K_2EDTA were immediately separated by centrifugation, aliquoted, and kept at -80 °C until required. The presence of Anti-HCV antibodies were tested for on all samples which were anti-HCV positive through ELISA testing, using a commercially available microparticle enzyme immunoassay kit (Axsym; Abbott Laboratories, Abbott Park, IL, USA and Elecsys, Roche Diagnostics, Mannheim, Germany)

2.2. HCV RNA isolation and detection

Magnetic particle-based HCV RNA extraction and HCV RNA detection and quantification were performed using commercial real-time PCR assay – QIAsypmhony + Rotorgene Q/artus HCV QS-RGQ (Qiagen GmBH, Hilden, Germany), COBAS Ampliprep/COBAS TaqMan HCV Test (Roche Molecular Systems, Inc. Pleasanton, CA, USA) and Abbott M2000 SP/Abbott RealTime HCV Amplification Kit (Abbott Molecular Inc. Des Plaines, IL, USA).

2.3. HCV NS3 region sequencing

Genotypic resistance testing was performed by population sequencing of the viral protease (codon 32 -185) using an in-house method with a commercial kit (Bosphore HCV drug resistance mutation sequencing kit v1.0, (Anatolia Geneworks, Istanbul, Turkey). The RT-PCR and the cycle sequencing thermal protocols were applied according to the manufacturer's instructions. PCR products were purified using the Bosphore PCR Product Purification Spin Kit (Anatolia Geneworks, Istanbul, Turkey) and sequencing was performed via Beckman Coulter CEQ 8000 Genetic Analysis System and the CEQ 8000 Genetic Analysis System Version 9 software (Beckman Coulter Inc., Brea, CA, USA).

2.4. Drug resistance mutation detection

Drug resistance mutation was analyzed by The Genafor/Arevir-geno2pheno drug resistance tool (Center of Advanced European Studies and Research, Bonn, Germany, http://coreceptor.bioinf.mpi-inf.mpg.de/). HCV D90208 was used as a reference strain for the HCV NS3 region.

3. Results

156 (87.6%) of the HCV genotype 1-infected patients were infected with subtype b. Out of 57 patients examined for

Table 1
Demographic characteristics of the study patients

Characteristic	Study group
Patient, n	178
Gender, F/M, n (%)	97 (54,5%)/81 (45,5%)
Age, median years (range)	56 (19 - 93)
HCV RNA, median IU/mL (range)	1.02+E7 (2.6+E3 - 1.0+E9)
ALT/AST, median U/L (range)	61 (8 - 280)/51 (6 - 220)
Sampling, region; city of Turkey	Marmara; Istanbul, Kocaeli, Edirne, Bursa, Sakarya, Yalova Black Sea; Zonguldak, Giresun East Anatolia; Erzincan Southeast Anatolia; Diyarbakir, Gaziantep, Urfa Central Anatolia; Ankara, Kayseri, Konya, Eskisehir Aegean; Izmir, Afyonkarahisar Mediterranean; Antalya, Mersin, Kahramanmaras
HCV genotype, n(%)	14 (7,9%)
1a	156 (87,6%)
1b	8 (4,4%)
1	
Patient under haemodialysis, n(%)	10 (5,6%)
HIV Co-infection, n(%)	1 (0,6%)
IL 28B polimorphism*, n(%)	34 (59,6%)
CT	9 (15,8%)
CC	14 (24,6%)
TT	

Abbreviations: F, female; M, male; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HIV, Human Immunodeficiency Virus
* IL28B polimorphism; rs12979860 C/T

interleukin 28B (IL28B) polymorphism, 34 (59.6%) patients were found to be CT, nine (15.8%) patients CC and 14 (24.6%) of them TT. The demographic and laboratory characteristics of the patients are shown in Table 1.

Amongst 33 (18.5%) of the 178 patients included in the current study, PI-associated amino acid substitutions were detected. In patients, where 22 different mutation patterns were detected, the two most common mutation patterns identified were T54S and R109K, respectively. Furthermore, amino acid substitutions were associated with telaprevir (TVR) resistance in 27 patients (15.2%), boceprevir (BOC) resistance in 26 (14.6%) patients, simeprevir (SMV) resistance in 11 (6.2%) patients and faldaprevir resistance in 13 (7.3%) patients. The PI-associated amino acid substitutions detected are presented in Table 2.

The patients examined were segregated into two groups; Group one consisted of patients with no PI-associated amino acid substitution (sensitive group), while Group 2 consisted of patients with at least one PI-associated drug resistance mutation (resistant group). Accordingly, 145/178 (81.5%) patients formed part of the sensitive group and 33/178 patients (18.5%) formed part of the resistant group. 48/145 patients in the sensitive group received one of the triple combination treatments containing TVR (n=39) or BOC (n=9). On the other hand, 7/33 of the patients in the resistant group received combination treatment containing TVR (Table 3).

The resistance mutation patterns amongst the resistant group (Group1) and the results following treatment are shown in Table 4. It became evident that a patient with a R155K mutation is non-responsive to the treatment, furthermore viral rebound developed during the treatment period, in one case both Q80K and N174S mutation patterns were detected, while in two separate cases V55A mutation patterns were detected (Table 4).

Table 2
Drug resistance mutation pattern in the study patients (n = 33)

Mutation pattern	Patient, n (%)	Drug	Resistance status
T54S	9 (5,1%)	TVR	Potantial resistant
		BOC, Faldaprevir	Resistant
V55A	3 (1,7%)	TVR, BOC	Resistant
Q80L	3 (1,7%)	Faldaprevir	Resistant
Q80H	1 (0,6%)	SMV	Resistant
Q80K	1 (0,6%)	TVR, BOC, SMV, Faldaprevir	Resistant
R109K	5 (2,8%)	TVR, BOC, SMV	Potantial resistant
R117C	1 (0,6%)	TVR, BOC	Potantial resistant
R117H	2 (1,1%)	TVR, BOC	Resistant
S122G	1 (0,6%)	SMV	Potantial resistant
L155I	1 (0,6%)	TVR	Resistant
I155L	1 (0,6%)	TVR	Resistant
R155K	1 (0,6%)	TVR, BOC, SMV, Faldaprevir	Resistant
R155G	1 (0,6%)	TVR, BOC	Resistant
R155T	1 (0,6%)	TVR, BOC	Resistant
A156G	1 (0,6%)	BOC, SMV	Resistant
A156S	1 (0,6%)	TVR, BOC	Resistant
A156T	1 (0,6%)	TVR, BOC, SMV	Resistant
G156S	2 (1,1%)	TVR, BOC	Resistant
I170V	1 (0,6%)	TVR, BOC	Resistant
I170T	1 (0,6%)	BOC	Resistant
N174F	1 (0,6%)	TVR, BOC	Resistant
N174S	3 (1,7%)	TVR, BOC	Potantial resistant

Telaprevir, TVR; boceprevir, BOC; simeprevir, SMV.

4. Discussion

In Turkey, HCV PIs drug resistance analysis was contextualized the first time in a surveillance study conducted at a national level in 2013 by the Viral Hepatitis Study Group of Turkish Society of Clinical Microbiology and Infection Diseases.[26] In the current

Table 3
Treatment response according to the resistance mutation in patients

Treatment response	No resistance mutation, n = 48	On the resistance mutation, n = 7
TVR/BOC, n(%)	39 (81,2%)/9 (18,8%)	7 (100%)/0 (0%)
Untolerated treatment by adverse effects, n(%)	15 (31,3%)	1 (14,3%)
SVR, n(%)	26* (54,2%)	2 (28,6%)
Relapsed, n(%)	3** (6,2%)	0 (0%)
Viral Rebound, n(%)	3*** (6,2%)	3 (42,9%)
Lack of virological response, n(%)	1 (2,1%)	1 (14,3%)

SVR, sustained virological response; TVR, telaprevir; BOC, boceprevir
* One of the sustained virological response obtained patients was a hemodialysis patient treated with TVR, PEG-IFN alfa 2a and 200 mg/day RBV combination for 24 weeks.
** In one of the patients, according to the drug resistance analyses performed after rebound developed during her/his telaprevir treatment, D168N (TVR possibly resistant, BOC resistant) mutation was detected.
*** In one of the patients, drug resistance was analyzed after a relapse developed following the telaprevir treatment and no resistance was detected.

Table 4
Telaprevir treatment response and the resistance mutation pattern detected in the patients (n = 7)

Patient no	HCV genotype	IL28 B polymorphism	Mutation characteristic			Treatment response
			Pattern	Resistance status	Fold change	
1	1b	CC	T54S	TVR partial resistant	1.9	SVR
2	1b	ND	R155G	TVR resistant	7.4	SVR
				BOC resistant	20	
3	1b	CC	Q80L	Faldaprevir resistant	1.2	Untolerated treatment by adverse effects
			R155K	TVR resistant	7.4	Lack of virological response
4	1a	ND		BOC resistant	4.7	
				SMV resistant	420	
				Faldaprevir resistant	360	
			I170T	BOC resistant	4.7	
			Q80K	TVR possibly resistant	14	
5	1a	ND		BOC possibly resistant		
				SMV resistant		Viral rebound
				Faldaprevir resistant		
			N174S	TVR possibly resistant	0.8	
				BOC possibly resistant	0.5	
6	1	CT	V55A	TVR resistant	3.1	Viral rebound
				BOC resistant	6.9	
7	1b	ND	V55A	TVR resistant	3.1	Viral rebound
				BOC resistant	6.9	

Abbreviations; ND, not determined, SVR, sustained virological response; TVR, telaprevir; BOC, boceprevir; simeprevir, SMV.

follow-up study it was revealed that 33 of the studied patients (18.5%) were resistant to at least one antiviral drug (Table 2). In various other studies conducted, both in American and European populations the percentage of DAAs-resistant variants in naive patients infected with HCV genotype 1 has been reported as 8.6%, and 71% in China.[16,27] In another study, conducted in 2014 Chen et al. stated that the global prevalence of DAA RAVs was at 58.7%.[28] In the current study although the NS3, NS5A and NS5B regions were analyzed RAVs were most frequently detected in the NS5A and NS3 regions. Once the results of these studies were compared with previous findings it became evident that the resistance rates varied dramatically amongst countries. In the forthcoming years, depending on the proliferation of CHC treatment with PIs, changes in the prevalence of drug resistance mutation can also be expected.

In our study, the two most frequently detected resistance mutations were T54S (5.1%) and R109K (2.8%). On the contrary, in a study conducted by Ye Wang et al. S122G (56.6%), which causes SMV resistance, was observed to be the most frequently detected mutation.[27] Our results indicate that a S122G mutation was detected in only one patient (0.6%), furthermore the number of the patients with resistance to DAAs TVR and BOC, (which are still in use in Turkey), were found to be 27 (15.2%) and 26 (14.6%), respectively. Although there have only been a limited number of studies conducted, the percentage of the pre-treatment TVR resistance mutations (V36L, T54A/S, V55A, Q80K/R, R155I/K/M/T, A156S, D168Q and V170T) detected in the world is reported to be between 4-28% [29-31]. Identification of the mutations which occur during treatment of CHC with antivirals may be necessary in understanding its contagiousness, comprehending its circulation and for the surveillance of its course.

In the near future, SMV is also expected to come into use in Turkey. In our study, the mutation patterns (Q80H, Q80K, R109K, S122G, R155K, A156G, A156T) causing resistance to SMV was detected in 11 (6.2%) of the xx patients examined (Table 2). Amongst the detected mutation patterns, Q80K and R155K were of particular interest, due to the fact that it resulted in resistance to the entire PI drug class. In previous studies, the prevalence of Q80K polymorphism in patients infected with HCV genotype 1a was found to be between 22-30%, comparatively this rate was found to be between 0.5-1% in those infected with genotype 1b.[32,33] If any Q80K mutation pattern is detected in advance in HCV type 1a patients it is recommended not to start SMV treatment.[34] In our study, it was observed that a patient with a R155K mutation was non-responsive to the treatment while a viral rebound developed during the treatment period in a patient with a Q80K mutation (Table 4). Our findings highlight the need for pre-treatment examination of patients for any Q80K and R155K mutations.

Previous studies demonstrate that there is a correlation between the pre-treatment of patients with PI mutations and treatment with DAAs.[35–37] In our study, while the SVR rate in the sensitive group was found to be 54.2%, the SVR rate in the resistance group was found to be 28.6%. Furthermore, while no response to the treatment could be obtained in 2.1% of the patients in the sensitive group the percentage of patients without any response to the treatment amongst the resistance group was 14.3%. Rebound developed during treatment in 42.9% of the patients in the resistance group, while this rate was determined as 6.2% in the sensitive group (Table 3).

Another disadvantage of HCV infection treatment with NS3/4A serine PIs is that some associated side effects were observed in a significant number of patients which may lead to early termination of the treatment.[13,14,20,21] According to the evaluation of TVR treatment, research findings obtained from a study conducted by Aygen et al. in Turkey, revealed that early termination as a result of side effects was observed in 9.9% of patients. This study also revealed that the most frequent side effect resulting in early termination was gastrointestinal associated side effects (63.6%).[38] Also, in another study conducted in Kocaeli/Turkey, 26.1% of patients could not continue TVR treatment due to side effects.[39] In our study, 16 (29.1%) out of the 55 patients who participated in the treatment could not continue their treatment due to side effects. Collectively these results suggest that there is a crucial need in Turkey for new easy-to-use, effective and reliable treatment options with a low side effect risk.

5. Conclusion

PIs resistance-associated amino acid substitutions can be detected in DAA naive and CHC patients prior to therapy and PI treatment, respectively. Our findings indicate that the PIs resistance-associated amino acid substitutions can be effective in clinical. HCV associated DAA resistance testing must remain as an integral part of the management of DAA naïve and experienced patients in Turkey. In addition, monitoring PIs resistance - associated amino acid

substitutions can provide useful information while preparing treatment plans and for continued tracking of the success rate associated with CHC treatments.

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