ORIGINAL ARTICLE

RESPONSE RATE OF SOFOSBUVIR BASED ANTIVIRAL THERAPY AMONG PATIENTS INFECTED WITH HEPATITIS C VIRUS GENOTYPE 3A

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ABSTRACT

Objective: To evaluate the efficacy of dual therapy of Sofosbuvir and Ribavirin among treatment-naive and non-responders of Interferon based antiviral regimens against HCV genotype 3a infected patients.

Material & Methods: A longitudinal study was conducted on 100 HCV genotype 3a infected patients from December 2016 to September 2017. Patients were initially divided into two groups including Group-A (n=50/100) as treatment naive, and Group-B (n=50/100) including non-responders. All patients received Sofosbuvir and RBV for 24weeks. HCV genotype was determined using Type-specific nested PCR based genotyping assay. Viral RNA was detected using qualitative or real-time PCR.

Results: After 4 weeks of treatment, HCV RNA was undetectable in 100 of Group A patients, while Group B patients achieved 92% RVR. SVR12 was 96% in treatment naïve Group A patients, while Group B patients achieved an SVR12 of 90%. There were no significant differences in the SVR12 rates between the two treatment groups (Pearson Chi-Square test, *p*-value >0.05). The association of RVR and SVR12 was found statistically significant (Pearson Chi-Square test, *p*- value<0.05).

Conclusion: Sofosbuvir and RBV is highly effective among the treatment-naive and previously treated chronic HCV genotype 3a infected patients.

Keywords: HCV genotype 3a, Sofosbuvir, SVR12.

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INTRODUCTION

Hepatitis C virus (HCV) is a hepatotropic RNA virus that causes progressive liver damage, which might result in liver cirrhosis and Hepatocellular Carcinoma (HCC)[1,2]. The frequency of HCV infection in Pakistan is high, with a reported national prevalence of 6.7% in 2014[3]. Results from earlier studies indicated that the use of IFN based combination therapies can lead to better treatment outcomes and reduction in HCV related morbidity and mortality[4]. However, the advent of IFN-free regimens of Direct-Acting Antivirals (DAAs) against HCV infection have resulted in higher SVR rates of over 90% and are associated with fewer side effects[5].

HCV has been classified into seven[1-7] major genotypes including multiple subtypes and quasispecies[6]. In Pakistan, the most prevalent HCV subtype infecting general population is 3a, with regional differences in the frequency distribution of other subtypes[7]. In KP of Pakistan, majority of infections are attributed to HCV subtype 3a[8]. HCV genotype determination not only helps predict response to therapy but also the type and length of the therapy administered[9]. Previous Standard of Care (SOC) based on a combination of RBV with either conventional or PEG-IFN for 24 weeks increased the probability of attaining SVR by 50-70% in case of HCV subtype 3a[10]. However, SVR rates for treatment of

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chronic HCV 3a patients that received prior treatment were only 40-50%[11]. In addition, response to IFN based combination therapy varied in case of different genotypes and among different ethnic groups and resistance has been observed even in the case of previously susceptible HCV genotype 3a[12]. As IFN based antiviral therapies are often associated with various side effects resulting from treatment, more effective therapeutic options with less adverse events are desired. The continuous discovery and development of agents that directly target various stages of viral life cycle are likely to provide HCV infected patients with effective IFN-free therapy[2]. In 2013, the first polymerase inhibitor Sofosbuvir was approved as a part of combination therapy for hepatitis C infection. Sofosbuvir is a potential candidate for treating chronic HCV infection, as it offers a few advantages over the current SOC, notably in patients with advanced liver disease or intolerant to IFN based therapies[13]. Moreover, when used in combination with RBV, it represents the first IFN-free therapy for chronic HCV infected patients. Ribavirin is an oral guanosine analog with broad-spectrum anti-viral activities such as direct inhibition of RNA polymerase, immunomodulatory activity and the introduction of lethal mutations in the HCV genome[14]. Sofosbuvir (SOF) in combination with RBV is highly effective against HCV genotype 2 and 3 infections with higher rates of SVR observed among patients with genotype 2 infection than among those with genotype 3 infection[15]. In phase 3 trials including HCV genotype 2 or 3 infected patients, dual therapy with SOF and RBV for 12 weeks achieved an SVR of 67% among treatment naïve patients and 78% among patients for whom IFN treatment was not an option. Among patients who experienced prior treatment failure, an SVR of 50% and 73% was observed among those who received 12 weeks and 16 weeks of SOF and RBV combination therapy respectively[16]. Dual therapy with SOF and RBV for 12 weeks is highly effective for genotype 2, whereas genotype 3 has proven to be more challenging and requires 24 weeks of therapy[10,17]. Although the efficacy of combination therapies containing SOF has been established in patients with HCV genotype 1, 2, 3 or 4 infections elsewhere including those with HCC and those co-infected with HIV, there is limited data for this regimen in Pakistani patients infected with HCV 3a. RNA viruses evolve along distinct patterns in different geographical regions of the world indicating the possibility of the variable outcome of similar antivirals in different ethnic populations. Keeping in view the phylogenetically distinct genetic architecture of Pakistani HCV 3a isolates and lower response rates of IFN-based antiviral regimens in our population, we designed this longitudinal study to characterize the treatment response of SOF and RBV dual therapy among treatment naïve and treatment experienced Pakistani patients infected with HCV subtype 3a.

MATERIALS AND METHODS

This longitudinal study was carried out from December 2016 to September 2017 at COMSATS Institute of Information Technology (CIIT) Islamabad, Khyber Teaching Hospital (KTH), Peshawar and Biotech. Research Laboratory Rawalpindi after approval by the institutional ethical committee (CIIT ethics board). Written informed consent was acquired from all the patients about participation and publication of results before acquiring their samples. All the procedures were carried out in accordance with the Declaration of Helsinki. Initially, 150 patients were selected, out of which 100 patients with confirmed HCV 3a subtype were followed for treatment response. Patients enrolled in the current study were divided into two groups as Group A comprised of all those never treated before, and Group B that received previous anti-HCV therapy. The study population comprised of both male and female patients belonging to six ethnically diverse divisions of the KP province, which is the third largest province of Pakistan with a population of 27.8 million and is administratively divided into 7 divisions.

Baseline investigations included ALT levels, viral load, Hemoglobin (Hb), neutrophil and platelet counts. Viral load and ALT levels were monitored in all groups of patients at every 4 weeks while other hematological investigations were carried out every 2 weeks.

All the confirmed HCV genotype 3a patients fulfilling the inclusion criteria received Sofosbuvir (Sovaldi, 400mg tablets, Ferozsons Laboratories Pakistan) and Ribavirin (XOLOX, 400mg tablets, Ferozsons Laboratories Pakistan) combination therapy. Sovaldi was given orally at a dose rate of 400mg/day for a period of 24 weeks. Ribavirin was given orally twice a day to a total dose of 800mg (body weight <75 Kg) or 1200mg (body weight >75 Kg) per day for a period of 24 weeks. RVR was defined as undetectable HCV RNA after 4 weeks of therapy while the end of treatment response (ETR) was assessed at the end of therapy at week 24. SVR12 was assessed by measuring HCV viral RNA at 12 weeks after the end of therapy.

Qualitative PCR for detection of active HCV infection was carried out using primers targeting the 5' UTR of the HCV genome as described earlier[8]. Viral RNA quantification was carried out using Sacace HCV quantitative analysis kit (Sacace Biotechnologies Caserta, Italy).

HCV genotype determination was carried using a modified Type-specific nested PCR based genotyp-

ing protocol described recently by our group (8). PCR amplified products were detected using 2% agarose gel. A 100 bp or 50 bp DNA ladder was used as a DNA size marker for identification of PCR products.

SPSS version 20 was used for data analysis. Qualitative variables for Group A and Group B patients were defined using percentages, whereas quantitative variables were defined using mean and standard deviation. Pearson Chi-square test was used to determine the association of variables of interest and p-value<0.05 was considered statistically significant.

RESULTS

RVR and SVR 12 rates

Among100 confirmed HCV 3a infected patients that were prospectively followed for response to dual therapy, 44 % were females and 56% were male patients. Among patients (n=50) enrolled in Group A, 42% (n=21) were females and 58% (n=29) were male patients out of which RVR was achieved in all females and male patients (100%), while SVR12 was achieved among 100% females and 93.1% male patients. Group B patients (n=50) included 46% females (n=23) and 54% male patients (n=27) out of which, 8.7% (2/23) females and 11.1% (3/27) males had a negative RVR as well as SVR12 (Fig. 1).

Overall results of this study indicated that all the patients (100%) enrolled in Group A achieved RVR, while Group B patients showed an RVR of 92% (Fig. 1). Of note, SVR 12 was observed higher (96%) among treatment naïve patients in Group A as compared to group B (90%) (Fig. 1). The ETR and SVR12 rates were found to be the same for the two groups. Although no significant difference was observed in the SVR12 rates of patients in group A and B (Pearson Chi-Square test, p-value>0.05), the association of RVR and SVR, however, was found statistically significant (Pearson Chi-Square test, p- value<0.05).

Table 1: Baseline parameters in Group A (n=50) and Group B patients (n=50)

Variable	Treatment naive (n=50) Group A	Treatment experienced (n=50) Group B	p-value
Age (Year)	44±10	41±9.8	0.821
ALT (IU/ml)	67±10.8	68±9.5	0.791
Viral Load (IU/ml)	1.8 x 106 ± 1.2 x 10^5	1.6 x 106 ± 1.1 x 10^5	0.562
Hb (g/dl)	13.8 ± 2.8	13.4 ± 2.5	0.182
Neutrophils (103/ μl)	42 ±9	43 ±9	0.854
Platelet (103/ μl)	189 ± 82	176 ± 88	0.595

ALT, Alanine Aminotransferase; Hb, Hemoglobin; Data shown as mean ± standard deviation. A p-value of <0.05 was considered statistically significant.

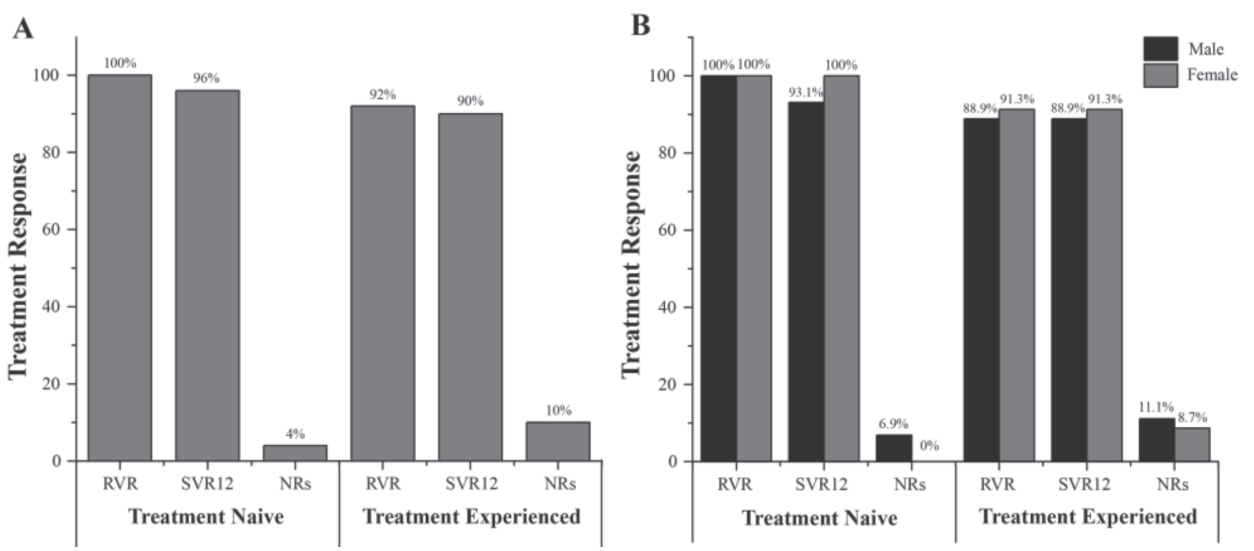

Figure 1: Frequency of RVR and SVR among treatment naïve and treatment experienced HCV 3a infected patients

Association with baseline characteristics

Mean age of all the patients included in the study was 41±10 (Range: 22-64 years). Mean age of patients included in Group A was low 41±9.8 as compared to 44±10 in Group B. Results indicated that patients of less than 40 years of age achieved a relatively higher SVR12 (94.34%) as compared to patients older than 40 years (91.5%).

Liver enzyme profiles and baseline characteristics including viral load, platelets, Hb and neutrophil counts are important in antiviral-response studies to assess disease severity, association with treatment response or treatment progress or initial treatment decisions. Various baseline characteristics of our study cohort are listed in Table 1. A higher ALT level (68±9.5) and relatively high baseline viral load ($1.8 \times 10^6 \pm 1.2 \times 10^5$) were observed among treatment naïve patients in Group A as compared to treatment-experienced patients in Group B (Table 1). However, the baseline viral load, Hb, ALT levels, Platelet and neutrophil counts did not differ significantly between the two treatment groups (Pearson Chi-Square test, *p*-value>0.05) (Table 1). Monitoring of viral load and ALT levels during the study period indicated the same trend with no significant association with treatment response among the treatment naïve and treatment experienced groups (Pearson Chi-Square test, *p*- value>0.05).

DISCUSSION

Distribution of HCV genotypes is geographically variable reflecting differences in the transmission modes, ethnicity, response to antiviral therapy and disease severity. HCV genotype 3a is being considered the most difficult genotype to treat and thus represents a major challenge in the evolving era of anti-HCV therapy[18,19]. Treatment of chronic HCV infection has evolved from IFN-based regimens to more effective and well tolerated INF-free treatments of DAA like Sofosbuvir and RBV[20]. Sofosbuvir is an oral nucleotide inhibitor targeting the key steps in the viral life cycle. When administered in a combination regimen with RBV, it is claimed to have achieved unprecedentedly high SVR among the chronically infected Hepatitis C patients. However, it is widely accepted that the Sofosbuvir and RBV combination therapy has suboptimal therapeutic efficacy in patient populations infected with hepatitis C genotype 1 and 3, particularly those with advanced hepatic fibrosis and those with a previous failure to IFN based therapy[21]. HCV 3a is highly prevalent in Pakistan accounting for 70-90% of all the HCV infections. As treatment efficacy of Sofosbuvir is genotype specific and have variable response rates against HCV variants, we designed this longitudinal study to address the therapeutic efficacy of the oral regimen of Sofosbuvir in combination with RBV in chronically infected HCV 3a patients from six ethnically diverse divisions of KP province of Pakistan.

Among 100 patients that were prospectively followed for response to dual therapy, RVR was achieved in all female and male patients (100%) of the treatment-naive Group A, while SVR12 was achieved among 100% female and 93.1% male patients. In the treatment-experienced Group B, 8.7% (2/23) of female and 11.1% (3/27) of male patients had a negative RVR as well as SVR12. Both RVR and SVR12 rates in the treatment-naïve, as well as treatment-experienced groups were comparatively higher in the case of female gender which has been reported earlier for IFN-based interventions[22]. Moreover, results indicated that patients <40 years of age achieved a statistically non-significant but relatively higher SVR12 (94.34%) as compared to patients older than 40 years (91.5%). However, it has been demonstrated that with the more recent generations of DAA, factors predictive of treatment response may not have the same significance and strength as reported earlier[23]. In the era of modern DAA, age does not significantly influence treatment response. This finding is also supported by recent studies demonstrating that the efficacy of IFN-free DAAs in patients over 70 is similar to that of younger age groups[24].

Baseline Viral kinetics, ALT and HCV genotype are the three strongest predictors of response to IFN-based antiviral therapies[23,25] but their importance for IFN-free regimens needs to be determined in different ethnic groups. In the present study, the observed baseline viral load was found relatively higher in Group A patients with SVR of 96%, whereas a relatively lower baseline viral load was observed in treatment-experienced patients enrolled in Group B with an SVR of 90%, however it was not significant just as the other parameters listed in Table 1. In the current DAA era, the baseline viral load and ALT levels appear to have little impact on the likelihood of achieving an SVR.

Viral load assessment at 4 weeks of therapy defined as RVR has proven useful in making therapeutic decisions about HCV infection[25]. In the present study, we observed a significant association between RVR and SVR (Pearson Chi-Square test, *P*-value<0.05). Treatment naïve patients in Group A achieved a better RVR of 100% and an SVR 12 of 96%, while those who received prior treatment in Group B achieved an RVR of 92% and an SVR12 of 90%. Previous results from both the POSITRON and FUSION studies showed that

Sofosbuvir given in combination with RBV resulted in a profound decrease (97% to 99%) in circulating levels of HCV RNA by week 4, among both HCV genotype 2 and 3 infected patients. Recently, SVR12 of 90% was observed by Nizammudin et al.[26] in a local study in Peshawar while assessing the efficacy of 24 weeks regimen of Sofosbuvir and RBV in treatment-naïve chronic HCV infected patients of KP[26], which is lower than the SVR12 rate observed in case of the treatment naïve group in our study. However, their study was limited to the selection of a small local ethnic group of only treatment-naïve patients with an unknown status of HCV genotype, while the current study focused only on HCV 3a infected treatment-naive as well as treatment-experienced patients of various ethnic backgrounds from six divisions of the KP province.

Response rates among patients with HCV genotype 3 infections have also been shown to vary according to treatment history and patient status with respect to cirrhosis. Observed SVR rates in Group A cohort is consistent with results from VALENCE study, a multicenter phase 3 trial in which the SVR rates observed were low with 12 weeks course of SOF and RBV, but in the case of treatment-naïve patients with genotype 3 who received a 24-week treatment course of SOF and RBV, the SVR12 rates were much higher. SVR12 was achieved in 95% and 92% of treatment-naïve patients and 87% and 62% in previously treated patients with or without cirrhosis[15].

Although we report SVR rates irrespective of the patient status of cirrhosis, it has been observed that presence of cirrhosis did not significantly impact results for the treatment-naïve genotype 3 patients with 24weeks treatment course. Comparable results were seen in a phase 2 trial study by Gane et al. reporting SVR of 100% in treatment-naïve patients treated with dual therapy of SOF and RBV[27]. In treatment-experienced cohort placed in Group B, observed SVR rates were higher than those reported previously. A history of prior treatment failure had a significant influence on the probability of response to IFN based antiviral therapy[28]. However, in the era of DAA, prior treatment failure with PEG-IFN does not appear to have the same impact on treatment response. We found an SVR rate of 90% in Group B patients that were NRs to dual therapy of either conventional or PEG-IFN. SVR rates reported previously in VALENCE trial were low (62%) when compared to SVR rates observed in our study. The difference in response rates of the same regimen can be attributed to the difference in ethnic population.

In FUSION trial the rate of SVR12 in patients with cirrhosis was especially low in the HCV genotype 3 group[29]. Studies from neighboring country India provided real-life experience results from a treatment cohort of Indian HCV genotype 3 patients that received a 24 weeks course of SOF and RBV, with SVR12 rates at 96±98% irrespective of previous treatment history or disease severity[30,31]. These reports indicate that oral treatment of chronic HCV infected genotype 3 patients with Sofosbuvir and RBV can result in better treatment outcomes. Resistance to dual therapy in these groups indicates that highly resistant types of HCV 3a also exist in our country which needs to be characterized thoroughly. Irrational use of previous regimens seems to have exerted enough selection pressure on HCV 3a to evolve into resistant HCV variants which could pose a challenge to the treatment strategies adopted in future.

CONCLUSION

Dual therapy of oral Sofosbuvir and RBV for 24 weeks resulted in high SVR rates and can be considered safe, tolerable and effective regimen both in treatment naïve and treatment experienced HCV 3a patients. Although some resistance to dual therapy existed in Pakistani population, this treatment can substitute PEG-IFN based antiviral therapy and may make possible treatment of a significant number of patients who are either unable to be treated with or who previously failed to have a response to IFN based antiviral therapy.

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AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under:

Ali I:	Conception,Study designing Planning, experimentation data analysis, manuscript Writing review, Facilitation for reagents and materials.
Gul A:	Conception Study designing data Compilation manuscript Writing.
Sahar AN:	Data Collection, Experimentation,data Comilatioin, manuscript.
Khan SN:	Data analysis, Statistical Analysis,Discussion,Critical Review.
Rahman S:	Experimentation, Manuscript Writing.
Khan IA:	Manuscript Writing ,Critical Review.
Jamil J:	Statistical Analysis Critical Review.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.