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Sustained virological response to antiviral drugs in treatment of different genotypes of HCV cirrhotic patients

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Abstract: Cirrhosis and liver cancer are both caused by hepatitis C virus (HCV) infection of the liver. Patients with HCV cirrhosis may be treated with one of many antiviral medications, depending on their specific genotype. Samples of cirrhotic HCV were obtained from 190 people at the Khyber Teaching Hospital and the Hayatabad Medical Complex in Peshawar, Pakistan. Multiplex and real-time PCR were used to assess the genotypes and viral loads of the samples, respectively. Sixty patients were given sofosbuvir plus daclatasvir with ribavirin, while the remaining 56 patients were given sofosbuvir with ribavirin for a period of 12-24 weeks. LFTs were also tracked both before and after therapy. Group I (sofosbuvir + daclatasvir) had a sustained virological response of 82.70 percent. Group II (sofosbuvir + daclatasvir with ribavirin) had an 86% sustained virological response, whereas group III (84% sustained virological response) received only ribavirin. When compared to other genotypes, genotype 3 showed the most impressive sustained virologic response (SVR) to the antiviral medicines. Based on the results of this trial, we propose sofosbuvir + daclatasvir ribavirin for the treatment of cirrhotic patients with various HCV genotypes since it produces the greatest sustained virological response.

Keywords: HCV, cirrhosis, genotypes, sofosbuvir, daclatasvir, ribavirin, RT-PCR.

INTRODUCTION

About 10 million persons in Pakistan are infected with Hepatitis C virus (HCV) (14, 24), making it the second-most populated nation with the infection. HCV is the fastest growing health risk in the world (Bartenschlager *et al*., 2018), and it is a main cause of chronic liver disease that may progress to fibrosis, cirrhosis and liver cancer (Di Pascoli *et al*., 2017). Hepatitis C virus (HCV) infection is the leading cause of hepatic carcinoma and cirrhosis, two forms of liver cancer with a combined 50% 5-year survival rate. Most transfusion-transmitted hepatitis is caused by HCV (Lanini *et al*., 2019).

The genomic size of HCV is 96,000kb, making it a single-strand RNA virus. Based on geographical and sequencing variation, the HCV may be divided into 7 genotypes. These genotypes are very important to consider while administering therapy (Thrift *et al*., 2017). HCV genotypes are further classified into the 67 subtypes that have been verified and the 20 that have not. Variation across HCV strains was found at 30-35% of nucleotide regions, with strains of the same subtype showing variation at 15% of nucleotide locations. Subtypes 1a, 1b, 2a and 3a of the virus have been shown to be responsible for a disproportionately high number of new infections in developing nations. Prior to the identification of HCV, it is believed that these "epidemic subtypes" spread rapidly via tainted blood, blood products, injectable medication consumption and other means (Ullah *et al*., 2020, Ullah *et al*., 2021).

HCV treatment has no direct, side-effect-free regimens. Pegylated interferon-ribavirin is the most commonly used treatment for HCV genotypes 1a and 1b, however it has a slow viral response rate and few adverse effects (Wani *et al*., 2022). HCV may be treated with new antiviral inhibitors. Oral combinations of direct-acting antivirals (DAAs) are the gold standard for chronic HCV treatment (Zeuzem *et al*., 2000). Headache, fatigue, vomiting, nausea, and fever are common adverse effects (Holmes *et*

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al., 2015). Sofosbuvir (SOF) is an oral direct antiviral that blocks HCV NS5B's RNA polymerase activity (Majumdar *et al*., 2016). SOF with ribavirin (RBV) increase sustained virologic response (SVR) rates to 70% in liver transplant patients with recurrent HCV infections (European Association, 2016). In the treatment of chronic HCV infection, the addition of the HCV NS5A inhibitor daclatasvir (DCV) to standard of care (SOF) with or without ribavirin has demonstrated encouraging antiviral activity (Charlton *et al*., 2013). Patients' levels of chronic liver damage are documented using a variety of grading systems and recommendations. A common method of quantifying liver damage is the Child Turcotte Pugh score or Child Criteria. Child class A, B and C were determined based on the score. Class A was given to children with scores between 5 and 6, B to those with scores between 7 and 9 and C to those with scores between 10 and 15 (Gambato *et al*., 2014). Patients with HCV cirrhosis in Khyber Pakhtunkhwa, Pakistan, were tested for HCV genotype and the present research indicates a sustained virological response to SOF with the combination of DCV RBV across all HCV genotypes, including mixed and untypable genotypes.

MATERIALS AND METHODS

This investigation hospitalised 190 HCV cirrhosis patients at the Hayatabad Medical Complex (HMC) or Khyber Teaching Hospital (KTH) in Peshawar, Khyber Pakhtunkhwa, Pakistan between 2019 and 2021 (Sulkowski *et al*., 2014). Each patient's serum was frozen at -80°C for analysis. All studies complied with WHO guidelines. Participants gave written consent before enrolling. The University of Peshawar's Centre for Biotechnology and Microbiology (COBAM) ethics committee approved the research (No. 156/M.Phil/Ph.D/(COBAM).

Polymerase chain reaction (PCR) and RNA isolation

The QIAamp Viral RNA Mini Kit (Cat No. 52904) was used to extract RNA from 200 l of serum, following the manufacturer's instructions. Manufacturing methodology (BIORON life science cDNA Kit) was used to produce the complementary DNA (cDNA) for qualitative detection of HCV. For cDNA synthesis, we mixed 4 l of Complete RT buffer (5X), 10mM dNTPs and the DNA polymerase enzyme. Reverse Primer OAS (10pmol/l) 1ul, RNAs inhibitors 0.5ul. RTase, M-MLV, 1ml (200U/ml) 20-50ng RNA in 1ul 10 l of deionized water (total volume brought to 20 l). 60 minutes at 37 degrees Celsius, 10 minutes at 70 degrees Celsius and another 60 minutes at 22 degrees Celsius made up the temperature profile for cDNA(Shahid *et al*., 2018). First-round PCR master mix components included 7.1ul of dH2O (final volume adjusted to 20ul), 6.9ul of master mix, 1ul each of OS and OAS primers (15pmol/ul) and 4ul of cDNA. Second-round PCR master mix components included dH2O (final volume adjusted to 20ul), Master Mix (final volume adjusted to 6ul), Inner Sense (OA) Primer (15 pmol/ul), Inner Antisense (IA) Primer (15pmol/ul) and first-round PCR product (4ul). Initial denaturation at 94 degrees Celsius for 2 minutes, followed by 35 cycles of (94 degrees Celsius for 30 seconds, 54 degrees Celsius for 30 seconds, and 72 degrees Celsius for 30 seconds), and finally a 10-minute extension at 72 degrees Celsius. The PCR products were seen under a UV trans-illuminator after being resolved on 1.2% agarose gel for 35-40 minutes (Janiak *et al*., 2018). The sample was verified to be HCV positive by the detection of the HCV 5'-UTR and HCV genotyping was subsequently conducted using multiplex PCR according to the method described(Shahid *et al*., 2018). The virus titer was determined using real-time PCR using the following methods, which were developed in accordance with the (Sacace Biotechnologies kit altona) process. First, maintain 50 degrees Celsius for 30 minutes. Stage 2, Maintain 95°C for 15 minutes Third Stage: 95°C for 20 seconds, then 60°C for 40 seconds You may do this for as many as 40 times.

STATISTICAL ANALYSIS

SPSS version 25.0 (on Windows 10) and Microsoft Office Excel 2018 (for statistical analysis) were used. Frequency, mean (standard deviation), range, percentage and bar charts were only some of the ways that various values were shown.

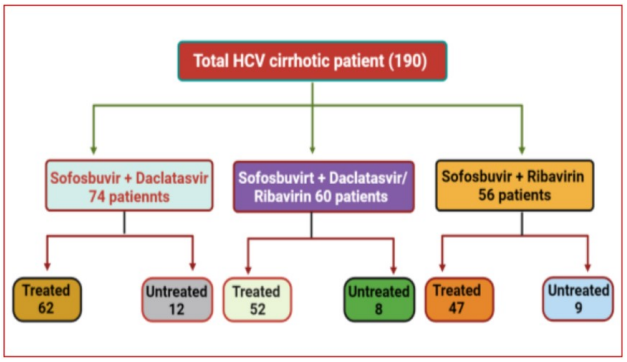

Fig. 1: Flow chart of antiviral drugs for HCV cirrhotic patients.

Plan of research

Patients were grouped by gender, genotype, and treatment. The first group received sofosbuvir (SOF) (400mg) and daclatasvir (DCV) (60mg) daily for 12 weeks (fig. 1), while the second group received SOF, DCV, and RBV (200mg) for 24 weeks. Patients with haemoglobin levels < 10g/dL or anaemia received larger RBV doses. Patients under 75 kilogrammes (kg) received 1,000 milligrammes (mg) of the antiviral drug daily, whereas those over 75kg received 1,200mg. All groups calculated SVR after 12 and 24 weeks. Fresh samples underwent liver function tests, including quantitative PCR.

Table 1: Demographic characteristic of 190 patients with HCV cirrhosis

Variable	SOF+DCV	SOF+DCV+RBV	SOF+RBV
Gender			
Male	48(64.86%)	35(58.33%)	34 (60.71%)
Female	26(35.13%)	25(41.66%)	22 (39.28%)
Age Years mean ± standard deviation (SD)	50.89±12.65	51.01±12.58	49.01±11.75
Child Turcotte Pugh Score			
Child class A	28(37.83%)	11(18.33%)	30 (53.57%)
Child class B	20(27.02%)	15(25.0%)	14 (25.00)
Child class C	26(35.13%)	34(56.66%)	12 (21.42)
ALT- IU/L mean ± standard deviation (SD)	95.0±26.47	100.90±27.11	94.81±25.34
AFP-ng/mL mean ± standard deviation (SD)	73.09±61.07	57.07±46.06	72.47 ± 59.93
Bilirubin mg/dl mean ± standard deviation (SD)	1.3±0.7	1.3±0.6	1.2±0.5
Albumin g/dl mean ± standard deviation (SD)	3.9 ±0.5	3.9 ±0.5	3.8 ±0.4
Viral load (IU/ml)	$<6.0\times10^6$	$<7.1\times10^6$	$<3.0\times10^6$

Symbol: SOF: sofosbuvir, DCV: daclatasvir, RBV: ribavirin, AFP: alpha fetoprotein, ALT, alanine aminotransferase.

Table 2: Antiviral therapy against different genotypes of HCV

Genotypes	SOF+DCV	SOF+DCV+RBV	SOF+RBV
1a	8	5	0
1b	3	0	0
2a	10	4	10
3a	41	17	38
3b	12	5	8
Mixed	0	7	0
Untypable	0	22	0
Total	74	60	56

Table 3: SVR of sofosbuvir plus daclatasvir after 12 and 24 weeks therapy for HCV genotypes

Patients classification				Patients treatment			
Genotypes	Compensate Cirrhosis	Decompensate Cirrhosis	Total	HCV +ive 12 weeks	HCV -ive 12 weeks	HCV +ive 24 weeks	HCV -ive 24 weeks
1a	7	1	08	03	05	02	06
1b	2	1	03	01	02	0	03
2a	6	4	10	03	07	02	08
3a	25	26	41	13	28	06	35
3b	8	4	12	05	07	02	10
Total= 74				25	49	12	62

Table 4: SVR of sofosbuvir plus daclatasvir with ribavirin after 12 and 24 weeks therapy for HCV genotypes

Patients classification				Patients treatment			
Genotypes	Compensate Cirrhosis	Decompensate Cirrhosis	Total	HCV +ive (Genotypes) 12 weeks	HCV -ive (Genotypes) 12 weeks	HCV +ive (Genotypes) 24 weeks	HCV -ive (Genotypes) 24 weeks
1a	0	5	05	02	03	0	05
2a	3	1	04	01	03	01	03
3a	10	7	17	04	13	02	15
3b	2	3	05	01	04	01	04
Mixed	3	4	07	02	05	01	06
Untypable	8	14	22	06	16	03	19
Total (60)				16	44	08	52

RESULTS

Based on the patients' treatment regimens, they were separated into three categories: SOF+DCV, SOF+DCV+RBV and SOF+RBV. In table 1 we see the demographics and other factors. Although the HCV genotypes of the various groups of cirrhotic individuals varied, table 2 shows that Genotype 3a was the most prevalent overall.

1st group: Sofosbuvir plus daclatasvir

All of the participants were given a full daily dosage of SOF (400mg) and DCV (60mg). In addition to gender and HCV genotype, patients were also categorized by their cirrhosis stage. There were 74 total patients, 28 female and 46male. There were 48 patients reported in the compensate stage (18 females and 30males) and 26 patients reported in the decompensate stage (10 females and 16 men) (fig. 2). (table 3) displays the various patients' HCV genotypes and their SVR results. In the beginning, 72 patients (genotypes) were given SOF + DCV for 12 weeks. Patients in the therapy group had their viral response to anti-viral medication monitored using real-time polymerase chain reaction at the conclusion of the 12-week treatment period. Sixty-six percent of the patients had undetectable levels of HCV RNA, while 34 percent of the non-respondents had detectable levels of the virus in their blood. These people were given 12 further weeks of treatment. At the end of 24 weeks of treatment, 62 patients (83%) achieved SVR, whereas 12 patients (17%) were still non-responders to therapy and died during final treatment.

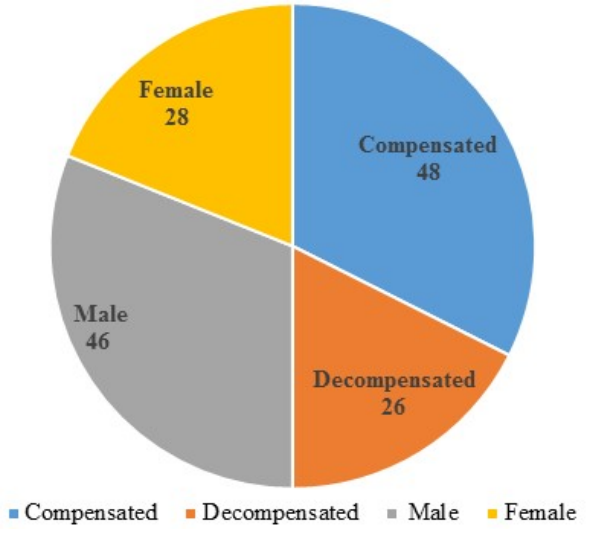

Fig. 2: Gender and degree of liver damage of group 1 patients.

2nd group: Sofosbuvir plus daclatasvir with ribavirin

Patients in this group were given the recommended daily doses of sofosbuvir (400mg) and daclatasvir (60mg) along with (200mg) of each drug. Patients were sorted into groups based on demographic characteristics such as gender, HCV genotype and liver cirrhosis stage. Twenty-five women and thirty-five men made up the total of 60 patients, with 11 women and 15 men in the compensation stage of cirrhosis and 14 women and 20 men in the decompensated stage (fig. 3). table 4 displays the various patient's SVR and HCV Genotypes. SOF with DCV with RBV was administered to 60 patients (genotypes) for 12 weeks. All patients with different HCV genotypes were tested using real-time polymerase chain reaction (PCR) after 12 weeks of therapy to see how well the anti-viral drugs were working. Seventy-three percent of the patients were negative for HCV RNA, whereas 27 percent were infected. These individuals were given further 12 weeks of treatment. Fifty-two patients (86%) achieved SVR after 24 weeks of treatment, whereas eight patients (14%) did not.

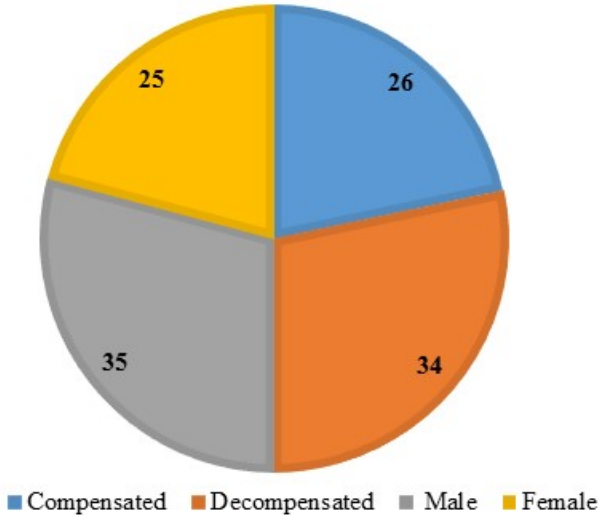

Fig. 3: Gender and Degree of Liver damage of Group 2 patients.

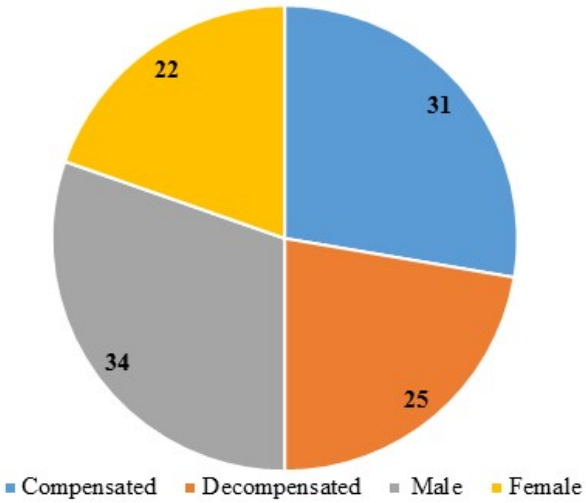

Fig. 4: Gender and Degree of Liver damage of Group 3 patients.

Table 5: SVR of sofosbuvir plus ribavirin after 12 and 24 weeks therapy for HCV genotypes

Patient classification				Patient treatment			
Genotype	Compensate Cirrhosis	Decompensate Cirrhosis	Total	HCV –ive 12 weeks	HCV +ive 12 weeks	HCV –ive 24 weeks	HCV +ive 24 weeks
2a	7	3	10	7	3	8	2
3a	18	20	38	28	10	33	5
3b	6	2	8	5	3	6	2
Total(56)				40	16	47	9

Table S1: Frequency of different complications in the HCV cirrhotic group

Complications	Frequency% (SOF+DCV+RBV)	Frequency% (SOF+DCV)	Frequency% (SOF+ RBV)
Anemia, Ascites, Arthralgia	3(1.9)	--	6(10.71)
Anemia, Ascites, Clubbing	2(1.2)	1(0.6)	--
Anemia, Ascites, Constipation	2(1.2)	1(0.6)	7(12.5)
Anemia, Ascites, Fatigue	1(0.6)	--	5(8.92)
Anemia, Ascites, Fever	8(4.9)	--	--
Anemia, Ascites, Hypertension	2(1.2)	--	5(8.92)
Anemia, Ascites, Myalgia	2(1.2)	4(2.5)	--
Anemia, Diabetes, Constipation	7(4.3)	1(0.6)	4(7.14)
Anemia, Fever, Arthralgia	3(1.9)	4(2.5)	--
Anemia, Fever, Myalgia	3(1.9)	5(3.1)	9(16.07)
Anemia, Jaundice, Arthralgia	1(0.6)	1(0.6)	1(1.78)
Anemia, Jaundice, Ascites	3(1.9)	--	--
Anemia, Jaundice, Fever	4(2.5	4(2.5)	--
Anemia, Jaundice, Nausea	2(1.2)	1(0.6)	1(1.78)
Anemia, Jaundice, Vomiting	2(1.2)	5(3.1)	2(3.57)
Anemia, Oedema, Myalgia	1(0.6)	--	1(1.78)
Anemia, Ascites, Epigastric	1(0.6)	2(1.8)	--
Anemia,, Ascites, Nausea	1(0.6)	--	3(5.35)
Diabetes, Hypertension, Oedema	1(0.6)	3(1.9)	--
Fever, Epigastric, Nausea	5(3.1)	4(2.5)	--
Fever, Jaundice, Hypertension	2(1.2)	1(0.6)	--
Hematemesis, Headache, Constipation	2(1.2)	--	2(3.57)
Jaundice, Oedema, Arthralgia	1(0.6)	15(9.3)	1(1.78)
Jaundice, Oedema, Ascites	1(0.6)	7(4.3)	--
Anemia, insomnia, Ascites	--	3(1.9)	3(5.37)
Headache, Vomiting, Fever	--	11(6.8)	6(10.71)
Total	60(100.0)	74(100.0)	56(100.0)

Symbol: (--) zero value

3rd Group: Sofosbuvir plus ribavirin

A total of 56 people in this study were given daily doses of 400 milligrams of sofosbuvir and 200 milligrams of ribavirin. fig. 4 displays the sex distribution, liver damage and HCV genotype of the patients. Table 5 displays the various patient's SVR and HCV Genotypes. For 12 weeks, 56 patients (genotypes) were given SOF plus RBV. All patients with different HCV genotypes were tested using real-time polymerase chain reaction (PCR) after 12 weeks of therapy to see how well the anti-viral drugs were working. Nearly three-quarters of patients have undetectable levels of HCV RNA, whereas 29% of non-responders have detectable levels of the virus in their blood. These individuals were given further 12 weeks of treatment. Overall, 47 patients (84%) achieved SVR after 24 weeks of therapy, whereas 9 patients (16%) remained non-responders to the medications owing to significant sequelae.

When comparing the different antiviral medication combinations, fig. 5 shows that SOF + DCV with ribavirin (2nd group) produced the best outcomes. Age, overall weakness, hyperglycemia, high blood pressure, a decrease in platelets, or problems in the epigastric region are only few of the many possible causes of therapeutic failure across all patient populations.

Table 6 in the appendices shows some of the most frequent problems that occurred before or during treatment throughout the whole population. Patients often

complained of excruciating discomfort in their stomachs and kidneys while they underwent therapy. Patients with HCV cirrhosis also often had low levels of hemoglobin, platelets and glucose. Hemoglobin, platelet and glucose levels returned to normal between 7 and 11 weeks into therapy for those in the decompensate stage of liver cirrhosis and between 5 and 7 weeks into treatment for those in the compensate stage. Anemia, ascites and a decrease in platelets are frequent treatment-related complications in the decompensated stage of cirrhosis.

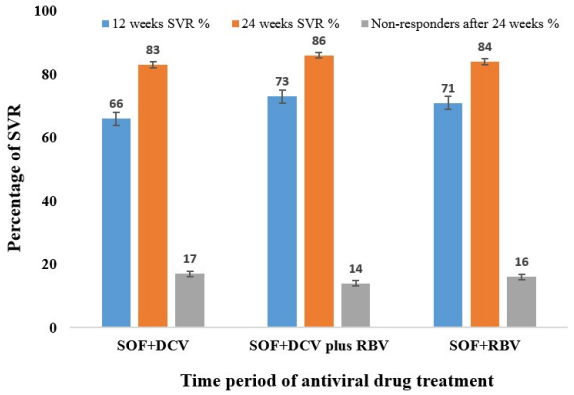

Fig. 5: Comparison of antiviral drug efficiency in responder and non-responder patients.

DISCUSSION

Due to the delayed onset of symptoms, hepatitis C virus (HCV) has earned the nickname "the silent killer." If not treated, HCV may lead to serious complications in the liver, including fibrosis, cirrhosis and even liver cancer. Vaccines against HCV are not yet commercially available. All HCV genotypes have seen a radical shift in how they are treated thanks to the advent of direct antiviral agents (DAAs). Interferon was once only effective in treating people with structurally normal liver, but today cirrhotic patients may demonstrate reversal with these direct medicines (Pol *et al*., 2012). In all cases of chronic, cirrhotic and non-cirrhotic HCV infection (Idrees *et al*., 2008), the novel treatment medicines daclatasvir and sofosbuvir with ribavirin are given for 12 and 24 weeks, respectively. The research found that the combination of sofosbuvir and daclatasvir was effective in treating cirrhotic individuals with HCV. Similar to the research by (Pellicile *et al*., 2014), the effectiveness of these combinatorial antiviral medications over 12 and 24 weeks showed a strong persistent virologic response (82%) (Foster *et al*., 2015). Patients with a wide range of HCV genotypes (60 total) were treated in the trial with sofosbuvir + daclatasvir with ribavirin. Significantly more than half of patients (63%) achieved SVR at 12 weeks, and by 24 weeks, 86% of patients were found to be virus-free and had gained SVR (Carrion, 2017).

A high SVR rate was achieved after 12 and 24 weeks of treatment for the majority of genotype 2, mixed and untypable patients in this research (Ilyas *et al*., 2011). In comparison to the first group of HCV genotypes, the effectiveness of the antiviral medicines was higher in this research. Calvaruso *et al*. (2019), who studied daclatasvir-based therapy for cirrhotic patients of varying genotypes, found results consistent with our study's. With the addition of both treatments, the SVR rate skyrocketed. Similar to our findings, Zafar *et al*. (2018). observed a persistent virological response in patients with untypeable genotypes who were treated with interferon alpha + ribavirin therapy for 24 weeks at normal dosages.

The most common HCV genotype in Pakistan is 3, which is highly drug-sensitive. Most participants in this research are of genotype 3, which is the most common (Liu *et al*., 2019). Genotype 3 responds to the medications most favorably and effectively of all the genotypes tested. Although some patients had major side effects from therapy, all patients were given treatment regardless of whether they were receiving SOF, DCV, or an RBV combo regimen. Pain, weariness and fever were more common in those given SOF + DCV with RBV than in those given SOF/DCV alone (Welzel *et al*., 2016). In a similar research, Poordad and Shah (Poordad *et al*., 2016; Shah *et al*., 2018). found that some patients either had fatal problems or stopped receiving treatment.

CONCLUSION

Patients with HCV genotypes were successfully treated with sofosbuvir + daclatasvir with ribavirin for 12 and 24 weeks, according to the research. Regardless of treatment duration or the addition of RBV, the SVR rate substantially increased in patients in group 2. Finally, when SOF and DCV are used along with RBV, they perform far better than the control group. Therefore, in this research, therapy with SOF with DCV and RBV is recommended for all HCV genotypes, including mixed and untyped.

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REFERENCES

Bartenschlager R, Baumert TF, Bukh J, Houghton M, Lemon SM, Lindenbach BD, Lohmann V, Moradpour D, Pietschmann T, Rice CM and Thimme R (2018). Critical challenges and emerging opportunities in hepatitis C virus research in an era of potent antiviral therapy: Considerations for scientists and funding agencies. *Virus Res.,* **15**(248): 53-62.

Calvaruso V, Mazzarelli C, Milazzo L, Badia L, Pasulo L, Guaraldi G, Lionetti R, Villa E, Borghi V, Carrai P and Alberti A (2019). Daclatasvir-based regimens in HCV

cirrhosis: experience from the Italian early access program. *Sci. Rep.*, **9**(1): 585.

Carrion AF (2017). Treatment of HCV in individuals with decompensated cirrhosis. *J. Liver Res. Disord. Ther.,* **3**(4): 00056.

Charlton MR, Gane EJ, Manns MP, Brown RS, Curry MP, Kwo PY, Fontana RJ, Gilroy R, Teperman LW, Muir AJ and McHutchison JG (2013). Sofosbuvir and ribavirin for the treatment of established recurrent hepatitis c infection after liver transplantation: preliminary results of a prospective, multicenter study: LB-2. *Hepatol.,* **58**(6): 1378A.

Di Pascoli M, Ceranto E, De Nardi P, Donato D, Gatta A, Angeli P and Pontisso P (2017). Hospitalizations due to cirrhosis: Clinical aspects in a large cohort of Italian patients and cost analysis report. *Digestive Dis.,* **35**(5): 433-438.

European Association for The Study of The Liver (2016). EASL recommendations on treatment of hepatitis C. *J. Hepatol.,* **66**(1): 153-194.

Foster GR, Afdhal N, Roberts SK, Brau N, Gane EJ, Pianko S, Lawitz E, Thompson A, Shiffman ML, Cooper C and Towner WJ (2015). Sofosbuvir and velpatasvir for HCV genotype 2 and 3 infection. *N. Engl. J. Med.*, **373**(27): 2608-2617.

Ilyas JA and Vierling JM (2011). An overview of emerging therapies for the treatment of chronic hepatitis C. C. *Clin. Liver Dis.*, **15**(3): 515-536.

Janiak M, Caraballo Cortés K, Perlejewski K, Kubicka-Russel D, Grabarczyk P, Demkow U and Radkowski M (2018). Next-generation sequencing of hepatitis C virus (HCV) mixed-genotype infections in anti-HCV-negative blood donors. *Phys. Ther. Rehabil*., 2018: 65-71.

Lanini S, Ustianowski A, Pisapia R, Zumla A and Ippolito G (2019). Viral hepatitis: Etiology, epidemiology, transmission, diagnostics, treatment and prevention. *Infect. Dis. Clin.,* **33**(4): 1045-1062.

Liu CH, Su TH, Liu CJ, Hong CM, Yang HC, Tseng TC, Chen PJ, Chen DS and Kao JH (2019). Sofosbuvir based direct acting antiviral therapies for patients with hepatitis C virus genotype 2 infection. *J. Gastroenterol. Hepatol.*, **34**(9): 1620-1625.

Majumdar A, Kitson MT and Roberts SK (2016). Systematic review: current concepts and challenges for the direct-acting antiviral era in hepatitis C cirrhosis. *Aliment. Pharmacol. Ther.,* **43**(12): 1276-1292.

Pellicelli AM, Montalbano M, Lionetti R, Durand C, Ferenci P, D'Offizi G, Knop V, Telese A, Lenci I, Andreoli A and Zeuzem S (2014). Sofosbuvir plus daclatasvir for post-transplant recurrent hepatitis C: potent antiviral activity but no clinical benefit if treatment is given late. *Dig. Liver Dis.*, **46**(10): 923-927.

Pol S, Ghalib RH, Rustgi VK, Martorell C, Everson GT, Tatum HA, Hezode C, Lim JK, Bronowicki JP, Abrams GA and Brau N (2012). Daclatasvir for previously untreated chronic hepatitis C genotype-1 infection: a randomised, parallel-group, double-blind, placebo-controlled, dose-finding, phase 2a trial. *The Lancet Inf. Dis.,* **12**(9): 671-677.

Shah A, Nizamuddin NU and Iqbal W (2018). Down staging of cirrhosis in all cirrhotic patients with hcv genotype 3-a infection, treated with 12-weeks triple therapy, including sofosbuvir, daclatasvir and ribavirin. *InMed. Forum,* **3**(29): 3.

Shahid I, Almalki WH, Ibrahim MM, Alghamdi SA, Mukhtar MH, Almalki SSR and Alhaidari MS (2018). Characterization of *in vitro* inhibitory effects of consensus short interference RNAs against non-structural 5B gene of hepatitis C virus 1a genotype. *Indian J. Med. Microbiol.,* **36**(4): 494-503.

Sulkowski MS, Gardiner DF, Rodriguez-Torres M, Reddy KR, Hassanein T, Jacobson I, Lawitz E, Lok AS, Hinestrosa F, Thuluvath PJ and Schwartz H (2014). Daclatasvir plus sofosbuvir for previously treated or untreated chronic HCV infection. *New Eng. J. Med.,* **370**(3): 211-21.

Thrift AP, El-Serag HB and Kanwal F (2017). Global epidemiology and burden of HCV infection and HCV-related disease. *Nat. Rev. Gastroenterol. Hepatol.,* **14**(2): 122-132.

Ullah A, Rehman IU, Ahmad J, Gohar M, Ahmad S and Ahmad B (2020). Hepatitis-C virus and cirrhosis: An overview from Khyber Pakhtunkhwa province of Pakistan. *Viral. Immunol.,* **33**(5): 396-403.

Ullah A, Rehman IU, Ahmad J, Odenthal M, Ahmad S, Nadeem T, Ali Q, Rizwan M, Khan MA, Hassan S and Ahsan H (2021). Phylogenetic analysis of the 5′ untranslated region of HCV from cirrhotic patients in Khyber Pakhtunkhwa, Pakistan. *Scient. Rep.,* **11**(1): 15023.

Wani MA, Agarwal NB and Khan MA (2022). Neurocognitive changes in chronic hepatitis c patients receiving combination therapy of interferon and ribavirin: A systematic review. *Curr Drug Ther.,***17**(5): 349-358.

Welzel TM, Petersen J, Herzer K, Ferenci P, Gschwantler M, Wedemeyer H, Berg T, Spengler U, Weiland O, van der Valk M and Rockstroh J (2016). Daclatasvir plus sofosbuvir, with or without ribavirin, achieved high sustained virological response rates in patients with HCV infection and advanced liver disease in a real-world cohort. *Gut.,* **65**(11): 1861-70.

Zafar A, Imran M, Zahoor S, Shah ZH, Ali M and Afzal MS (2018). Prevalence and treatment of untypable HCV variants in different districts of Punjab, Pakistan.*Viral Immunol.*, **31**(6): 426-432.

Zeuzem S, Feinman SV, Rasenack J, Heathcote EJ, Lai MY, Gane E, O'Grady J, Reichen J, Diago M, Lin A and Hoffman J (2000). Peginterferon alfa-2a in patients with chronic hepatitis C. *New Eng. J. Med.,* **343**(23): 1666-1672.