JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Diagnostic approach to elucidate the efficacy and side effects of direct-acting antivirals in HCV infected patients

Sonia Younas[1,2], Hamid Mukhtar[3], Umar Farooq Gohar[3], Abdullah Alsrhani[4], Badr Alzahrani[4], Kashaf Junaid[4], Muhammad Usman Qamar[5], Hasan Ejaz[4]

[1] HKU-Pasteur Research Pole, School of Public Health, LKS Faculty of Medicine, The University of Hong Kong, Hong Kong, China
[2] Department of Microbiology and Molecular Pathology, Al Razi Healthcare Lahore, Pakistan
[3] Institute of Industrial Biotechnology, Government College University Lahore, Lahore, Pakistan
[4] Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Jouf University, Al Jouf, Saudi Arabia
[5] Department of Microbiology, Faculty of Life Sciences, Government College University Faisalabad, Pakistan

Abstract

Introduction: The conventional interferon therapy of hepatitis C virus has been substituted substantially with sofosbuvir and daclatasvir due to constraints in efficacy and tolerability. This study aimed diagnostically to monitor the effectiveness and side effects of direct-acting antivirals in the management of HCV infections.

Methodology: This prospective study was conducted on HCV-infected patients treated with sofosbuvir and daclatasvir. Different serological, biochemical, hematological, and molecular techniques were used for the assessment of patients. Only treatment-naive patients aged ≥ 18 to 75 years received 12 weeks of treatment. The primary endpoint was a sustained virologic response with undetectable HCV RNA in the patients' serum at the end of the treatment.

Results: We identified 229 cases of confirmed HCV infections by PCR, 94.3% of which had genotype 3. The study population comprised 66% females and 34% males with a median age of 42.2 ± 10.6 SD. Ninety-three percent of the patients accomplished SVR at week 12. The combined therapy of SOF/DAC achieved the highest efficacy rate (92.6%) among the different HCV genotype 3 patients. A statistically significant relationship was observed between low baseline viral load ($p < 0.001$; 95% CI = 1.2-3.1) and HCV genotype 3 with minor side effects, including lethargy, headache, nausea, insomnia, diarrhea, and fever.

Conclusions: HCV-infected patients can be treated well with an interferon-free SOF/DAC regimen, tolerated with generally mild adverse effects with a higher SVR.

Key words: Hepatitis C virus; sofosbuvir; daclatasvir; HCV genotypes; direct-acting antiviral drugs; sustained virologic response.

J Infect Dev Ctries 2021; 15(10):1489-1496. doi:10.3855/jidc.12912

(Received 29 April 2020 – Accepted 12 February 2021)

Introduction

Chronic hepatitis C (HCV) infection is a leading cause of persistent liver disorder, cirrhosis, and liver cancer, which could be a crucial indication for liver transplantation [1]. Every year nearly 700,000 patients die from untreated chronic HCV, making this infection a global health issue. Antiviral therapy may reduce the burden of HCV infections; but, due to the lack of apparent symptoms, the majority of patients remain unaware of their disease [2]. Approximately 71 million people worldwide suffer from this bloodborne viral infection, and there is no vaccine available to prevent infection [3]. Chronic HCV infection develops in about 80% of those exposed to the virus [4]. The occurrence rate of HCV infections varied worldwide and was highest in Egypt, followed by Pakistan as the second-highest county globally [5].

The transmission of HCV is mainly due to substandard and poor health system-related practices. Reused or unsterile syringes, blood transfusion centers, unlicensed dental and organ transplant clinics play a primary role in transmitting hepatitis [6]. Most HCV infections have genotype 3, followed by 1, 2, and 4 [7]. Quantification of serum HCV RNA levels, liver enzymes, and HCV-specific antibodies (anti-HCV) in a patient blood specimen, used to detect the HCV infection. Ribavirin and peg-interferon (alfa-2a or alfa-2b), a combined regimen was used to treat the HCV

infections until 2011 to achieve a sustained virologic response (SVR) of 40-80% [8]. The use of ribavirin and peg-interferon combined therapeutic regimen is associated with substantial adverse effects that have shifted to new direct-acting antivirals (DAAs) [9,10].

Sofosbuvir (SOF) and daclatasvir (DAC) are new antiviral agents with good profile, lower toxicity, convenient usage, and fewer drug interactions. These antiviral drugs have higher efficacy and safety profiles for liver cirrhotic and non-cirrhotic patients [11]. SOF is a pan-genotypic nucleotide analog, an NS5B polymerase inhibitor, and is used in combination with other DAAs for HCV therapy [12]. DAC is a complex replication inhibitor of HCV NS5A with pan-genotypic activity and a pharmacodynamic profile that enables a single daily dosage [13]. A dose of 400 mg SOF and 60 mg DAC is recommended once a day for three months. SOF/DAC antiviral regimens have an affirmative safety profile, undoubtedly changing treatment options with superior efficacy and milder side effects. This combined therapy duration is shorter than the conventional interferon therapy and attains a high SVR level at three months of treatment [12]. In 2016, roughly half of countries, including Pakistan and Egypt, began using DAAs for chronic hepatitis C care, and this number is increasing with time. Current availability by several competitors of the generic DAAs has reduced HCV therapy costs in Pakistan [14].

The HCV treatment goal is to reduce complications and fatality by eliminating the infection [15]. The achievement of SVR shows that the virus has been eliminated from the body, and the viral RNA is undetectable after the completion of therapy and remains undetected afterward [16]. This study aimed to evaluate the effectiveness, side effects of SOF/DAC in various HCV genotypes and monitor the association of different diagnostic markers.

Methodology

Ethical approval and study design

The current prospective study was permitted ethically by the Al-Razi Healthcare Diagnostic Center, Lahore, Pakistan, and carried out according to the ethics guidelines of the Helsinki Declaration [17].

Selection criteria of the study population

We selected HCV-infected cases of treatment-naive patients from January to August 2018 who had never been given HCV therapy with interferon-containing regimens or those with any DAAs. All the patients were ≥ 18 to 75 years of age. The patients who had previous treatment history, liver transplant recipients, antiviral drug abusers, pregnant females, HIV, and HBV co-infected were excluded from the study.

Patient data collection

The patients who received 400 mg SOF and 60 mg DAC therapy once daily were monitored for SVR at 12 weeks (SVR_{12}) of treatment. During the therapy, different side effects noticed include fever, headache, lethargy, diarrhea, insomnia, and anxiety. The baseline serology, biochemistry, hematology, and molecular analysis with regular follow-up laboratory investigations were performed.

Baseline diagnosis

We processed a total number of 300 blood samples collected from suspected cases of HCV infection. The specimens processed for initial screening and automated analyzer Cobas P800 performed confirmation of HCV-specific antibodies [18]. The platelet count determination was carried out on the XE-5000 hematology analyzer (Sysmex, Chuo-ku, Japan). Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were performed on fully automated Modular Cobas P800. PreciControl Multi 1 and PreciControl Multi 2 were used as quality control of the assays [19].

Polymerase chain reaction (PCR)

Cobas AmpliPrep was used to extract, and denaturation of the HCV genome, and the real-time PCR performed HCV viral RNA quantification. HCV RNA concentrations measured to the lowest quantification (25 IU/mL) and detection (10 IU/mL) limit with Cobas TaqMan HCV test version 2.0 [20]. The standard used for quality control contained hepatitis C viral sequences, identical to primer attachment sites. The distinct pattern between HCV standard amplicon and target was achieved by a unique probe binding region. Each sample and control at a known copy number was included with the standard. The molecular steps started with the preparation of the specimen, reverse transcription, amplification, and detection. The RNA titer in the test samples was calculated in the Cobas TaqMan analyzer by comparing the HCV signals to the standard signals for individual specimens and control.

Genotyping

The HCV genotyping assay was accomplished using the AMPLIQUALITY HCV-TS (AB Analitica, Advanced Biomedicine, Padova, Italy) kit based on the reverse hybridization method. The biotin-labeled

amplicon of the viral 5'-UTR region hybridized to the HCV genotype-specific oligonucleotide probes immobilized on a nylon strip. Positivity of the stained control band was used to confirm the efficiency of the conjugate bonds, the substrate's reaction, and a reference for the alignment of the transparent film.

Statistical analysis

SPSS version 24 and GraphPad Prism were used for statistical analysis and data expression. We compared continuous variables by independent t-test and Chi-square and Fisher exact test used for the categorical variables (significant p-value < 0.05).

Results

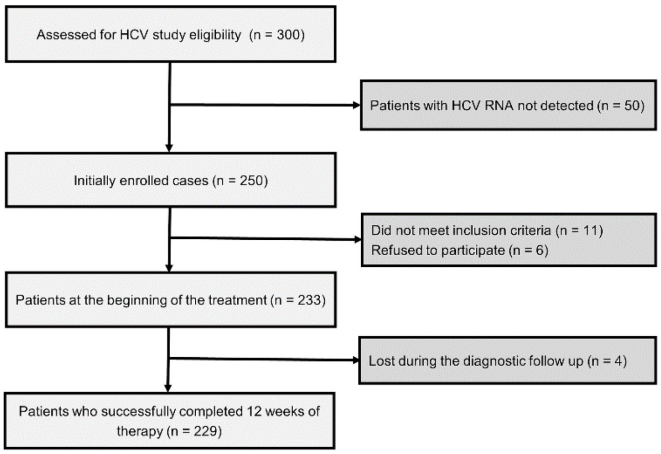
Out of 300, HCV screened patients, 250 were positive for PCR. A total of 17 patients who did not meet inclusion criteria and non-willing drug abusers were excluded from the study. A total of 233 patients who started HCV treatment followed up until week 12 of treatment using various diagnostic tests. The diagnostic follow-up tests could not be performed on the four patients who absconded during the treatment, and the final analysis was accomplished on 229 cases (Figure 1). Most of the patients in this study were from 40-50 years of age, with a mean age of 42.2 ± 10.6 SD. There were 150 (66%) enrolled females and 79 (34%) male patients. HCV genotyping results confirmed 216 (94.3%) cases of genotype 3 and 13 (5.7%) other genotypes. The baseline viral load was observed among HCV patients with a mean concentration of 6.02 log10 IU/ml. The baseline serum ALT levels were deranged in 186 (81.2%), while serum AST levels elevated in 175 (76.4%) cases. A low platelet count of < 150×10^9/µl was found in 26 (11.4%) cases (Table 1).

Table 1. Demographic features and baseline investigations of the patients at the beginning of therapy (n = 229).

Characteristic	Patient number n (%)
Mean age, years (SD)	42.2 ± 10.6 (18 to > 75)
Sex	
Female	150 (66)
Male	79 (34)
HCV genotypes and viral load	
Genotype 3	216 (94.3)
Genotype 1	10 (4.4)
Genotype 2	02 (0.9)
Genotype 4	01 (0.4)
Mean baseline HCV RNA, log_{10} IU/mL (range)	6.02 log_{10} IU/mL (4.11-9.32 log_{10} IU/mL)
Liver enzymes	
Serum ALT Mean ± SD	65.46 ± 48.55 IU/L
< 32	43 (18.8)
> 32	186 (81.2)
Serum AST Mean ± SD	56.81 ± 42.5 IU/L
< 33	54 (23.6)
> 33	175 (76.4)
Platelets	
Platelets $\times10^9$/L Mean ± SD	244.8 ± 79.6
< 150	26 (11.3)
150-400	201 (87.8)
> 400	02 (0.9)

HCV: Hepatitis C virus; ALT: Alanine aminotransferase; SD: Standard deviation; AST: Aspartate aminotransferase

Figure 1. Consort flow diagram of the cases selected for the study.



SVR_{12} was used to observe the response of the treatment with SOF/DAC. Out of 229 patients, the combined therapy of SOF/DAC achieved SVR in 212 (92.6%) patients; however, only 17 (7.4%) cases were found as non-SVR. The serum ALT, AST, and platelet count baseline characteristics had no statistical association with SVR cases (Table 2). Statistically significant relationship between low baseline viral load (p < 0.001; 95% CI = 1.2-3.1) and HCV genotype 3 (p = 0.01; 95% CI = 1.71-22.53) observed in SVR patients. Patients who achieved SVR had a mean viral load of 5.94 log_{10} IU/mL ± 0.94 SD, while the non-SVR cases had a higher mean viral load of 6.71 log_{10} IU/mL ± 0.55 SD (Figure 2).

A comparative biochemical and hematological analysis of serum ALT, AST, and platelet count at Week 0 (baseline) and after 12 weeks of antiviral usage showed statistically significant normal levels of ALT from 65.46 ± 48.55 IU/L to 26.57 ± 16.13 IU/L (p < 0.01) and AST from 56.81 ± 42.5 IU/l to 25.04 ± 15.38 IU/L (p < 0.01). A significant statistical difference was also seen in baseline platelet count and at week 12, with a p-value of 0.02 (Figure 3).

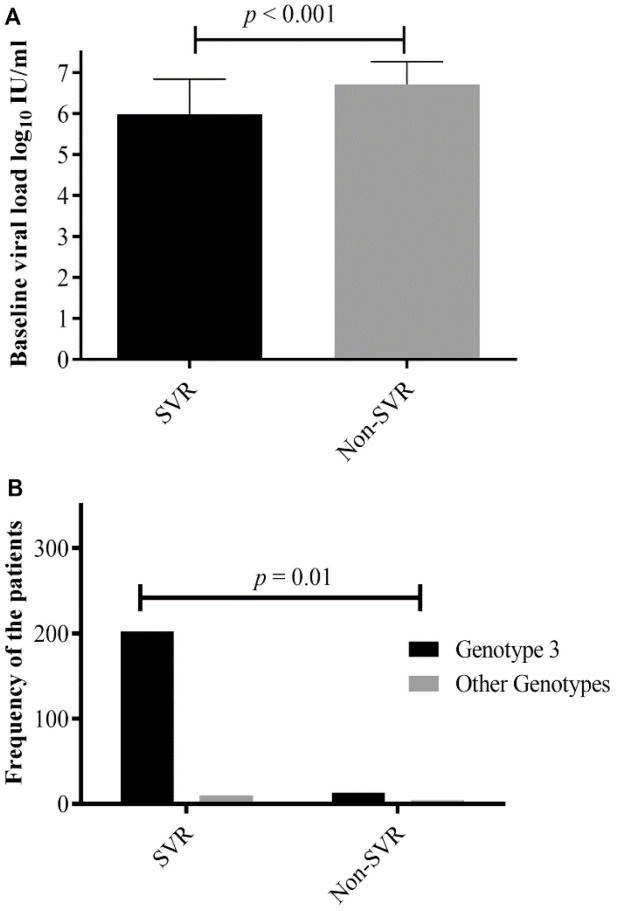
No cases of mortality or significant side effects were seen in patients after the treatment. However, the side effects associated with the SOF/DAC treatment include 32 (14%) cases of lethargy, 25 (10.9%) headache, 22 (9.6%) nausea, 17 (7.4%) insomnia, 12 (5.2%) fever, and 9 (3.9%) diarrhea (Figure 4).

Table 2. Relationship of SVR with baseline characteristics.

Characteristics		SVR (n = 212)	Non-SVR (n = 17)	*p*-value (95% CI)
Viral load log10 IU/mL (Mean ± SD)		5.94 ± 0.94	6.71 ± 0.55	< 0.001 (1.2-3.1)
Liver enzymes				
ALT (Mean ± SD)	26.57 ± 16.13			0.44 (0.4-8.17)
	< 32	41 (19.3%)	2 (11.8%)	
	> 32	171 (80.7%)	15 (88.2%)	
AST (Mean ± SD)	25.04 ± 15.38			0.99 (0.31-3.21)
	< 33	50 (23.5%)	4 (23.5%)	
	> 33	162 (76.4%)	13 (76.5%)	
Platelets				
Platelet count (Mean ± SD)		246.4 ± 78.75	225.3 ± 91.60	0.30 (-61–18.54)
	< 150	24 (11.3%)	2 (11.8%)	
	150-400	186 (87.8%)	15 (88.2%)	
	> 400	2 (0.9%)	0	
HCV genotypes				
Genotype	3	203 (95.7%)	13 (76.5%)	0.01 (1.71-22.53)
	1	07 (3.3%)	3 (17.6%)	
	2	1 (0.5%)	1 (5.9%)	
	4	1 (0.5%)	0	

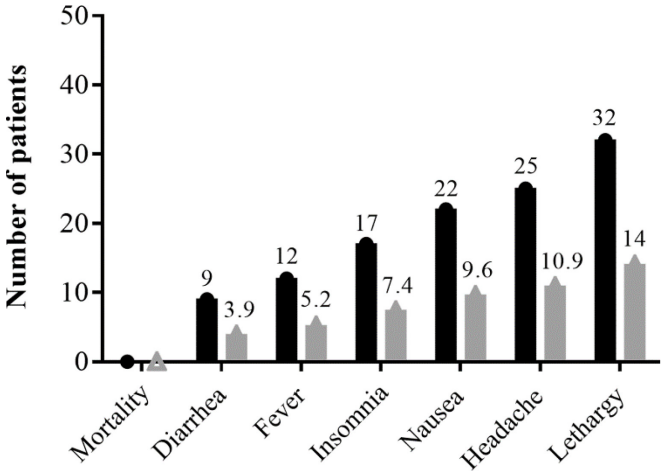
SVR: Sustained virologic response; SD: Standard deviation; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; CI: Confidence interval.

Figure 2. Association of sustained viral response with baseline viral load and genotype of HCV.



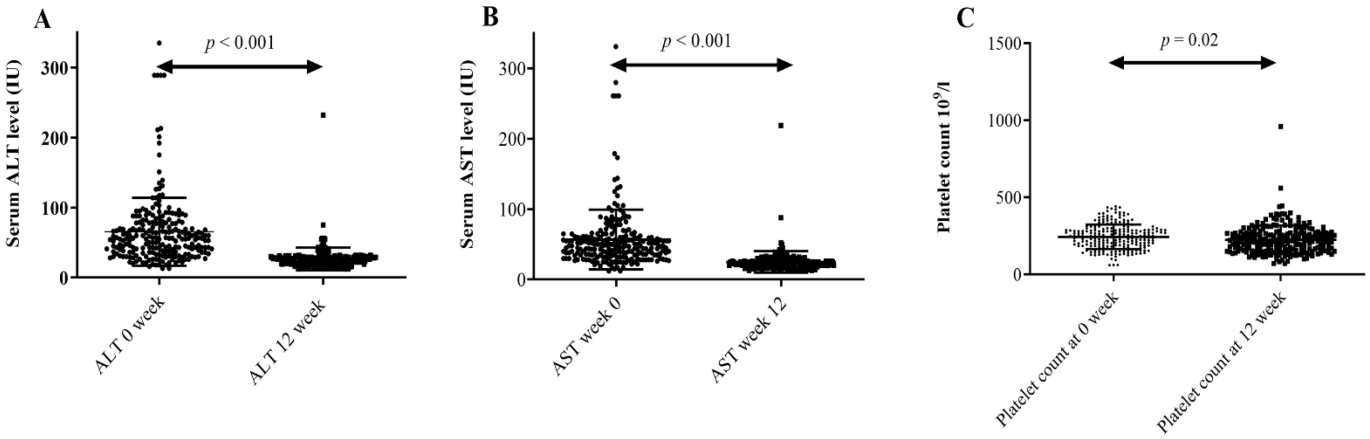
(A) Statistically significant (p < 0.001; 95% CI = 1.2-3.1) baseline viral load log10 IU/mL in SVR patients by independent T test (B) Statistically significant (p = 0.01; 95% CI = 1.71-22.53) with genotype 3 in SVR cases by Chi Square Test. SVR: Sustained virologic response; CI: Confidence interval.

Figure 4. Adverse effects observed during the treatment (n = 229).



Each black bar represents the frequency, and grey expresses the percentage of adverse effects associated with SOF/DAC therapy. SOF/DAC: Sofosbuvir and daclatasvir

Figure 3. Comparison of serum biochemical and hematological analysis at the beginning and end of treatment.



(A) Statistically significant ($p < 0.001$) healthy levels of ALT at the end of therapy. (B) Statistically significant ($p < 0.001$) normal serum levels of AST at the end of therapy. (C) Statistically significant ($p < 0.02$) healthy levels of platelet count at the end of treatment. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

Discussion

Interferon therapy has been the cornerstone of HCV treatment in the past two decades, which had limitations due to the suboptimal rates of SVR and lack of tolerability. The standard injectable interferon and ribavirin therapy of chronic HCV has shifted to a combination of DAAs [9]. Now interferon-free regimens for the cure of chronic HCV infections are superseded with remarkable improvement with SVR.

In this study, 12 weeks of therapeutic SOF/DAC combination resulted in high efficacy rates in treatment-naive patients with different genotypes. We observed SVR_{12} in 92.6% of patients treated with SOF/DAC with mild side effects. Two studies conducted in 2016 and 2014 reported an overall 90% and 92% efficacy rate with SOF/DAC, respectively [16,20]. This combined drug therapy manifested 83% and 94% efficacy among advanced liver cirrhotic and liver transplant recipients [21]. These studies support the current findings in which the 92.6% SVR was achieved in HCV-infected patients. A similar observation was seen in a study on two types of HCV groups (treatment-naive and treatment-experienced) with an efficacy rate of 90% and 86%. A recent study reported very high SVR in patients with genotype 3a infection in treatment-experienced (100%) and treatment-naive (99%) [14]. The results are slightly different from our findings, which recorded the efficacy rate against genotypes 1, 2, 3, and 4. The combination of SOF/DAC was given for three months that demonstrated a higher efficacy rate. The higher efficacy and good tolerability are confined to HCV genotype 3 patients and in compensated cirrhosis or advanced fibrosis [22].

We confirmed 250 HCV-positive patients by the real-time PCR among 300 suspected HCV cases, but only 229 were subjected to treatment due to inclusion criteria. Kim *et al*. analyzed 354 serum samples positive for anti HCV ELISA out of which real-time PCR confirmed 202 HCV-positive patients [23]. The reliability and sensitivity of antibodies-based tests depend on the level of viremia [24].

The demographics data presented the mean age of the patients who suffered from HCV infection was 42.2 ± 10.6. In comparison, Charlton *et al.* reported an average of 59 years from the age group of 18 to 82 years in HCV-infected patients [25]. We report a higher number of HCV-positive cases amongst females (66%) in comparison to male patients (34%). A study conducted on the management of chronic hepatitis C in Karachi found that 62% of the patients were females [26]. A similar observation was seen in a study from Pakistan in which a higher number of HCV cases were reported among females [27]. High illiteracy, lack of awareness, and other ritual practices are significant obstacles in controlling HCV infections in Pakistan. The adherence to therapy becomes difficult, owing to low socio-economic factors. The plausible explanation could be that females' perception of disease is based on beliefs that they will only cure with injectable. Unqualified healthcare providers and dentists frequently use injections for ailments that disseminate HCV infections. Moreover, visits to parlors where cleanliness and sterilization conditions are poor, ear and nose piercing from shops with unsterilized equipment could contribute to higher disease progression among females. The age and gender distribution of HCV are

similar to the findings published in a recent study from Gujrat, Pakistan [14].

The most prevalent genotype was genotype 3, representing 94.3 % of the patients, which is in accordance with the global prevalence and distribution. The patients with genotype 3 showed a higher SVR rate as compared to genotype 1 and 2 at week 12 of treatment. The global prevalence of genotype 3 reported 54.3 million (30.1%) in HCV patients [28]. Genotype 3 has been reported as the most prevalent type in Pakistan in different studies [7,29]. A recent study on genotype distribution in Pakistan showed genotype 3 (73.9%) was the prevalent one, followed by genotype 1 (9.7%) and genotype 4 (0.3%) [30]. The regimen was effective and well-tolerated among different genotypes, showed the pan-genotypic activity of the drugs as it encompasses a broad spectrum of activity against all HCV genotypes. The baseline parameters analyzed in our study were found to be higher than the normal before antiviral therapy. The post-treatment biochemical and hematological results in most of the cases returned to normal. Comparable findings observed in another study showed that 50% of the patients had more than 1.5 times elevated alanine aminotransferase levels [31]. We noticed similar results with a raised ALT and AST levels among HCV patients prior to antiviral therapy.

The platelet count was reasonable in most cases of this study, which is congruent with other studies. We assessed that the patients who achieved the SVR had a mean serum HCV RNA concentration of 5.94 $\log_{10}$ IU/ml ± 0.94 SD. The raised HCV RNA level > 10^8 IU/ml in HCV patients varies among different patients [32]. Promising effectiveness of antiviral drugs observed in the treatment of hepatitis C with a fixed dose of 400 mg SOF and 60 mg DAC given to patients for 24 weeks [33]. In another study, 24 weeks of fixed and daily dosage therapy of SOF/DAC exhibited excellent results in patients with HCV infection [20]. The same treatment regimen with a shorter duration of 12 weeks showed a favorable response in HCV-infected patients in the current study. Patients showed excellent therapeutic tolerance, contrary to interferon-based regimens. Discontinuation of medication due to side effects in interferon-free SOF therapies are unusual. The current study did not find any case of cessation of treatment due to any of the significant side effects. We observed minimal side effects of SOF/DAC during the treatment, and patients responded well to the therapy. Some minor side effects, including fatigue, nausea, headache, and insomnia, were reported in a US study with a good tolerability profile [11]. We could not find any published Pakistani data on the importance of molecular analysis and blood parameters to elucidate the effectiveness and side effects of DAAs in patients with chronic hepatitis C infections. Our study had limitations in sample size, constraints in detecting subgenotypes, and a lack of HCV RNA detection during different treatment weeks. The educational awareness programs among the community, patients, and healthcare workers could significantly reduce the burden of the disease [34].

Conclusions

DAAs such as SOF/DAC was found to be superlative regimens that knocked off interferon-based therapy and can achieve an excellent SVR in cases of HCV infections. This combination therapy has an excellent tolerability profile with generally mild adverse effects, and there were no significant changes in hematological parameters. The regimen reduces the elevated blood aminotransferases in patients, notably, who achieve SVR after completing treatment. SOF/DAC regimen demonstrated 92.6% efficacy and well-tolerability among the patients affected with different HCV genotypes, particularly against genotype 3, which is more prevalent in Pakistan. Monitoring the baseline characteristics is helpful in better management of patients to achieve SVR. Further studies with large sample sizes, different ethnic groups, and a significant number of cases infected with other genotypes of HCV are required.

References

1. Lozano R, Naghavi M, Foreman K, Lim S, Shibuya K, Aboyans V, Abraham J, Adair T, Aggarwal R, Ahn SY (2012) Global and regional mortality from 235 causes of death for 20 age groups in 1990 and 2010: a systematic analysis for the Global Burden of Disease Study 2010. Lancet 380: 2095-2128.
2. World Health Organization (WHO) (2019) Hepatitis C, fact sheet. Available: http://www.who.int/en/news-room/fact-sheets/detail/hepatitis-c. Accessed 9 July 2019.
3. World Health Organization (WHO) (2016) Guidelines for the screening, care and treatment of persons with chronic hepatitis C infection. Available: https://apps.who.int/iris/handle/10665/205035. Accessed 15 April 2016.
4. Nelson PK, Mathers BM, Cowie B, Hagan H, Des Jarlais D, Horyniak D, Degenhardt L (2011) Global epidemiology of hepatitis B and hepatitis C in people who inject drugs: results of systematic reviews. Lancet 378: 571-583.
5. Mohamoud YA, Riome S, Abu-Raddad LJ (2016) Epidemiology of hepatitis C virus in the Arabian Gulf countries: Systematic review and meta-analysis of prevalence. Int J Infect Dis 46: 116-125.
6. Omata M, Kanda T, Yokosuka O, Crawford D, Al-Mahtab M, Wei L, Ibrahim A, Lau GK, Sharma BC, Hamid SS, Chuang

WL, Dokmeci AK (2015) Features of hepatitis C virus infection, current therapies and ongoing clinical trials in ten Asian Pacific countries. Hepatol Int 9: 486-507.
7. Gower E, Estes C, Blach S, Razavi-Shearer K, Razavi H (2014) Global epidemiology and genotype distribution of the hepatitis C virus infection. J Hepatol 61: S45-57.
8. Tsubota A, Fujise K, Namiki Y, Tada N (2011) Peginterferon and ribavirin treatment for hepatitis C virus infection. World J. Gastroenterol 17: 419-432.
9. Ahmad T, Yin P, Saffitz J, Pockros PJ, Lalezari J, Shiffman M, Freilich B, Zamparo J, Brown K, Dimitrova D, Kumar M, Manion D, Heath-Chiozzi M, Wolf R, Hughes E, Muir AJ, Hernandez AF (2015) Cardiac dysfunction associated with a nucleotide polymerase inhibitor for treatment of hepatitis C. Hepatology 62: 409-416.
10. Wuerth K, Magel T, Conway B (2019) Sofosbuvir and velpatasvir in the treatment of chronic hepatitis C. Future Virol 14: 715-727.
11. Lawitz E, Mangia A, Wyles D, Rodriguez-Torres M, Hassanein T, Gordon SC, Schultz M, Davis MN, Kayali Z, Reddy KR (2013) Sofosbuvir for previously untreated chronic hepatitis C infection. N Eng J Med 368: 1878-1887.
12. Lawitz E, Sulkowski MS, Ghalib R, Rodriguez-Torres M, Younossi ZM, Corregidor A, Dejesus E, Pearlman B, Rabinovitz M, Gitlin N (2014) Simeprevir plus sofosbuvir, with or without ribavirin, to treat chronic infection with hepatitis C virus genotype 1 in non-responders to pegylated interferon and ribavirin and treatment-naive patients: the COSMOS randomised study. Lancet 384: 1756-1765.
13. Gao M, Nettles RE, Belema M, Snyder LB, Nguyen VN, Fridell RA, Serrano-Wu MH, Langley DR, Sun J-H, O'boyle Ii DRJN (2010) Chemical genetics strategy identifies an HCV NS5A inhibitor with a potent clinical effect. Nature 465: 96-100.
14. Butt Z, Shah SMA (2019) Daclatasvir plus Sofosbuvir with or without ribavirin in patients with chronic Hepatitis C genotype 3a in Pakistani population. A real world experience. Pak. J Med Sci 35: 409-413.
15. Bamford CG, Mclauchlan J (2019) Comparative host genomics: how has human evolution affected our immune defence against hepatitis C virus? Future Virol 14: 125-128.
16. Leroy V, Angus P, Bronowicki JP, Dore GJ, Hezode C, Pianko S, Pol S, Stuart K, Tse E, Mcphee F, Bhore R, Jimenez-Exposito MJ, Thompson AJ (2016) Daclatasvir, sofosbuvir, and ribavirin for hepatitis C virus genotype 3 and advanced liver disease: A randomized phase III study (ALLY-3+). Hepatology 63: 1430-1441.
17. World Medical Association (WMA) (2013). WMA declaration of Helsinki – ethical principles for medical research involving human subjects. Available: https://www.wma.net/policies-post/wma-declaration-of-helsinki-ethical-principles-for-medical-research-involving-human-subjects/. Accessed 24 December 2020.
18. Colin C, Lanoir D, Touzet S, Meyaud-Kraemer L, Bailly F, Trepo C, HEPATITIS Group (2001) Sensitivity and specificity of third-generation hepatitis C virus antibody detection assays: an analysis of the literature. J Viral Hepat 8: 87-95.
19. Wolffram I, Petroff D, Bätz O, Jedrysiak K, Kramer J, Tenckhoff H, Berg T, Wiegand J (2015) Prevalence of elevated ALT values, HBsAg, and anti-HCV in the primary care setting and evaluation of guideline defined hepatitis risk scenarios. J Hepatol 62: 1256-1264.
20. Sulkowski MS, Gardiner DF, Rodriguez-Torres M, Reddy KR, Hassanein T, Jacobson I, Lawitz E, Lok AS, Hinestrosa F, Thuluvath PJ, Schwartz H, Nelson DR, Everson GT, Eley T, Wind-Rotolo M, Huang SP, Gao M, Hernandez D, Mcphee F, Sherman D, Hindes R, Symonds W, Pasquinelli C, Grasela DM, Group AIS (2014) Daclatasvir plus sofosbuvir for previously treated or untreated chronic HCV infection. N Engl J Med 370: 211-221.
21. Poordad F, Schiff ER, Vierling JM, Landis C, Fontana RJ, Yang R, Mcphee F, Hughes EA, Noviello S, Swenson ES (2016) Daclatasvir with sofosbuvir and ribavirin for hepatitis C virus infection with advanced cirrhosis or post-liver transplantation recurrence. Hepatology 63: 1493-1505.
22. Nelson DR, Cooper JN, Lalezari JP, Lawitz E, Pockros PJ, Gitlin N, Freilich BF, Younes ZH, Harlan W, Ghalib R (2015) All-oral 12-week treatment with daclatasvir plus sofosbuvir in patients with hepatitis C virus genotype 3 infection: ALLY-3 phase III study. Hepatology 61: 1127-1135.
23. Kim YS, Lee HS, Ahn YO (1999) Factors associated with positive predictability of the anti-HCV ELISA method with confirmatory RT-PCR. J Korean Med Sci 14: 629-634.
24. Kirisci O, Calıskan A (2019) Threshold value of the anti-HCV test in the diagnosis of HCV infection. J Infect Dev Ctries 13: 914-919. doi: 10.3855/jidc.11657.
25. Charlton M, Gane E, Manns MP, Brown RS, Jr., Curry MP, Kwo PY, Fontana RJ, Gilroy R, Teperman L, Muir AJ, Mchutchison JG, Symonds WT, Brainard D, Kirby B, Dvory-Sobol H, Denning J, Arterburn S, Samuel D, Forns X, Terrault NA (2015) Sofosbuvir and ribavirin for treatment of compensated recurrent hepatitis C virus infection after liver transplantation. Gastroenterology 148: 108-117.
26. Capileno YA, Van Den Bergh R, Donchunk D, Hinderaker SG, Hamid S, Auat R, Khalid GG, Fatima R, Yaqoob A, Van Overloop C (2017) Management of chronic Hepatitis C at a primary health clinic in the high-burden context of Karachi, Pakistan. PLoS One 12: e0175562.
27. Rana NA, Munir B, Hussain N, Imtiaz N, Gondal MA, Parvaiz F (2020) Seroprevalence, biochemical investigation and risk factor assessment for HBV and HCV infection in hospital based patients of Islamabad, Pakistan. J Biores Manag 7: 10-18.
28. Messina JP, Humphreys I, Flaxman A, Brown A, Cooke GS, Pybus OG, Barnes E (2015) Global distribution and prevalence of hepatitis C virus genotypes. Hepatology 61: 77-87.
29. Umer M, Iqbal M (2016) Hepatitis C virus prevalence and genotype distribution in Pakistan: Comprehensive review of recent data. World J Gastroenterol 22: 1684-1700.
30. Khan A, Nadir A, Mushtaq MH, Junaid K, Khan AM, Ali H, Waqar F, Khan TA, Khan AA (2020) Molecular epidemiology and genotype distribution of hepatitis C in Pakistan; a multicenter cross-sectional study. Infect Genet Evol 84:104372.
31. Koff R (2014) the efficacy and safety of sofosbuvir, a novel, oral nucleotide NS 5B polymerase inhibitor, in the treatment of chronic hepatitis C virus infection. Aliment Pharmacol Ther 39: 478-487.
32. Sandoughdaran S, Alavian SM, Sharafi H, Behnava B, Salimi S, Mehrnoush L, Karimi Elizee P, Keshvari M (2015) Efficacy of prolonged treatment with pegylated interferon (Peg-IFN) and ribavirin in thalassemic patients with hepatitis C who relapsed after previous Peg-IFN-based therapy. Hepat Mon 15: e23564.

33. Bunchorntavakul C, Reddy KR (2015) Review article: the efficacy and safety of daclatasvir in the treatment of chronic hepatitis C virus infection. Aliment Pharmacol Ther 42: 258-272.
34. Azatyan V, Yessayan L, Shmavonyan M, Melik-Andreasyan G, Perikhanyan A, Porkshenyan K (2019) Evaluation of IL-2, IL-10, IL-4 and ɤ-interferon levels in the oral fluids of patients with hepatitis C, B and HIV. J Infect Dev Ctries 13: 069S-074S. doi: 10.3855/jidc.10919.

Corresponding author
Dr. Hasan Ejaz, PhD
Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, King Khalid Road, Jouf University, Sakaka, Al Jouf 72388, Saudi Arabia.
Phone: +966 557625174
E-mail: hetariq@ju.edu.sa, hasanmicro@gmail.com

Conflict of interests: No conflict of interests is declared.