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ORIGINAL RESEARCH

Naturally Occurring Resistance Associated Substitutions in Non-Cirrhotic, Treatment Naive HCV–HIV Co-Infected Patients Does Not Affect the Treatment Response for Anti-HCV Antiviral Therapy

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Purpose: Limited literature on the prevalence of baseline resistance associated substitutions (BL-RAS) among HCV–HIV co-infected patients and their association with treatment outcomes is available especially from India. Hence, the present study aimed to study naturally occurring RAS among non-cirrhotic HCV–HIV co-infected patients and their impact on the response to anti-HCV therapy.

Patients and Methods: In this retrospective study, archived blood samples of 80 HCV–HIV co-infected patients, before anti-HCV therapy initiation, were tested for substitutions at the drug acting sites (NS5a and NS5b) in the HCV genome by direct PCR sequencing.

Results: BL-RAS were seen in 19 (23.7%) patients. As well as BL-RAS, all patients were given sofosbuvir (SOF) 400 mg+ daclatasvir (DCV) 60 mg for 12 weeks. Overall, sustained virological response (SVR) was achieved in 63 (78.8%) patients, in 13 with BL-RAS and in 50 without BL-RAS. All the SVR failure cases (n=17) were retreated with SOF (400 mg) +DCV (60 mg)+ ribavirin (RBV) for 24 weeks. SVR was eventually attained in 14 (82.3%) patients, in 4/6 (66.6%) with BL-RAS and in 10/11 (91%) without BL-RAS. On univariate analysis, age more than 30 years (OR: 11.6; 95% CI: 3.0–45.5, p-value<0.001) and female gender (OR: 8.6; 95% CI: 1.1−69, p-value <0.009) were found to be significant factors associated with the attainment of SVR.

Conclusion: BL-RAS are common in HCV–HIV co-infected patients. The existence of BL-RAS, however, did not affect the attainment of SVR among non-cirrhotic, treatment naive HCV–HIV co-infected patients.

Keywords: HCV–HIV co-infection, drug resistance, direct acting antiviral, resistance associated substitutions

Introduction

Globally, around 2.3 million people are co-infected with the hepatitis C virus (HCV) and the human immunodeficiency virus (HIV).[1] In India the prevalence of HCV–HIV co-infection is around 3–5% but it varies in different geographical regions and in different patient populations.[2] The highest prevalence is seen in the Northeast region, around 11–13%, and among intravenous drug users (IVDU).[2] HCV–HIV co-infection accelerates the rate of hepatitis C disease progression, leading to early cirrhosis and

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liver-related complications, thereby increasing mortality rates.[3,4] With the advent of direct-acting antiviral (DAA) therapy for HCV, sustained virological response (SVR) is achieved at similar rates among co-infected patients as in HCV mono-infected patients.[5–7] Failure to attain SVR despite adequate DAA duration and strict adherence is a multi-factorial issue and is influenced by both host and viral factors.[8] Naturally occurring resistance associated substitutions (RAS) in the HCV genome at the drug acting sites before treatment initiation, ie baseline (BL- RAS), are an important virological factor that might influence treatment outcome.[9,10] Limited literature determining the prevalence of BL-RAS among HCV–HIV co-infected patients and their association with treatment outcome is available especially from India.[11,12] Therefore, in the present study we evaluated the impact of BL-RAS among HCV–HIV co-infected patients on the attainment of SVR.

Patients and Methods

A total of 80 HCV–HIV co-infected non-cirrhotic adult patients were included in this retrospective study (Figure 1). All the patients before initiation of HCV therapy were given standard anti-retroviral treatment and had achieved HIV viral suppression as per the national guidelines.[13] None of the patients had previously received anti-viral therapy for HCV. Irrespective of the genotype (Gt), all patients received sofosbuvir (SOF) 400 mg+ daclatasvir (DCV) 60 mg for 12 weeks as per the national guidelines.[13] Follow-up records until completion of the treatment were obtained from the hospital information system (HIS). Virological cure was determined by performance of SVR at 12 weeks after the end of treatment by conducting an HCV RNA test on the blood (plasma) sample. Baseline (before initiation of HCV therapy) blood samples were retrieved from −80°C and RAS testing was done. Plasma samples of patients who did not attain SVR

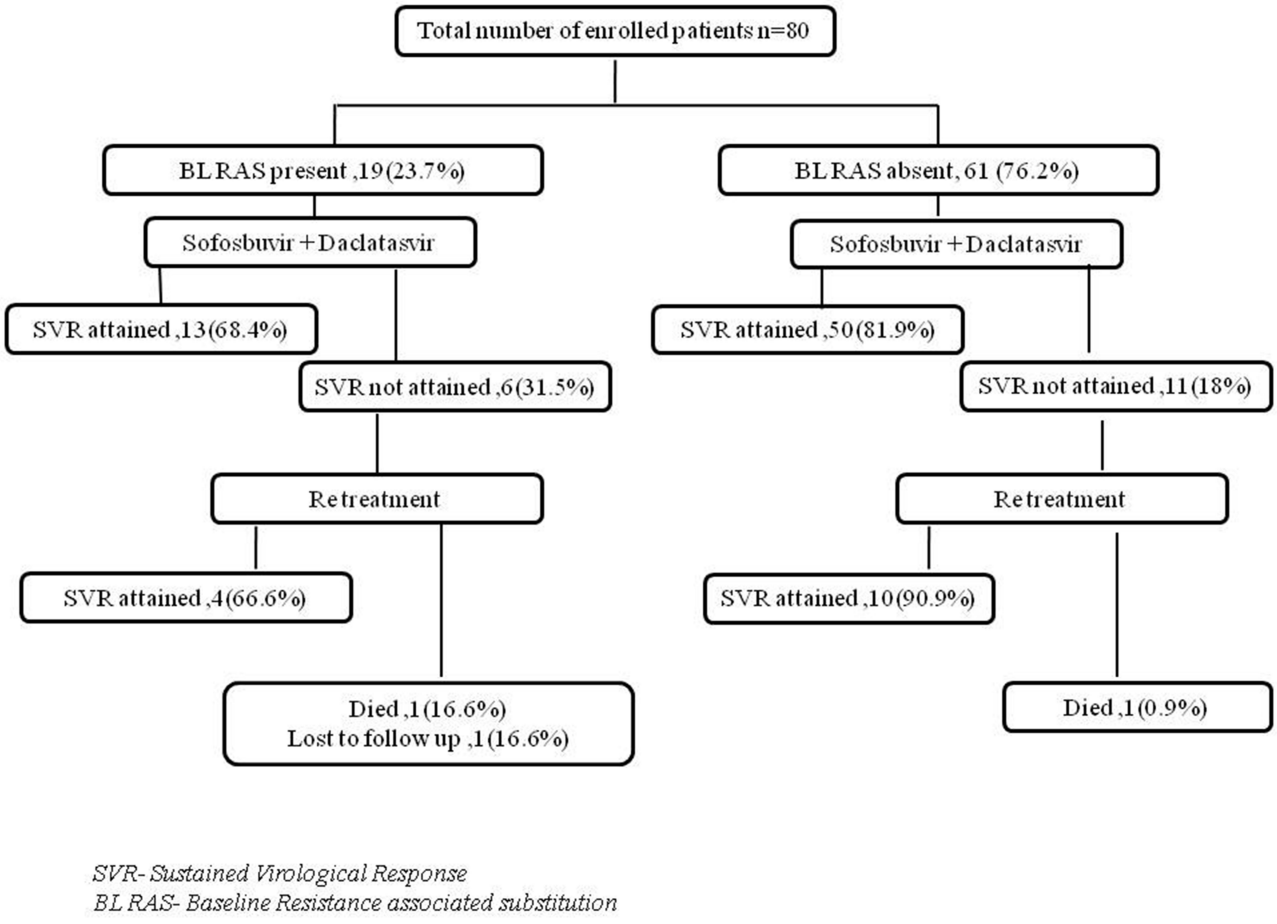

Figure 1 Details of the enrolled population.

were also retrieved and tested for RAS. The study was approved by the Institutional Review Board of the Institute of Liver and Biliary Sciences (IEC No. IEC/2017/49/NA06) and conducted in accordance with the Declaration of Helsinki. The study was performed on anonymised, de-identified archived samples, hence the necessity for patient consent forms was waived by the ethics committee.

HCV RAS Testing

Once thawed, plasma samples were subjected to RAS testing and genotyping by direct PCR sequencing as per the protocol described earlier.[14] Viral RNA isolation was done using the High Pure Viral Nucleic Acid Kit (Roche Diagnostics, GmbH, Mannheim, Germany). cDNA was prepared using Quantitect reverse transcription kit (Qiagen, GmbH, Germany). cDNA was subjected to PCR using in-house designed primers. Parts of NS5a and NS5b DAA target regions incorporating all important sites at which known substitutions are present were amplified by single-round PCR reaction using in-house designed primers.[14] For the NS5a region, different primer sets were used to amplify samples of Gt1 (F 5′-ACRCACTAYGTGCCBGAGAG-3′, R 5′- RAYCTGGCAHGGGCAYTTNA-3′) and Gt3 (F 5′-CRACNCAYTAYGTYCCYGA-3′, R 5′-CGRTGRAGYCTBACYCCRTC-3′) generating a PCR amplicon of size 554bp and 602bp, respectively. For the NS5b region, pan-genotypic primer set (F 5′-ACYACCATYATGGCNAARARYGAGGT-3′ and R 5′-TAYCTRGTCATRGCCTCCGTGAAGRC-3′) were used generating an amplicon size of 631bp. PCR was performed using a Phusion high-fidelity DNA polymerase (Thermoscientific Inc., Waltham, MA). Amplified products were purified by gel-excision using a QIAquick Gel Extraction Kit (QIAGEN, GmbH, Mannheim, Germany). Sanger sequencing was performed with primers as denoted in the Big Dye Terminator v3.1 Cycle Sequencing Kit on an ABI Prism 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA).

RAS Analysis

Sequences were proofread and aligned using the ClustalX version in the BioEdit program v7.2.5 (Carlsbad, CA). RAS analysis was done using a geno2pheno (HCV) online tool.[15] When no RAS were detected, amplicons were designated as wild-type and, wherever RAS was detected, it was denoted at that particular amino acid position. Substitutions were reported as per the tool, specific for the genotype, subtype and against the drug used in the study.[15]

Statistical Analysis

Statistical analysis was done using the Statistical SPSS software, version 21.0 (Chicago, IL, USA). Continuous variables were expressed as mean ± SD or median where appropriate and categorical variables were expressed as a percentage. Univariate analysis was performed with various factors and final attainment of SVR in the study group. The odds ratio (OR) was calculated with a 95% confidence interval (CI). All statistical tests were two-tailed, and results with a p-value of <0.05 were considered statistically significant.

Results

Baseline characteristics of the study population (n=80) are described in Table 1. Mean HIV viral load in all patients was <50 copies/mL at the time of initiation of anti-HCV treatment. Median CD4 count was 341 (IQR: 218–506) cells/mm^3. Gt6 was the commonest genotype seen in the study, followed by Gt3; 3b 6xa were the commonest subtypes seen in Gt3 and Gt6, respectively. BL-RAS was seen in 19 (23.7%) patients.

Overall, SVR was attained in 63 (78.8%) patients. Comparison of several factors in the SVR attained and

Table 1 Baseline Characteristics of the Studied Population (n=80)

Characteristics	
Mean age (SD)[a] years	34.1 (9.2)
Gender n (%) Male Female	 57 (71.2%) 23 (28.8%)
Median baseline CD4 count cells/mm^3 (IQR) [b]	341 (218–506)
Mean HCV viral load in $\log_{10}$IU/mL (SD)	6.1 (0.97)
Mean HIV viral load in $\log_{10}$ IU/mL	<1.6
Genotype n (%) Genotype 1 [1a] Genotype 3 [3a/3b/3g] Genotype 6 [6n/6v/6u/6xa]	 12 (15%) 32 [12/18/2] (40%) 36 [15/2/2/17] (45%)
BL-RAS[c] Present Absent	 19 (23.7%) 61(76.2%)

Notes: [a]SD, standard deviation; [b]IQR, interquartile range; [c]BL-RAS, baseline resistance associated substitutions.

Table 2 Factors Associated with Treatment Failure

Factors	SVR Attained (n=63)	SVR Not Attained (n=17)	Unadjusted OR[a] (95% CI)	p-value
	n(%)	n(%)		
Age				
<30 years	18 (56.2)	14 (43.8)	1	<0.001
>30years	45 (93.8)	3 (6.2)	11.6 (3.0–45.5)	
Gender				
Male	41 (71.9)	16 (28.1)	1	0.009
Female	22 (95.6)	1 (4.4)	8.6 (1.1–69.1)	
Genotype (Gt)				
Gt1	10 (83.3)	2 (16.7)	2.8 (0.5–15.3)	0.107
Gt3	18 (64.3)	10 (35.7)	1	
Gt6	31 (86.1)	5 (16.9)	3.4 (1.0–11.7)	
BL-RAS at NS5a				
No	50 (80.8)	11 (19.2)	1.9 (0.6–6.4)	0.282
Yes	13 (68.4)	6 (31.6)	1	
CD4 count (cells/mm^3)				
<350	28 (82.4)	6 (17.6)	1	0.495
≥350	35 (76.1)	11 (23.9)	0.68 (0.2–2.1)	

Note: [a]OR, odds ratio.

non-attained groups was carried out (Table 2). On univariate analysis, age more than 30 years (OR: 11.6; 95% CI: 3.0–45.5, p-value <0.001) and female gender (OR: 8.6; 95% CI: 1.1−69, p-value <0.009) were found to be significant factors associated with the attainment of SVR. However, no significant association was seen in the study between HCV Gt, existence of BL-RAS and CD4 counts with respect to the attainment of SVR.

SVR failure was seen in 17 (21.2%) cases; 11 did not have any BL-RAS, while 6 had BL-RAS (Figure 1). SVR failure cases belonged to Gt3 in 10 (58.8%) cases, subtype 3b in 4 cases and subtype 3a in 6 cases. Gt6 was seen in 5 (29.4%) cases, with subtype 6xa in 3 cases and subtype 6n in 2 cases. Gt1 was seen in 2 cases and both were subtype 1a. All the SVR failure cases were reinitiated on SOF (400 mg) +DCV (60 mg)+ weight-based ribavirin (RBV) for 24 weeks, following which SVR was eventually achieved in 14 (82.3%) cases; 2 patients died and 1 was lost to follow-up.

Different types of BL–RAS seen in the study as per Gt and subtype are shown in Table 3. All BL-RAS were seen in the NS5a region and none were observed in the NS5b region of the virus. Thirteen (68.4%) patients, despite BL-RAS, attained SVR. In 6 patients with non-attainment of SVR after retreatment, eventually SVR was attained in 4 (66.6%). BL-RAS were not seen in 61 patients and, of those, 50 (81.9%) attained SVR.

Of patients with BL-RAS, 17/19 (89.4%) belonged to Gt3, with 15/17 (88.2%) of subtype 3b and 2 of subtype 3a. The most common BL-RAS was dual RAS at 30K and 31M position observed in 16 (84.21%) patients. Among patients with BL-RAS, SVR was not achieved in 6 (31.5%), and BL-RAS continued to be seen until SVR failure in 4 (66.6%) of them. No new RAS developed at the time of SVR failure in any of the patients included in the study (Table 3).

Discussion

The present study, which is the first of its kind from India, demonstrated a 23.7% prevalence of BL-RAS in an HCV–HIV co-infected group. Earlier studies conducted with this co-infected group showed a prevalence of around 3–16.9%.[10,11,16] Success of DAA therapy is usually seen at similar rates among HCV–HIV co-infected patients as in HCV mono-infected patients. In approximately 2–5% cases, where SVR is not attained, many factors have been associated with treatment failure.[8] Non-adherence to treatment, drug interactions between HIV and HCV drugs, severity of underlying liver disease, genotype of the HCV virus

Table 3 Distribution of Baseline Resistance Associated Substitutions (BL-RAS)

S. No	Genotype	Subtype	BL-RAS at NS5a [a]	BL- RAS at NS5b[a]	Prediction for Drug Resistance[c]	SVR[b]	SVR RAS at NS5a	SVR RAS at NS5b	After Reeatment
1.	3	3b	31M,30K	None	Resistant	Attained	-	-	-
2.	3	3b	31M,30K	None	Resistant	Not attained	None	None	SVR attained
3.	3	3b	31M,30K	None	Resistant	Attained	-	-	-
4.	3	3b	31M,30K	None	Resistant	Attained	-	-	-
5.	3	3b	31M,30K	None	Resistant	Attained	-	-	-
6.	6	6xa	93A	None	Not known	Attained	-	-	-
7.	3	3b	31M,30K	None	Resistant	Attained	-	-	-
8.	3	3b	31M,30K	None	Resistant	Attained	-	-	-
9.	3	3b	31M,30K	None	Resistant	Attained	-	-	-
10.	3	3b	31M,30K	None	Resistant	Attained	-	-	-
11.	3	3b	31M,30K	None	Resistant	Attained	-	-	-
12.	3	3b	31M,30K	None	Resistant	Not attained	31M,30K	None	Died
13.	3	3b	31M,30K	None	Resistant	Not attained	31M,30K	None	SVR attained
14.	3	3a	31M,30K	None	Resistant	Not attained	None	None	SVR attained
15.	1	1a	30H	None	Not known	Attained	-	-	-
16.	3	3b	31M,30K	None	Resistant	Attained	-	-	-
17.	3	3b	31M,30K	None	Resistant	Not attained	31M,30K	None	SVR attained
18.	3	3a	93H	None	Resistant	Not attained	93H	None	Lost to follow-up
19.	3	3b	31M,30K	None	Resistant	Attained	-	-	-

Notes: [a]BL-RAS, baseline resistance associated substitutions; [b]SVR, sustained virological response; [c]as per the information of online tool (reference 15).

and substitutions in the HCV viral genome at the drug acting site are a few of the important causative factors.[8] There is a lack of literature concerning BL-RAS in HCV–HIV co-infected patients being compared with a cure for HCV.[17] Therefore, in the present study we evaluated BL-RAS and their association with treatment outcomes in this group. Although in the present study we did find that a greater percentage of patients without BL-RAS attained a cure in comparison to those who had BL-RAS (Figure 1), it was not a significant association. This may be because fewer cases failed the treatment (n=17) than attained a cure (n=63). Similar results are reported for the HCV mono-infected patients.[10,18]

We found that all BL-RAS were seen in the NS5a region of the genome and none in the NS5b region. Earlier studies had shown the existence of BL-RAS even in the NS5b region. The absence of BL-RAS in the NS5b region in our study could be attributed to the fact that most of our patients belonged to Gt6 and Gt3 and only known substitutions were taken into consideration based on the information available in the geno2pheno tool. Gt6 has shown an inherent property of very minimal occurrence

of BL-RAS in HCV mono-infected patients.[19] In Gt3 of HCV, non Gt3a subtypes have shown more occurrence of naturally occurring substitutions in the viral genome.[20] A similar finding was also observed in our study, where most of the BL-RAS were seen in the Gt3b subtype.

Overall, the SVR rate seen in our study was 78.8% smaller than we anticipated based on the existing literature. The limitation of the study was its retrospective nature, whereby several factors like strict adherence to treatment or development of any other co-morbidity during the course of treatment could not be taken into account. In the present study two factors – patient age >30 years and male gender – were found to be significantly associated with treatment failure. Male gender has already been reported as an important factor in treatment failure in HCV mono-infected cases.[9,16]

We also did not find any significant association between Gt of the virus and treatment failure; maximum treatment failure in our study belonged to Gt3. Gt3 has already been considered the most challenging Gt to treat. But retreatment with an SOF-based regimen was successful in most of our patients. In the present study, all SVR failure cases (n=17) were reinitiated on SOF+DCV+ weight-based RBV for 24 weeks and SVR 12 was attained in 14; 2 patients died and one was lost to follow-up. Our results highlight the importance of retreatment with SOF+DCV+ weight-based RBV for 24 weeks in SVR failure cases. Higher SVR rates have been shown to result from SOF-based retreatment in non-responders to prior SOF-based therapy.[21]

There are certain limitations in our study. As it was a retrospective study, information available from hospital records was analysed and only known RAS were considered. We also used a simple population sequencing technique to look for RAS, which restricted the detection rate to only those present at a rate of >15% in a patient. But as there is a scarcity of data regarding RAS testing and its usefulness in a HCV–HIV co-infected group, findings from our study could contribute a great dea.

Conclusion

Our study highlights that BL-RAS are common in HCV–HIV co-infected patients and mostly seen in Gt3, especially subtype 3b. However, the existence of BL-RAS did not affect the attainment of SVR among non-cirrhotic, treatment naive patients, and retreatment among SVR failures with SOF+DCV+ weight- based RBV for 24 weeks can make them attain SVR easily.

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Disclosure

All authors disclose no potential conflicts of interest (financial, professional, or personal) that are relevant to the manuscript.

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