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Efficacy of Sofosbuvir plus Ribavirin in treatment of cirrhotic patients with HCV genotype 2 and 3 infections in Pakistan

Aminullah, Irshad ur Rehman*, Saeed Ahmad, Jamshaid Ahmad, Bashir Ahmad

Centre of Biotechnology and Microbiology, University of Peshawar, Peshawar-25000, Pakistan

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Abstract

Hepatitis C virus (HCV) is one of the leading causes of liver cirrhosis. Adjunctive therapy of ribavirin and interferon is commonly used against HCV infection, but it has wide diverse side effects. Currently, there is an urgent need for the development of new alternate therapy for combating HCV infections. This study is aimed to assess the efficacy of sofosbuvir plus ribavirin treatment in HCV cirrhotic patients (genotypes 2 and 3), viral drug response and to prevent progression of cirrhosis by eradication of HCV**.** Sample of 62 HCV cirrhotic patients were collected from the Gastroenterology ward of local hospital. Multiplex and real-time PCR were used to determine the genotypes and viral load respectively. The subjects were divided into two groups; A and B; A (HCV genotype 2), B (HCV genotype 3) infections. Group A and B received sofosbuvir (400 mg daily) plus ribavirin (200 mg twice daily) for 3 and 6 months respectively. The blood samples were further analyzed for the presence of viral RNA.Sustained Virologic Response (SVR) was shown by 88.70% of HCV cirrhotic patients. Group A patients (genotype 2) disclosed a good response to sofosbuvir (SVR 92%) as compared to group B (genotype 3; 88%). The SVR of group B patients was 70% and 88% after 12 and 24 weeks of treatment respectively. Sofosbuvir plus ribavirin may be prescribed for the treatment of HCV genotype 2 (3-months) and genotype 3 patients (6-months) with liver cirrhosis.

* **Corresponding Author:** Dr. Irshad Ur Rehman ✉ irshad_rehman@yahoo.com

Introduction

Hepatitis C virus is the causes of acute and chronic hepatitis. In the initial stages, the virus causes acute hepatitis, associated with no symptoms, mild illness or high-grade illness. If untreated, acute hepatitis develops into a chronic liver disease (Bethea *et al.,* 2000; Kanda *et al.,* 2013). Progression rate of chronic hepatitis to liver cirrhosis or cancer is high, with the global death toll of 399000 per year. Currently, all over the world, 71 million people live with chronic HCV infection reported by the World Health Organization (2018). Egypt has the highest prevalence (22%) of HCV infection, while 2 to 5 million Europeans (Rockstroh *et al.,* 2012) and 4 million Americans have hepatitis C infection (Mukhtar *et al.,* 1919).

The occurrence of hepatitis C in Pakistan is 5% (Afridi *et al.,* 2013) and in the Khyber Pakhtunkhwa, it is 5-9% (Khan *et al.,* 2014), contributing to liver cancer (Munaf *et al.,* 2014).

The clearance rate of acute HCV infection is 20 to 40% while in 40 to 60% cases it leads to chronic stages such as fibrosis, cirrhosis, and HCC as the reason of unawareness in the people about HCV (Tang et Grise, 2009; Kemp *et al.,* 2018).

In cirrhosis, blood pressure is raised due to the blockade of blood flow through the liver. This is because scar tissues replace normal liver parenchyma that hinders blood flow through portal vessels. Inflammation-related damage to liver parenchyma activates stellate cells which produce my fibroblasts that lead to fibrosis and hepatic blood flow obstruction (Hammer et McPhee, 2014).

In addition, stellate cells also secrete metalloproteinases inhibitors like tissue inhibitors metalloproteinase 1 and 2. Metalloproteinases lyse fibrotic materials in the extracellular matrix. Transforming growth factor beta1 (TGF-β1), a fibrotic response and connective tissues proliferation stimulator is also produced from the stellate cells (Iredale, 2003; Puche *et al.,* 2013).

The recommended therapy of chronic hepatitis is boceprevir and telaprevir (protease inhibitors) but it cannot be used for all the patients with HCV contagion. Similarly, treatment with interferon and ribavirin also has various side effects (Soriano *et al.,* 2012; Chung, 2012). Sofosbuvir (SOF) and simeprevir (SIM) are the new therapeutic drugs, highly effective, direct-acting antivirals (DAAs) (Rockstroh, 2013; Schmidt *et al.,* 2014) approved by the US FDA (http:// files.easl.eu/easl, 2014)**.** Sofosbuvir is an oral medicine used for cure of chronic HCV infection and direct acting nucleotide polymerase inhibitor (Bourlière *et al.,* 2011). Sofosbuvir works as an analogue of the nucleoside triphosphates, that are used in HCV genome replication and terminates polymerase chain reaction by binding to the conserved active site (NS5B) of the polymerase.

This effect of the sofosbuvir is nonobligatory and non-specific and works for most of HCV genotypes (Lam *et al.,* 2010; Sofia *et al.,* 2010).The FDA accepted sofosbuvir (SOF) with a combination of ribavirin and for triple therapy with injected pegIFN and ribavirin of HCV genotypes. Theprescription dose is from 12 to 24 weeks (Tucker, 2013; Wang *et al.,* 2016).

This study investigates the effectiveness of sofosbuvir plus ribavirin treatment for hepatitis C genotype 2 and genotype 3 patients with cirrhosis.

Materials and methods

Sample collection and RNA extraction

Blood samples of 62 cirrhotic patients were collected from Gastroenterology ward HMC Peshawar, Khyber Pakhtunkhwa, Pakistan during January 2015 to May 2016 and serum was stored at -80℃. Written informed consent was obtained from all the recruited persons, containing demographic, clinical characteristics and estimated infection time. Approval of the study was granted from the Ethics Committee, COBAM, and University of Peshawar, Pakistan.

RNA was isolated from 150 ul serum by RNA extraction kit (Favor-gene Viral Nucleic Acid Extraction Kit I, Cat. No. FAVNK 001-2). Viral RNA

load was determined by real-time Polymerase Chain Reaction instrument (Rotor gene 6000, Real-time PCR). The complementary DNA (cDNA) sequence was synthesized using outer antisense primer (OAS) by means of manufacturing protocol (BIORON life science cDNA Kit. 105100).The outer antisense primer for qualitative PCR was the reverse primer in the first round of Nested PCR amplification of 5′-untranslated region of HCV and in the 2nd round, inner antisense (IAS) primer was used. After the detection of 5′-UTR of HCV, the sample was confirmed as HCV positive and the HCV genotyping was performed.

Multiplex PCR for genotyping

Forward and reverse primers in the multiplex PCR were adapted from Idrees *et al*., (2011).The 4 ul cDNA sample was amplified in the first round of multiplex PCR using forward or outer sense primer (5'TTGTGGTACTGCCTGATAGGG'3) and reverse or outer antisense primer (5' GGATGTACCCCATGAGGATCG'3). The PCR cocktail used in the 1st round was 7.1 ul PCR water, 6.9 ul master mix, 1 ul of each primer.

The PCR conditions were; initial denaturation of 94°C for 2 min followed by 35 rounds of 94°C denaturation step for 30 sec, annealing step of 54°C (30 sec) and extension step of 72°C for 45 sec with the final extension at 72°C for 10 min. Similarly, 4 µL of the first round PCR product was further amplified in round 2nd genotype-specific primers (1a, 1b, 2a, 2b, 3a, 3b, 4a, 5a, and 6a) in two mixtures. Mixture-A consisted of antisense primers: 1a, 3a, 4a, 5a, and 6a while mixture-B was the composition of antisense primers: 1b, 2a, 2b, 2c, and 3b.

The reaction mixture was composed of 5.5 ul PCR water, 4.5 ul master mix (Bioron life science) and 1 ul each primer to make total 20 ul volume followed by 35 rounds of 94°C initial denaturation step for (2 min), denaturation step for 94°C (1 min), annealing step of 64°C (1 min) and an extension step of 72°C (1 min) with the final extension at 72°C for (1 min). PCR products were seperated on 1.2% agarose gel for 35 minutes and the PCR products were visualized using UV trans-illuminator.

Study design

The study of sofosbuvir was conducted for the first time in KP, for the cure of HCV cirrhotic patients. HCV genotype 2 and 3 patient's infections were classified into two group in this study. Both groups (A and B) received sofosbuvir once daily and ribavirin twice per day. The group A and B patients received oral sofosbuvir (400 mg daily) plus ribavirin (200 mg twice daily) for 12 weeks and 24 weeks respectively. The dose was given to the patients according to the weight such as 1000 mg dose per day with a body weight of less than 75 kg and 1200 mg with a body weight of ≥75 kg.The SVR of group B patients was checked after 12 weeks of treatment and again after 24 weeks fresh qualitative PCR, full blood counting (FBC) for platelets and SGPT tests were also performed. Side effects observed in most of the patients during the treatment included fever, vomiting, pain, anemia, epigastric pain etc. In all the patients with HCV cirrhosis, the compensate stage cirrhosis patients were 37 and decompensate stage was 25. The biochemical tests were performed and all the adverse events were noted.

Statistical analysis

The data was analyzed through IBM SPSS 2017 for Windows and MS excel. The age wise genotyping variables and treatment with antiviral drugs were given in the form of mean and standard deviation (SD).

Results

All the recruited patients received a complete dose of sofosbuvir plus ribavirin. The patients were classified on the basis of gender, the type of HCV genotype involved and the stage of cirrhosis. Out of 62 patients, 37 patients (10 females and 27 male) were in the compensated stage of cirrhosis while 25 patients (12 females and 13 male) were reported in the decompensated stage of cirrhosis. Child Turcotte Pugh Score was used on the base of severity of liver disease analysis and scoring (Table 1).

Table 1. Demographic characteristics and etiology of HCV cirrhotic patients.

Gender	All patients	
Male	40 (35.5%)	
Female	22 (64.5%)	
Cirrhosis		
Compensate stage	37 (59.7%)	Male-27 Female-10
Decompensate stage	25 (40.3%)	Male-13 Female-12
Child turrcotte pugh score		
Child class A (5-6)	32 (51.61%)	
Child class B (7-9)	19 (30.64)	
Child class C (10-15)	11 (17.74)	
ALT- IU/L		
Mean ± Standard deviation	97.0±26.35	
AFP-ng/Ml		
<40	36 (58)	Male 24 Female 12
41-150	16 (26)	Male 11 Female 5
>150	10 (16)	Male 8 Female 6
Mean ± Standard deviation	75.96 ± 64.93	
Platelets		
Platelets - 10^3/mL	Decreased	
Haemoglobin g/dL		
Mean ± Standard deviation	11.5 ± 1.7	
Glucose		
Male/Female	Low or decreased	
Genotypes		
2a	12 (19.4%)	
3a	41 (66.1%)	
3b	09 (14.5%)	
Viral load (Iu/ml)		
10,00000 -30,00000	33 (53.2%)	
>30,00000-60,00000	22 (35.5%)	
>60,00000	07 (11.3%)	

ALT: Alanine transaminase, AFP: Alfa-Fetoprotein.

Frequency distribution of the HCV cirrhotic patients on the base of age wise, genotypes and gender base were shown in the (Fig. 1, 2, 3). All the HCV cirrhotic patients had a sustained the virologic response to the treatment of sofosbuvir plus ribavirin. In this study, the group B patients (HCV genotype 3) were

predominant (50) as compared to genotype 2 (12), indicating the increased genotype 3 infection in Khyber Pakhtunkhwa, Pakistan.

The antiviral drugs sofosbuvir plus ribavirin were given to the HCV genotype 2 patients for 3 months and genotype 3 patients for 6 months. After the completion of three months of treatment, blood samples of both the groups were subjected to real time PCR to monitor the viral response of anti-viral treatment. Ninety-two percent of the patients with genotypes 2 showed no detectable HCV RNA and 8% of the non-respondents had positive serum viral RNA.

In 70% of the patients with HCV genotype 3 infection (group B), no HCV RNA was detected after three months of treatment. All the group B patients were treated with sofosbuvir plus ribavirin for another three months. After six months of treatment, 44 patients (88%) had sustained the virologic response while 6 patients (12%) were still non-respondents to the drugs (Table. 2).

Table 2. Treatment with sofosbuvir plus ribavirin after three months and six months.

Patient classification					Patient treatment			
S/no	Genotype 2a	Genotype 3a	Genotype 3b	HCV +ive (Genotypes-2) 12 weeks	HCV –ive (Genotypes-2) 12 weeks	HCV +ive (Genotypes-3) 12 weeks	HCV –ive (Genotypes-3) 12 weeks	HCV –ive (Genotypes-3) 24 weeks
1	3	4	2	-	3/3	-	6	6/6
2	6	24	4	-	6/6	9	19	26/28
3	2	12	3	-	2/2	5	10	12/15
4	1	1	-	1	0/1	1	-	0/1
		Total (62)			11/12			44/50

Symbol. (-) No patient.

The main reason of the patients with no response to the treatment regimen could be due to different complications such as aging, general weakness, diabetes, sugar, high blood pressure, decreasing of platelets etc. The level of alanine transaminase before the treatment of the patients was in the range of 74 to 296 IU/L. All the liver function tests were in the normal range after the treatment.

The most common complications before and during treatment were fever, headache, fatigue, insomnia, nausea, diabetes, constipation, anemia, myalgia, and vomiting etc. (Figure 4). Some of the patients reported severe abdominal and kidney pain during the three months (genotype 2) and six months (genotype 3) treatment. Other commonly observed side effects were the decreased level of hemoglobin, platelets, and glucose in the HCV cirrhotic patients. In compensate stage of liver cirrhosis, the level of hemoglobin, platelets, and glucose become in the normal ranges after 4 to 6 weeks of treatment, while in the decompensate stage, the level becomes in the normal range after 8 weeks of treatment. The commonly observed side effects in the decompensate stage of cirrhosis include anemia, ascites, fever, and low platelets level during the treatment.

Discussion

To date, nostudy has been reported on sofosbuvir therapy in Khyber Pakhtunkhwa, Pakistan. In the present study, 62 HCV cirrhotic patients with genotype 2 (group A) and 3 (group B) infection were treated with oral antiviral drugs sofosbuvir plus ribavirin for three and six months respectively.

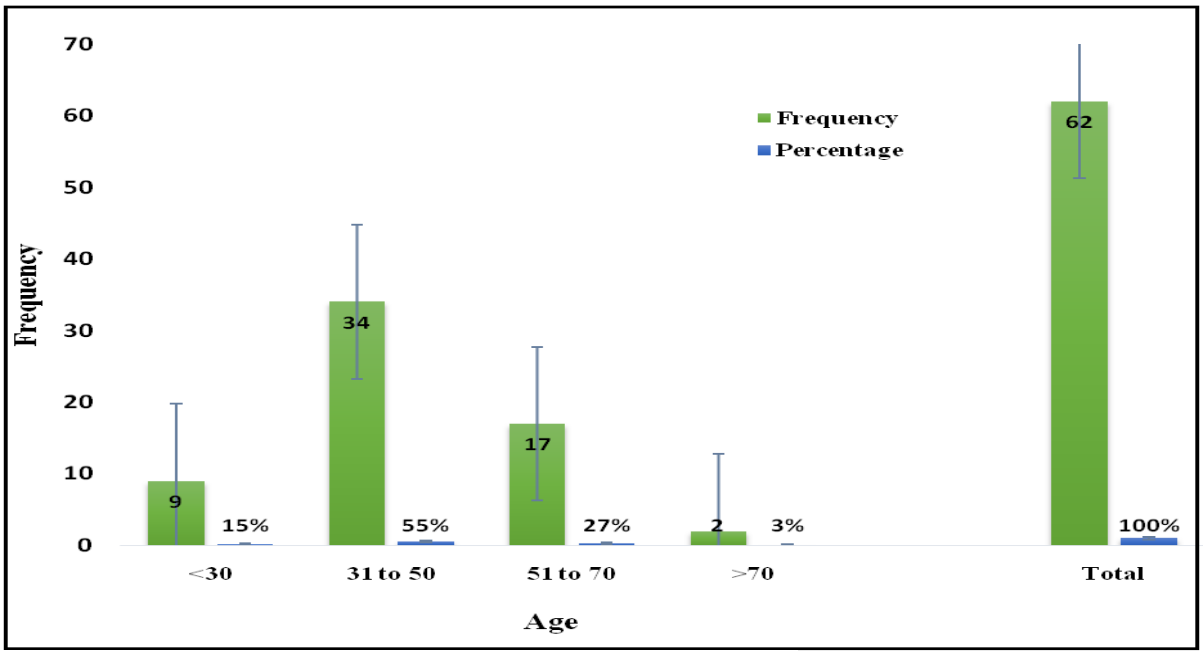

Fig. 1. Frequency distribution of HCV cirrhotic patients on the base of Age.

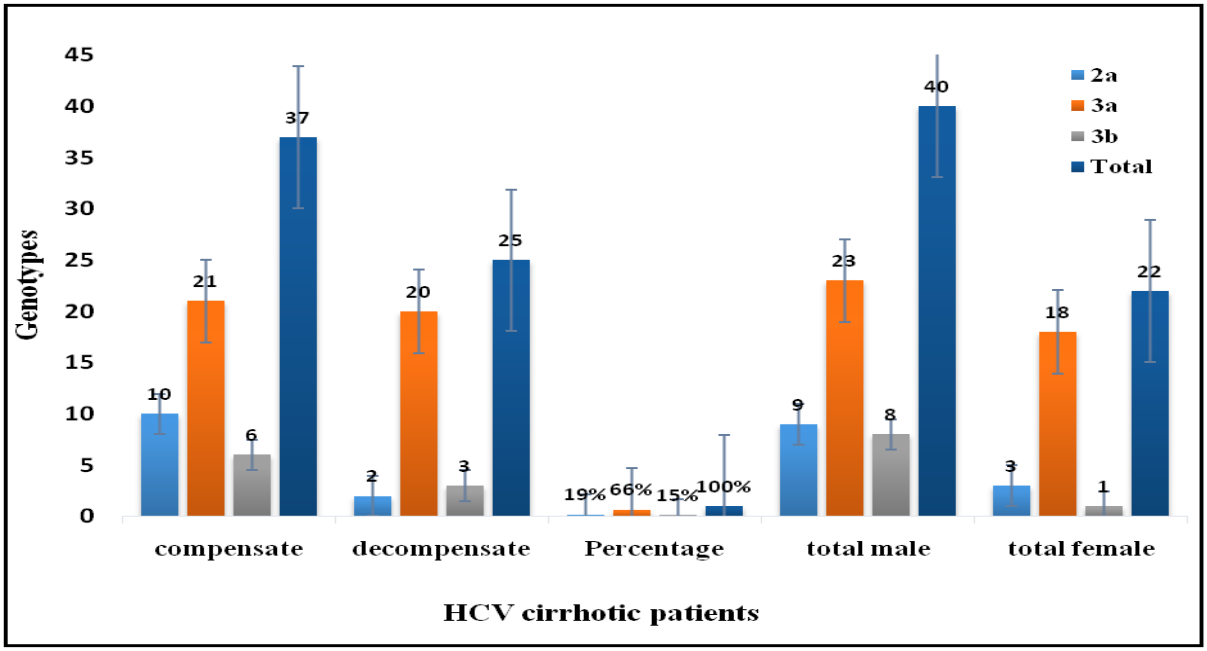

Fig. 2. Classification of HCV cirrhotic patients on the base of genotypes.

After six months of treatment, 88% of the patients (55) had undetectable HCV RNA. In patients having genotype 2 infection, 11 patients (92%) had sustained the virologic response after the three months of treatment. After six months treatment of genotype 3 patients with sofosbuvir plus ribavirin, 44 patients (88%) had untraceable RNA of the virus. After the initial treatment of HCV genotype 3 patients for three months, 35 patients (70%) had sustained the virologic response. Extending the treatment duration of HCV genotype 3 patients for another three months resulted in the increased sustained the virologic response of 44 patients (88%). The drug response rate to genotype 3 was lower as compared togenotype 2 patients. So, sofosbuvir extended treatment of 24 weeks significantly improved the rate of SVR in genotype 3 cirrhotic patients. The lower response rate of the HCV genotype 3 patients as compared to genotype 2 is yet to be elucidated by Wyles, (2012), Jacobson *et al.,* (2013) and Lawitz *et al.,* (2013).

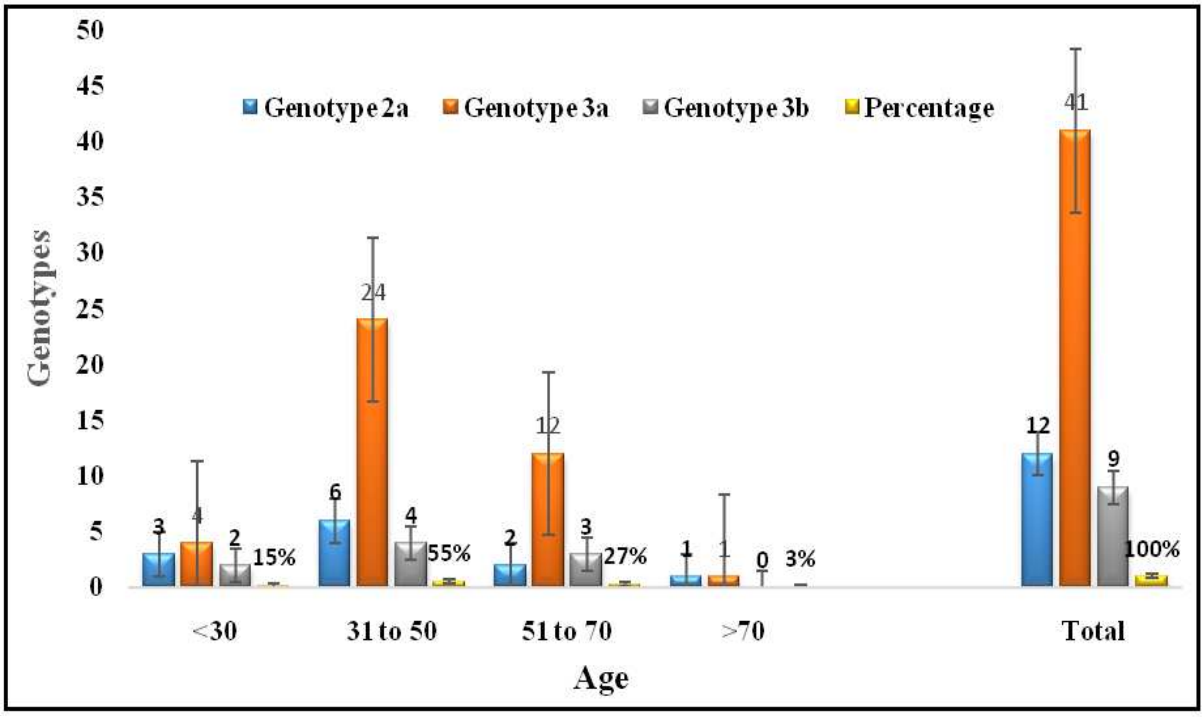

Fig. 3. Distribution of HCV genotypes on the base of Age wise.

In this study, the sofosbuvir plus ribavirin treatment was found non-effective in one of the genotype 2 patient (8%) and six genotype 3 patients (12%). Early cessation of the drug in patients with genotype 2 and 3 infections was low.

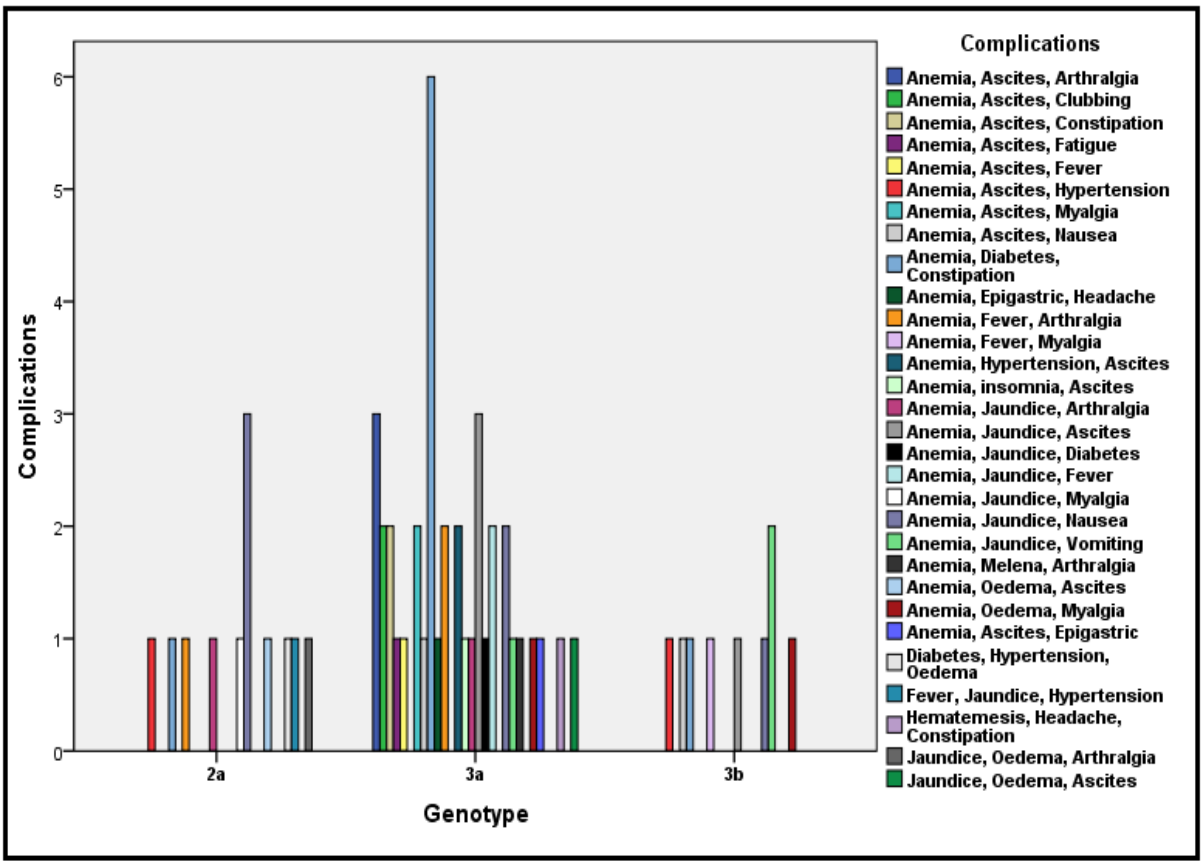

Fig. 4. Common complications HCV cirrhotic patients during treatment.

The fewer early cessation cases were due complications related to ribavirin therapy. Common side-effects of the sofosbuvir plus ribavirin therapy observed were anemia, arthralgia, ascites, abdominal pain, constipation, diabetes, epigastric pain, fever, fatigue, hematemesis, hypertension, headache, insomnia, jaundice, melena, myalgia, nausea, oedema, vomiting in these patients. Similar side effects related to sofosbuvir plus ribavirin therapy were reported by McHutchison*et al.*, (1998) and Fried*et al.,* (2002).

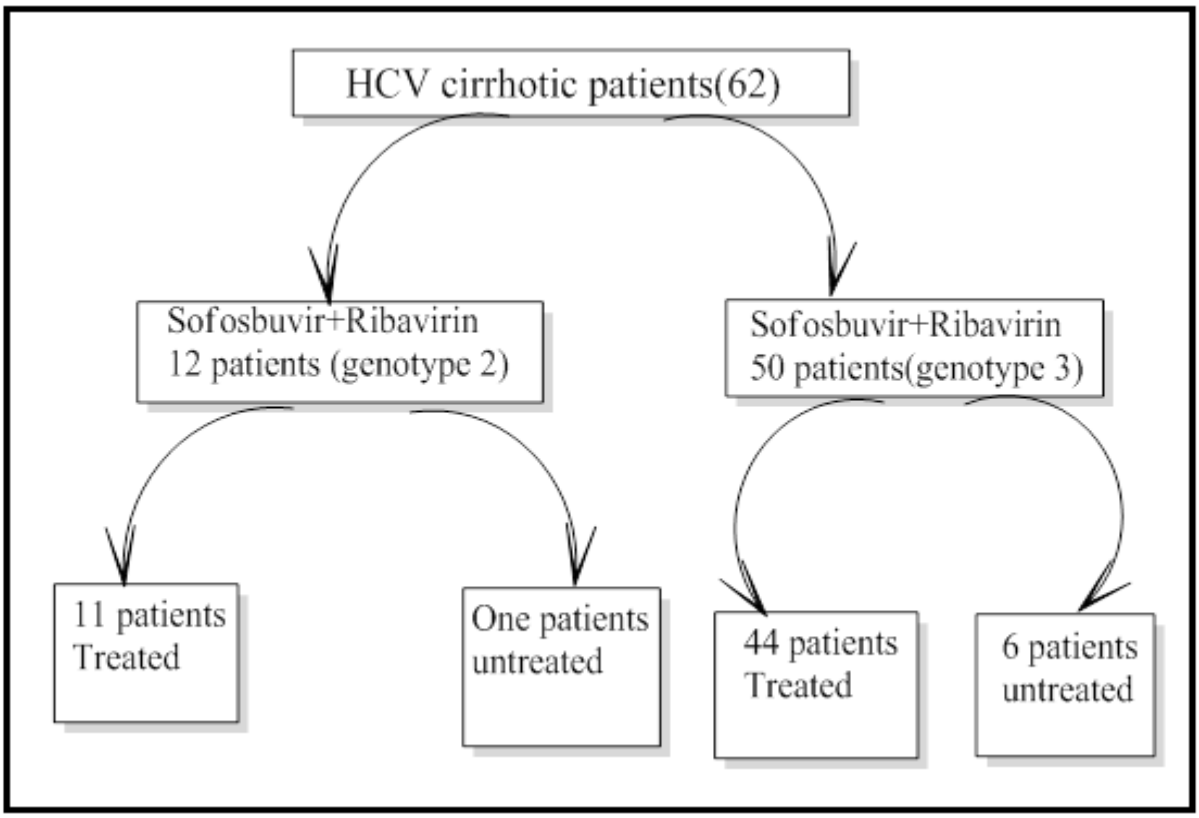

Fig. 5. Graphical summary. Antiviral drugs for HCV patients.

Recently, Gayem *et al.,* (2018) reported the effects of sofosbuvir plus ledipasvir and sofosbuvir plus valaprevir on the 112 patients with compensate stage cirrhosis and the SVR was reported 89 and 92% respectively. Akhane *et al.*, (2018) described the efficacy of sofosbuvir plus ribavirin in the treatment of chronic hepatitis C patients and was recorded the rapid viologic response (81.3%), end of treatment response (99.2%) and SVR (96.6%). The main side effects observed in these patients was anemia and similar observation was noted in our study.

Pakistan has a historic high HCV epidemic as approximately 1 in 20 individuals are infected with the virus. Liver diseases associated with HCV infections are increasing in the country. Healthcare procedures account for most of the transmissions in the risk populationsstated by Afsheen*et al.*,(2017) and Kanaani*et al.*,(2018).According to the last ten years data, HCV genotype 3a is the common genotype responsible for HCV infections in about 62-70% of the patientsdescribed by Butt*et al.*,(2009)and is one of the most prevalent genotype in Nepal, Bangladesh andIndiastudied by Tokita *et al.,* (1994),Das*et al.,* (2002) andNarahari, (2009).

The genotype 3 virus infection rate was higheras compared with genotype 2 virus and high rate of genotype 3 was observed in our study. Similar high prevalence of genotype 3 infection was reported in the studies ofIdress *et al.,* (2011), Afridi *et al.*,(2014) and Akhtar *et al.*,(2015).

Conclusion

Hepatitis C virus is the main cause of liver cirrhosis. There is a continuous increase of reported HCV cirrhosis patients in Khyber Pakhtunkhwa, Pakistan. Treatment of genotype 2 and genotype 3 with sofosbuvir plus ribavirin for three and six months respectively may be effective for HCV cirrhotic patients. This study further necessitates studies on sofosbuvir in combination with ribavirin or with other antiviral agents in various HCV patients of other populations.

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Ethical approval

Ethical approval and informed consent were obtained from the Ethics Committee of the Center of Biotechnology and Microbiology, University of Peshawar.

Conflict of interest

No conflicts of interest were reported by the authors.

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