Journal of Population Therapeutics & Clinical Pharmacology

RESEARCH ARTICLE
DOI: 10.53555/jptcp.v30i19.3642

DRUG RESPONSE OF UNTYPABLE HCV ISOLATES TO CURRENT DIRECTLY ACTING ANTIVIRAL (DAA) REGIMEN IN PAKISTAN

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Abstract:
An increased prevalence of diagnostically untypable HCV isolates has been reported for the past few years in Pakistan and the efficacy of DAA-based treatment regimens can be significantly affected by HCV genotypes and subtypes. The Current study aimed to evaluate the drug response of diagnostically untypable isolates to existing DAA based treatment regimens in Pakistan. Drug response was evaluated by an observational study including 192 patients infected with an untypable genotype with 101 patients receiving Sofosbuvir-Daclatasvir (SOF-DCV) and 91 receiving Sofosbuvir-Ribavirin (SOF-RBV) for 12 weeks and by monitoring Rapid virologic response (RVR) at 4 weeks of initiating therapy, end of treatment (EOT) response at 12 weeks and sustained virologic response at 12 weeks (SVR12) after EOT. Overall, SVR12 of untypable genotypes against the current treatment regimen was 83.3% (160/192). The untypable genotype responded significantly well to SOF-DCV as compared to SOF-RBV with an SVR12 rate of 91.0% and 74.7% respectively. The proportion of non-responders to SOF-DCV (6.9%) was significantly less as compared to non-responders to SOF-RBV (19.7%). Also, relapse rates were significantly high in the group treated with SOF-RBV (5.4%) as compared to SOF-DCV (1.9%) ($p<0.05$). Efficacy of both regimens varied significantly with age ($p>0.05$) but did not vary with gender. A Pangenotypic SOF-DCV treatment regimen is found as an effective regimen for these circulating untypable genotypes.

Keywords: HCV, Untypable isolates, DAAs, Sofosbuvir, Ribavirin, Daclatasvir, Drug response, RVR, SVR, EOT

1. Introduction

Hepatitis C virus (HCV) is an enveloped, positive sense, single-stranded RNA virus of the family Flaviviridae responsible for causing 58 million chronic HCV infections (WHO, 2021; Martinez & Franco, 2020; Mumtaz et al., 2020; Shi & Suzuki, 2018; Li et al., 2015; Kim et al., 2013). HCV exhibits a high genetic heterogeneity driven by a high level of viral replication, error prone nature of RNA-dependent RNA polymerase and the immune pressure of the host. The estimated mutation rate for HCV is $1.51.5 \times 10^{-3}$ to 2.0×10^{-3} nucleotide substitutions per site per genome per year. All these factors lead to a high heterogeneity exhibited as uninterruptedly evolving quasispecies in the same host and in the form of globally circulating genotypes and their subtypes (Martinez & Franco, 2020, Echeverría et al., 2015; Domingo et al., 2012; Ribeiro et al., 2012; Cuevas et al., 2009). HCV has been classified into 8 genotypes and 86 subtypes based on this diversity in the nucleotide sequences with significant differences in their geographical distribution patterns (Hedskog et al., 2019; Borgia et al., 2018; Schnell et al., 2018; Smith et al., 2014). Classification of the *Hepatitis C virus* into genotypes has clinical significance and is critical for elucidating viral evolution, their spread, and transmission routes from endemic areas. Diverse geographical patterns of spread can provide epidemiological markers to trace the source of an outbreak (Sharafi et al, 2015; Humphreys et al, 2012; Wasitthankasem et al, 2015). Recently developed pangenotypic DAAs are effective against all the genotypes dramatically increasing the sustained virologic response in chronic hepatitis C (CHC) with an SVR of above 95% in clinical trials (Kanda et al., 2019; Mera et al., 2019; Terrault et al., 2016; Feld et al., 2015). However, responses of genotypes vary with these DAAs, and the emergence of resistance associated variants and resistance associated substitution (RAS) have been documented in clinical trials and real world settings with CHC (Xie et al., 2022; Hezode et al., 2018; Wei et al., 2018; Sarrazin et al., 2016). In Pakistan, genotype 3a is the predominant type, however, for the past few years an increase in the prevalence of untypable isolates have been reported and highlighted in numerous studies (Qamar et al., 2021; Haqqi et al., 2019; Khan et al., 2019; Wahid et al., 2019; Zahid et al., 2019; Zafar et., 2018; Afzal et al., 2014; Ali et al., 2014; Waqar et al., 2014; Rauf et al., 2013; Safi et al., 2012; Ali et al., 2011; Idrees et al., 2011; Mahmood et al., 2011; Waqar et al., 2011; Ahmad et al., 2011). Exploration of these diagnostically untypable isolates and their drug responses to existing DAA based treatment regimen in Pakistan is needed to provide data regarding the efficacy of these regimens for patients infected with untypable isolates. It is also required to improve the existent treatment regimens along with finding alternative therapies and the need for RAS screening in case of virological failure (Zafar et al., 2018; Afzal et al., 2014). Moreover, with the second largest global HCV burden (10 million infections), Pakistan is a high-focus country for the global reduction of HCV by 2030 under the global health sector strategy (GHSS). Pakistan has developed its National Hepatitis Strategic Framework (NHSF) from 2017–21 in line with GHSS, and through national and provincial Hepatitis Control programs, the government is providing free of cost diagnosis and DAA based treatment. Elimination of HCV requires massive screening and subsequent treatment of the infected populations. Consequently, the distribution of different genotypes particularly rapidly increasing untypable genotypes has fundamental implications concerning translational efforts aimed at eradicating HCV particularly related to HCV treatment. Therefore, the current study has been designed to evaluate the efficacy of existing treatment regimens on these untypable isolates.

2. Material and Methods

2.1. Study Design and Inclusion criteria

An observational study was conducted in different hospitals in coordination with the physicians treating CHC**.** Viral RNA positive serum samples referred by collaborating physicians were collected along with informed consent and basic information regarding age and gender. A total of 1035 samples were collected and genotyped by following Ohno et al, (1997) protocol. Out of 1035 samples, 204 samples were found untypable. Out of these 204, 192 patients aged ≥15 years, non-cirrhotic, treatment naïve, with no other comorbidities including coinfection with Hepatitis B Virus, HIV or renal disease receiving treatment for HCV were enrolled in this study.

2.2. Treatment Regimen

Patients were divided into two groups: one group was treated with sofosbuvir (SOF) (400 mg/day) and daclatasvir (DCV) (60 mg/day) for 12 weeks (SOF-DCV) and other group was treated with SOF (400mg/day) and ribavirin (RBV) according to body weight (1,200 mg/day for > 75kg and 600mg/day for < 75kg) for 12 weeks. Required pretreatment, ongoing and post treatment assessments as suggested by the physician were recorded using patients reports.

2.3. Treatment Efficacy

Efficacy of the drug was analyzed by using Rapid Virologic Response (RVR), End of treatment (EOT) response and Sustained Virologic Response (SVR12). Rapid Virologic Response was defined as a negative PCR (undetectable HCV RNA) at week 4 of receiving treatment, EOT was defined as a negative PCR at week 12 on completion of treatment and SVR12 was a negative PCR at 12 weeks after completion of treatment.

2.4. Statistical Analysis

Pearson Chi-Square test (χ^2) was used for analysis of all the variables (SPSS version 17) and results were interpreted at 5% level of significance.

3. Results

3.1. Treatment Efficacy of Untypable isolates

All of 192 patients, including 51.5% (99/192) males and 48.4% (93/192) females, were receiving HCV treatment for the first time (treatment naive). Age of the patients ranged from 15-80 years. All patients had elevated ALT levels (9.08- 69.00 U/L) and hemoglobin levels (9.6-13.57 g/dl). One hundred and one (n=101) patients received treatment with Sofosbuvir-Daclatasvir (SOF-DCV) and 91 patients received Sofosbuvir-Ribavirin (SOF-RBV) treatment. Overall, Sustained Virological Response (SVR) of untypable genotypes against current treatment regime was 83.3% as 160/192 patients achieved SVR, 12 weeks after completion of treatment. Untypable genotype responded significantly well to treatment regime SOF-DCV as compared to SOF-RBV as SVR rate was high in SOF-DCV treated patients (91%) as compared to SOF-RBV patients (74.7%). Moreover, proportions of non-responders to SOF-DCV 6.9% (7/101) were significantly less as compared to non-responders to SOF-RBV 19.7% (18/91). Also relapse rate were significantly high in the group treated with SOF-RBV 5.4% (5/91) as compared to SOF-DCV 1.9%. (2/101) ($p<0.05$, $\chi^2=10.586$, $p=0.03$) (Table 1 and figure 1).

Table 1 Efficacy of different treatment regimens against untypable HCV isolates

Parameters	Sofosbuvir-Daclatasvir n=101		Sofosbuvir-Ribavirin n=91	
	Frequency	Percentage	Frequency	Percentage
RVR	80	79.2%	51	56.0%
EOT	94	93.0%	73	80.2%
SVR	92	91.0%	68	74.7%
Relapse	2	1.9%	5	5.4%
Non responders	5	4.9%	18	19.7%

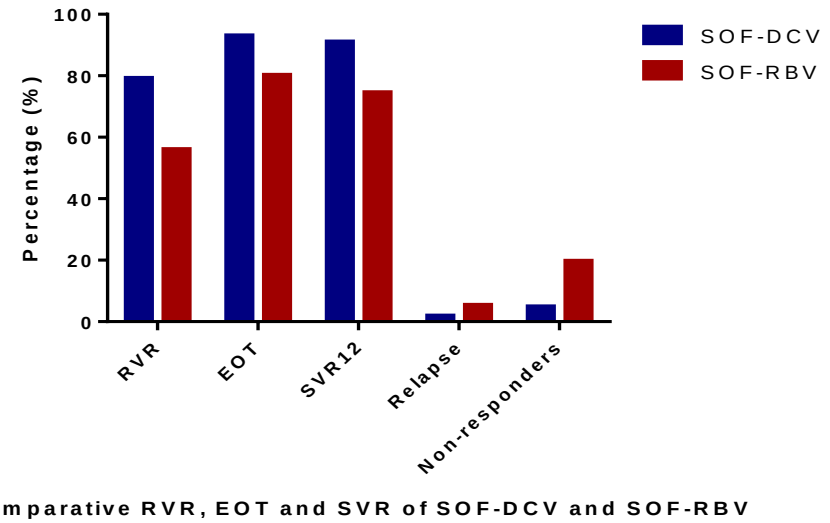

Figure 1: Comparative RVR, EOT, SVR, relapse and non-responders of SOF-DCV and SOF-RBV

3.2. Treatment efficacy of sofosbuvir-daclatasvir

Out of 101, 79.2% (80/101) of patients achieved an RVR on completion of four weeks of treatment while 94% (94/101) of patients achieved an EOT response on completion of 12 weeks of treatment, and 91% (92/101) achieved an SVR12 after 12 weeks of completion of their treatment. HCV relapsed among only 1.9% (2/101) patients while 5.9% (6/101) patients did not respond to any of the drugs. One patient from this group died due to a road accident and one did not follow up (Table 1).

3.2.1. Gender based treatment efficacy of sofosbuvir-daclatasvir

Rapid Virological Response (RVR) was achieved by 84.3% (43/51) of males and 74% (37/50) of females. Sustained Virological Response was achieved by 94.1% (48/50) of males and 88% (44/51) of females. Four percent of females relapsed 4% (2/50) as 3 out 50 females had positive PCR after 12 weeks. Amon patients who did not respond to this treatment regimen 6% (3/50) were females and 3.9% (2/51) were males. (Table 2, Figure 2). No significant difference was observed in treatment efficacy of this treatment regimen between males and females ($p>0.05$, $\chi^2=1.358$, $p=0.8$).

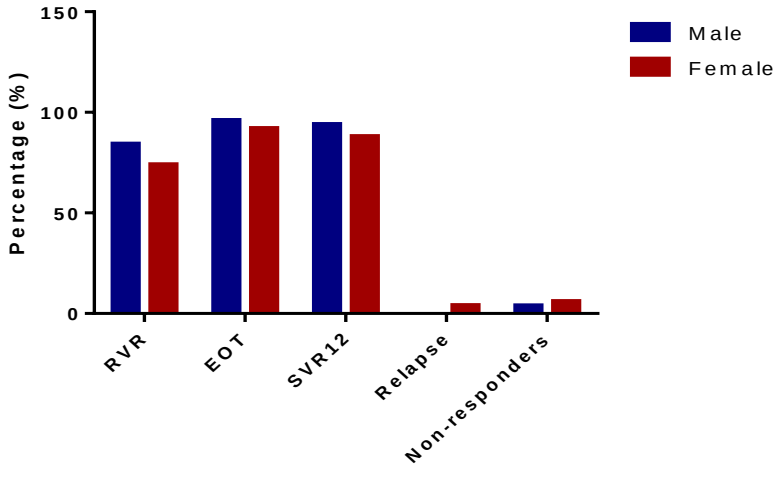

Figure 2: Gender based comparative RVR, EOT and SVR of SOF-DCV in patients infected with untypable HCV genotype and it also shows the relapse rate and non-responders among male and female patients

3.2.2. Age based treatment efficacy of sofosbuvir-daclatasvir

Among age groups, significantly high number of patients 85.7% (35/91) from age group >50 years achieved RVR as compared to 20% (2/10) in age group ≥50 years. Likewise, proportion of patients achieving EOT and SVR was significantly high in age group >50 where 95.6% (87/91) achieved EOT and SVR as compared to age group ≥50 years where 70% (7/10) of patients achieved EOT and 50% (5/10) achieved SVR. None of the patients from young age group >50 years relapsed. However, relapse rate was significantly high in age group ≥50 years where 20% (2/10) patients relapsed (Table 3 Figure 3). A significant number of patients from age group ≥50 years 30% (3/10) did not respond to this treatment regimen as compared to 2.1% (2/91) from age group >50 years ($p<0.05$, $\chi2=68.513$, $p=0.001$).

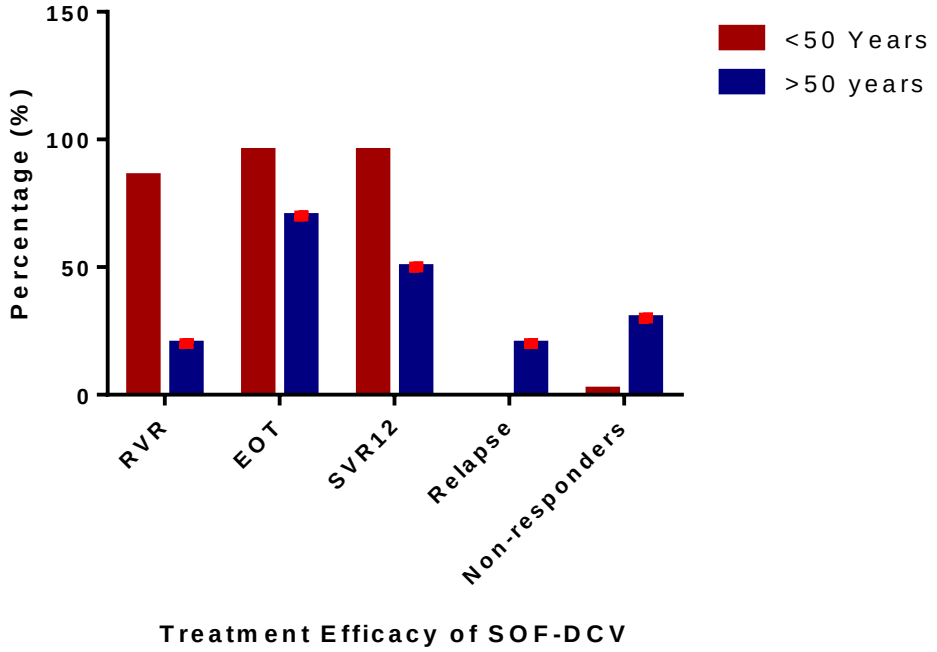

Figure 3. Age based efficacy of SOF-DCV against untypable isolates

Table 2. Gender based efficacy of SO-DCV and SOF-RBV

	SOF-DCV				SOF-RBV			
Parameter	Males n=51		Females n=50		Males n=48		Females n=43	
	F	%	F	%	F	%	F	%
RVR	43	84.3	37	74	21	43.7	30	69.7
EOT	49	96	46	92	38	79.1	35	81.3
SVR	48	94.1	44	88	35	72.9	33	76.7
Relapse	0	0	2	4	2	4.1	3	6.9
Non-Responders	2	3.9	3	6	10	20.8	8	18.6

3.3. Treatment Efficacy of Sofosbuvir-Ribavirin

Out of 91 patients ,56% (51/91) achieved RVR at four weeks while 80.2% (73/91) of patients achieved EOT response on completion of 12 weeks of treatment. About 74.7% (68/91) achieved a SVR12 after 12 weeks and 5.4% (5/91) cases were relapsed after 12 weeks of treatment. While 19.7% (18/91) were found to be non-responders (Table 1 Figure 1).

3.3.1. Gender based treatment efficacy of sofosbuvir-ribavirin

Rapid Virological Response (RVR) was achieved by 43.7% (21/48) males and 69.7% (30/43) females and SVR was achieved by 72.9% (35/48) males and 76.7% (33/43) of females. achieved

SVR as compared to 72.9% (35/48) males. Relapse rate in male was 1% (2/48) and in females it was found to be 6.9% (3/43) as 2 out of 48 men and 3 out of 43 of females had positive PCR after 12 weeks whereas. Among non-responders 20.8% (10/48) males and 18.6% (8/43) females (Table 2, Figure 4). No significant difference was observed between efficacy of SOF-RBV between males and females ($p>0.05$, $\chi^2=2.151$, $p=0.7$).

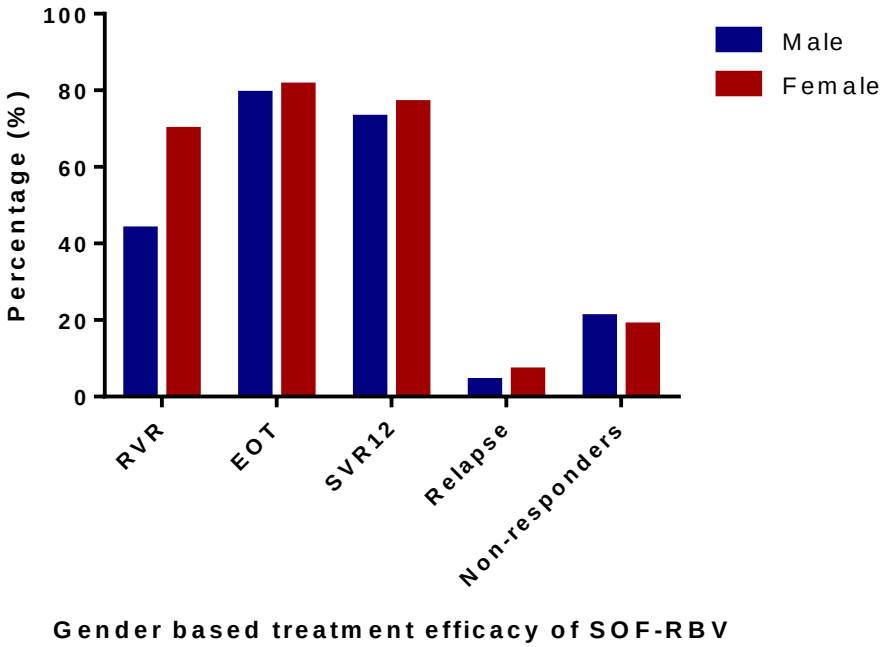

Figure 4. Gender based comparative RVR, EOT and SVR of SOF-RBV in patients infected with untypable HCV genotype and it also shows the relapse rate and non-responders among male and female patients

3.3.2. Age based treatment efficacy of sofosbuvir-ribavirin

Among age groups, significantly high number of patients 64.1% (50/78) from age group >50 years achieved RVR as compared to 7.6% (1/13) in age group ≥50 years. Likewise, proportion of patients achieving EOT and SVR was significantly high in age group >50 where 85.8% (67/78) achieved EOT and 80.7% (63/78) attained SVR as compared to age group ≥50 years where 33.3% (6/13) of patients achieved EOT and 38.1% (5/13) achieved SVR. Relapse rate was found to be significantly high among patients of age group ≥50 where 7.6% (1/13) patients relapse as compared to 5.1% (4/78) of patients from age group >50 (Table 3, Figure 5). A significant number of patients from age group ≥50 years 53.8% (7/13) did not respond to this treatment regimen as compared to 14.1% (11/78) from age group >50 years ($p<0.05$, $\chi2=17.938$ $p=0.02$).

Table 3. Age based efficacy of SOF-DCV and SOF-RBV against untypable HCV isolates

Drug	Parameter	Age group			
		>50 Years N=91		50 ≤Years N=10	
SOF-DCV		Frequency	Percentage	Frequency	Percentage
	RVR	78	85.7%	2	20%
	EOT	87	95.6%	7	70%
	SVR	87	95.6%	5	50%
	Relapse	0	0	2	20%
	Non-Responders	2	2.1%	3	30%
Drug	Parameter	Age group			
SOF-RBV		>50 Years		50 ≤Years	

		N=78		N=13	
		Frequency	Percentage	Frequency	Percentage
	RVR	50	64.1%	1	7.6%
	EOT	67	85.8%	6	33.3%
	SVR	63	80.7%	5	38.1%
	Relapse	4	5.1%	1	
	Non-Responders	11	14.1%	7	53.8%

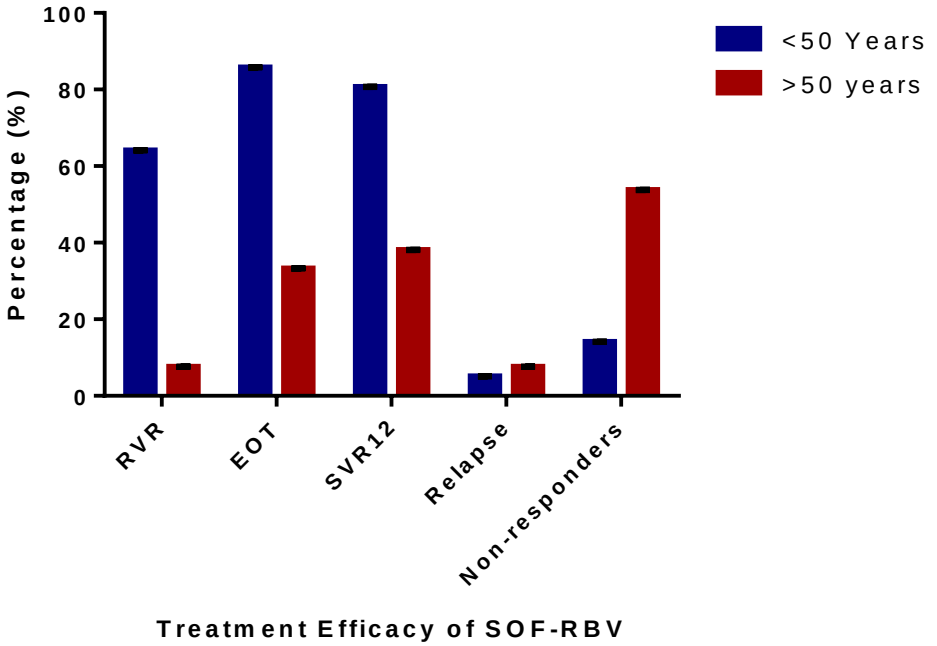

Figure 5. Age based efficacy of SOF-RBV against untypable isolates

4. Discussion

HCV genome exhibit high heterogeneity owing to its very high mutation rate and numerous studies have documented that progression, complexity, clinical manifestations and therapeutic response is determined by genotype. In Pakistan, genotyping is routinely done by using Ohno et al, (1997) method which cannot detect mixed and untypable genotypes (Zahid et al., 2020). Emergence of large number of untypable isolates particularly presence of these isolates among non-responders and relapsers is alarming and little information about genetic diversity of HCV isolates in these patients is available (Ahmad et al., 2011). In Pakistan, different studies have highlighted the need of characterization of increasing number of untypable isolates to solve this mystery of untypable isolates (Zafar et., 2018; Afzal et al., 2014). In current study patients with untypable genotype were treated with generic sofosbuvir-based interferon-free DAAs and. The SVR12 rate for SOF-RBV in our study is less as compared to previous studies and clinical trials including VALENCE, POSITRON and FUSION for SOF-RBV for genotype 2 and 3 (Dalgard et al., 2017 Lawitz et al., 2015; Zeuzem et al., 2014; Jacobson, 2013). Our results are comparable to Yen et al, (2022) who have found an overall SVR rate of 96.5% in HCV-infected patients with mixed-genotype or undetermined (untypable) receiving pan-genotypic DAA therapy. A study has reported a 100% SVR rate in patients infected with mixed genotypes using pan-genotypic regimens and found an overall SVR rate of 96.5% in HCV-infected patients with mixed and undetermined genotypes receiving pan-genotypic DAA therapy but number of untypable samples in their study was small as compared to our study (Ding et al., 2021). Our results are in line with Wahid et al, (2019) who have reported that efficacy of DAAs is less in elderly population of Pakistan and clinicians needs to prescribe SOF-DCV instead of SOF-RBV. A study has documented excellent EOT of 98.7% in patients

treated with SOF-RBV regimen irrespective of previous treatment history, genotype, age, and viral load (Jamil et al., 2018). Our results are comparable to ALLY-3 phase III trials which have reported a SVR12 rate of 90.1% in treatment naive patients with genotype 3 treated with SOF-DAC (Nelson et al., 2015). In this study about 19.7% of patients treated with SOF-RBV and 4.9% of patients treated with SOF-DCV did not respond to treatment and 1.9% of patients treated with SOF-DCV and 5.4% of patients treated with SOF-RBV relapsed. It can be explained on the basis of resistance due to RAS, lower SVR rates are associated with presence of clinically important RAS before treatment (basal RAS). It is generally believed that HCV treatment outcome is significantly affected by the presence of low frequency variants within viral populations (Zeuzem et al., 2017; Sarrazin et al., 2016). Most of the relapsed patients have clinically important RAS detectable by Sanger sequencing at the time of virological failure but there are only few reports regarding their selection from low frequency variants following their emergence as a result of DAA pressure. This highlights the need of monitoring of RASs which can remain undetected by Sanger sequencing (Dietz et al., 2018; Di Maio et al., 2018; Perales et al., 2018). In this study 20.8% patients treated with SOF-DCV and 44% treated with SOF-RBV did not achieve RVR and continued to have detectable RNA, however, almost 57% of these achieved SVR. A study Carver et al, (2020) has documented high SVR rates in patients having persistent viremia as well as RVR. For relapsers and non-responders treatment duration can be extended, alternative combinations of DAAs can be used. Mostly, extending therapy for 24 weeks can help in achieving SVR in patients. However, RAS screening can also be considered for such patients. There are few limitations to our study, treatment experience patients, cirrhotic patients and patients with coinfections were not included in study. Overall, untypable isolates have shown a better response to pangenotypic SOF-DCV and this combination can be successfully used to treat patients with untypable as well as all genotypes. However, patients with virologic failure need to be carefully assessed for RAS associated with particular treatment regimen.

5. Conclusion

Current pangenotypic therapy SOF-DCV is very effective for circulating genotypes as an SVR of 91% can be achieved using Sofosbuvir and Daclatasvir for 12 weeks in patients with untypable isolates without determination of genotype. And SOF-RBV needs to be avoided without determination of genotype. Diagnostics and treatment regimens need to be updated. More directly acting antivirals should be added in national *Hepatitis C*ontrol program. Screening of RAS in non-responders and relapsers needs to be encouraged.

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