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Original Article

Safety and efficacy of Sofosbuvir therapy in chronic hepatitis C patients of Peshawar, Khyber Pakhtunkhwa, Pakistan

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Abstract

This study is designed to monitor the rapid virological response (RVR) for 8 weeks and sustained virological response (SVR) for 12 weeks during Sofosbuvir therapy in chronic hepatitis C patients in Peshawar, KPK, Pakistan. A total of 162 hepatitis C positive patients were enrolled in this study. After RNA isolation, Quantitative PCR was done to get viral load, and Ohn *et al*., 1997 protocol were used for hepatitis virus different genotypes. Statistical analysis was performed using SPSS. A Sample *t*-test was applied to compare gender and age. Chi Square test was applied to compare the RVR and SVR. In total 162 positive hepatitis C patients, 90 were male and 72 were female. High rate 153(94%) of RVR and 158(97%) SVR were achieved during 8 and 12 weeks of sofosbuvir therapy. High SVR and RVR were achieved in the <30 and 31-40 age groups. High EVR 125(99%) by genotype 3 were achieved at 8 weeks of therapy and high SVR 126(100%) by genotype 3 and 28(93%) by genotype 1 were achieved at 12 weeks of sofosbuvir therapy. The current study reveals that high RVR and SVR rates were obtained by shortening the SOF therapy's treatment time up to 8 and 12 weeks. We got a 98.7% response rate at the end of the treatment.

Keywords: efficacy, hepatitis C virus, Sofosbuvir, therapy, Peshawar, Pakistan

1. Introduction

Globally three percent of the world population is infected from hepatitis C virus of which seventy-one million people have chronic hepatitis C viral infection (World Health Organization [WHO], 2017). Hepatitis C virus infection can develop fibrosis in the liver, which can progress to cirrhosis, decompensation in the liver, and then cause cancer in the liver (Feld *et al*., 2915). Overall, there are already 399,000 deaths associated with HCV, primarily due to cirrhosis that cannot be recovered and is mostly considered the last stage of hepatic Carcinoma (Ashfaq *et al*., 2011). Hepatitis C virus genotypes and subtypes have been recognized worldwide; six genotypes were deeply analyzed for epidemiological, therapy, and vaccine development (Akbar *et al*., 2009). Currently, there are seven to eight genotypes of hepatitis C infection. Expanded classification of hepatitis C virus into seven genotypes and 67 subtypes: updated criteria and genotype assignment web resource (Smith *et al*., 2014). Identification of a novel hepatitis C virus genotype from Punjab, India: expanding classification of hepatitis C virus into eight genotypes (Borgia *et al*., 2018). Hepatitis C is prevalent and distributed differently in different parts of the world (Morozov, & Lagaye, 2018). Genotypes 1, 2, and 3 are distributed throughout the globe. A Subtype of genotype 1, 1a and 1b are most commonly present in Europe, United States, and

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Australia while subtype 1b is most common in China and Japan (Zein *et al*., 1996). In Europe, Japan and North America, genotype 1 is the most predominant (Rivas-Estilla *et al*., 2008). Genotype 3 is the most common in Asian countries and Nepal. In 2016, in Pakistan, the prevalence rate of the hepatitis C virus was six percent, but in 2017 this surged to 8.4% (Sebastiani *et al*., 2019).

Besides the pattern of the distribution of genotype, the viral load pre-treatment is also a major determinant in hepatitis C treatment and also a prognostic factor in treatment and recurrence rate (Roffi *et al*., 2001). The recommended treatment option for hepatitis C patients between 2000 and 2011 was Interferon plus Ribavirin (Waheed, Bhatti, & Ashraf, 2013). Treatment response of hepatitis C virus patients depends on a variety of factors, including a number of age of patients, condition of the liver, number of viruses, and genotype of hepatitis C virus. There was little response with some adverse effects with the combined therapy (Aziz, Raza, Waheed, Gill, & Gill, 2012; Crotty, Cameron, & Andino, 2001). Sofosbuvir is an FDA-approved HCV polymerase pyrimidine analog that has shown good potency, high reaction to mutations and low side effects of oral treatment (Waheed, Bhatti, & Ashraf, 2013). Sofosbuvir is a phosphoramidite medicine transformed in the hepatocytes to its active form. The active form imitates physiological nucleotides and includes the developing RNA strand which results in RNA chain termination suppression of RNA synthesis (Aziz *et al*., 2011). In this research, 162 hepatitis C virus positive patients received Sofosbuvir treatment for 12 weeks. The study's goal was to monitor the rapid virological response (RVR) for 8 weeks and sustained virological response (SVR) for 12 weeks during Sofosbuvir therapy. We also examined the effect of gender, age groups and genotypes on the treatment outcome of SOF. Complications associated with sofosbuvir (SOF) treatment in chronic hepatitis C patients were also examined during end of therapy.

2. Materials and Methods

2.1 Area of study

A prospective non-randomised study was carried out from April to June 2018. This study was conducted on chronic hepatitis C virus positive patients of both genders who were ready for using Sofosbuvir (SOF) therapy 400mg once a daily for treatment all these patients who were registered at Khyber Teaching hospital, Peshawar.

2.2 Study design

This study were designed to check the safety and efficacy rate of Sofosbuvir therapy in chronic hepatitis C patients by shortening treatment duration to 8 and 12 weeks. Data were collected from all those subjects who have positive HCV viral load result after the quantitative PCR test. Enrollment criteria of the patient are; patients which had a chronic infection of hepatitis C virus, Non-Cirrhotic and had not used any past treatment. Those patients were not included who were previously treated with interferon therapy, both treatment; relapse and non-responders were also excluded from the study.

2.3 Sample collection

Data was collected and information regarding patients with HCV positive was obtained using predetermined questionnaires. The key criteria of the questionnaire included names of patients, gender, age, CBC and test of liver, etc. In this study, a total of 162 chronic HCV-positive patient samples were included. Nearly 3 ml of blood has been obtained with the approval of the patient or the guardian of the patient. Serum isolation from blood, which has been transferred to the Zoology Department of Molecular Biology and Virology Laboratory, University of Peshawar and kept at -20°C.

2.4 RNA extraction

RNA was extracted from the blood plasma by using Trizol (Thermofisher) reagent.

2.5 HCV RNA PCR quantitative test

Quantitative PCR was carried out to find out the positivity and viral load of hepatitis C patients. Extracted RNA were then carried out by different steps to get the result of Quantitative PCR. Master mix were prepared using PCR tube and centrifugation of tube were briefly done. After the centrifugation, the PCR tube were inserted into Roter Gene Machine for the amplification process. Positivity was checked for internal control (I.C). The findings of all internal controls are positive, indicating that the results are genuine. Quantification of HCV was performed three times: before treatment began, at eight weeks, and twelve weeks.

2.6 HCV genotyping

Genotyping was performed on all HCV-positive samples. By using Ohno *et al* protocol six different genotypes of HCV were identified (55). The process of reverse transcription for the synthesis of cDNA using viral RNA (10 L) as a template was carried out with 200 units of M–mL Virus RT. Forward primer (5′-GGGAGGTCTCGTAGACC GTGCACCATG-3′) and reverse primer (5′ GAGACGGG TATAGTACCCCATGAGAGTCGGC-3′) were used to amplify 5 µL of synthesized cDNA in the first round of PCR. Two second-round PCRs were run for each first-round PCR sample. Mix 1, which included primers for genotypes 1b, 2a, 2b, and 3b, was used in a one-second round. Mix 2 was used to complete another second round, which included genotype-specific primers for 3a, 4, 5a, 1a, and 6a. In all PCRs, Taq DNA polymerase (Invitrogen) was employed to amplify the template cDNA. After electrophoresis on a 2% agarose gel with a DNA marker of 100 bp ladder, the amplified products of the second round of PCR were observed on a gel documentation system. By comparing the resulting band size to the HCV genotype-specific PCR band, the HCV genotype of each sample was confirmed.

2.7 Statistical analysis

To determine the percentage gender, age, genotype, and treatment impact of patients with hepatitis C, statistical

analysis was performed using SPSSv23 (IBM SPSS Statistics Inc). Sample *t*-test was applied to compare the gender and age. Chi Square test was applied to compare the RVR and SVR.

3. Results

The baseline characteristics of the study are shown in Figure 1. A total of 162 chronic hepatitis C patients completed 12 weeks of sofosbuvir therapy, out of which 90 (56%) were male and 44 (44%) were female. Patients were also distributed based on of age group, with an age range of 28 to 68. The age was divided into four groups; <30, 31-40, 41-50, and >50. HCV genotypes were also determined during the study, showing the highest prevalence rate of genotype 3 followed by 1, 4, and untypable genotypes. Rapid virological response at 8 weeks and sustained virological response at 12 weeks of sofosbuvir therapy in chronic hepatitis C patients showed a high rate of 97% of SVR at 12 weeks of therapy as compared to RVR at 8 weeks of therapy.

Gender wise effect of sofosbuvir (SOF) treatment on chronic hepatitis C patients were checked in this study. Both RVR for 8 weeks and SVR for 12 weeks were studied in gender. A total of 162 patients were included in this study. Out of 162 patients, 90(56%) were male and 72(44%) were female. During treatment 84(93%) male patients and 69(96%) female patients receive RVR at 8 weeks and 6(7%) male and 3(4%) female patient were detected positive. Sustained virological response (SVR) were achieved in 87(97%) male and 71(99%) female patients and only 3(3%) male and 1(1%) female patient had detectable HCV RNA viral load at 12 weeks (Table 1)

The age range was between 20 and 68 years and four groups were arranged. Age-wise EVR and ETR of 162 individuals were also assessed during the study. During the treatment patient having ages less <30 and 31-40 received RVR at 8 weeks' therapy and only 2(4%) patients and 7(12%) patients from 41-50 age and >50 age group were detected positive. SVR were also checked after 8 weeks of therapy and 44(98%) from 41-50 and 57(95%) patients from age group >50 were detected negative and Only 1(2%) from group 41-50 and 3(5%) from age group >50 were detected positive during 12 weeks' therapy (Table 2).

RVR and SVR were also checked in the different genotype of chronic hepatitis C patients. Out of 162 patients, 28(93%) patient from genotype 1, 125 (99%) patients from genotype 3 were detected negative during 8 weeks of therapy and only 2(7%) patient from genotype 1, 1(1%) patients from genotype 3, 3(100%) from genotype 4 and 3(100%) patient had untypable genotype were positive at 8 weeks of therapy. ETR were also checked in which 28(93%) patients from genotype 1, 126(100%) patient from genotype 3, 2(67%) from genotype 4, 2(67%) from Untypable were detected negative and 2(7%) from genotype 1, 1(33%) patient from genotype 4 and 1(33%) patient had untypable genotype were detected positive HCV RNA at the end of 12 weeks' treatment (Table 3).

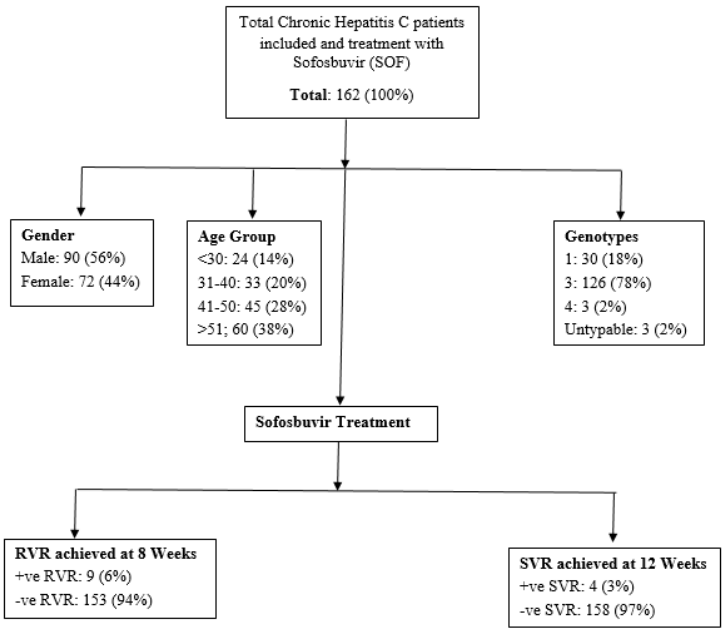

Figure 1. Showing baseline characteristics of chronic hepatitis C patients

Table 1. Gender wise effect of SOF therapy on chronic hepatitis C patients

Gender	Frequency (%)	RVR achieved (%)		SVR achieved (%)	
		+ve	-ve	+ve	-ve
Male	90(100)	6(7)	84(93)	3(3)	87(97)
Female	72(100)	3(4)	69(96)	1(1)	71(99)
Total	162(100%)	9(6%)	153(94%)	4(3%)	158(97%)

Table 2. Age wise effect of SOF therapy on chronic hepatitis C patients

Age	Frequency (%)	RVR achieved (%)		SVR achieved (%)	
		+ve	-ve	+ve	-ve
<30	24(100)	--	24(100)	--	24(100)
31-40	33(100)	--	33(100)	--	33(100)
41-50	45(100)	2(4)	43(96)	1(2)	44(98)
>50	60(100)	7(12)	53(88)	3(5)	57(95)
Total	162(100%)	9(6%)	153(94%)	4(3%)	158(97%)

Table 3. Genotype wise effect of SOF therapy on chronic hepatitis C patients

Genotype	Frequency (%)	RVR achieved (%)		SVR achieved (%)	
		+ve	-ve	+ve	-ve
1	30(100)	2(7)	28(93)	2(7)	28(93)
3	126(100)	1(1)	125(99)	--	126(100)
4	3(100)	3(100)	--	1(33)	2(67)
Untypable	3(100)	3(100)	--	1(33)	2(67)
Total	162(100%)	9(6%)	153(94%)	4(3%)	158(97%)

Adverse effect were also observed in chronic hepatitis C patients during 24 weeks of sofosbuvir therapy. Out of 162 chronic hepatitis C patients, the most common adverse effect 135(83%) were fatigue, 99(61%) had Nausea, 105(65%) had diarrhea, 84(52%) had a headache, 132(81) had muscle spasm and 90(55%) had Insomnia (Figure 2).

4. Discussion

The research was conducted in Peshawar, Khyber Pakhtunkhwa, Pakistan, to evaluate the efficacy of sofosbuvir treatment on chronic hepatitis C patients. A total of 162(100%) patients were enrolled in this study after detecting positive HCV Quantitative PCR of every individual. Gender-wise effects of SOF therapy were also studied. RVR was obtained in 94% of patients, whereas SVR was achieved in 97% of patients. Only 4(3%) patients did not achieve SVR out of 162 patients in which 3(3%) were male and 1(1%) were female. A High rate of EVR 99% and ETR 98.7% were also achieved in patients which are treated with sofosbuvir and ribavirin therapy (Jameel, Waheed, Malik, & Durrani, 2018). A clinical trial by Zeuzem *et al*., (2014) also shows 85% of SVR in hepatitis C patients using DAA therapy. Our results were also similar to Akhter *et al*., 2016 who administered DAA therapy and achieved 96.5% ETR at 24 weeks. SVR12 was attained in 98.6% of all patients investigated; whereas females had a better SVR12 than males, and the majority of relapsers were males, this difference is not clinically significant (Akhter *et al*., 2016). Similarly, several investigations have shown that female sex and less hepatic fibrosis are independently linked to higher SVR rates. In addition, fertile women with genotypes 2 or 3 have a greater therapeutic response (Conjeevaram *et al*., 2006; Floreani *et al*., 2011; McHutchison *et al*., 2009; Roberts *et al*., 2010; Saif-Al-Islam *et al*., 2020). These investigations, however, have been predicated on interferon therapy. Yang *et al*., on the other hand, observed SVR be high in patients receiving DAA therapy regardless of age, gender, or degree of hepatic fibrosis (Yang *et al*., 2019).

Patients age were an important factor, to check the RVR and SVR rate among chronic hepatitis C patients, they are categories into different age group; <30, 31-40, 41-50, and >50. During the treatment patient having age less <30 and 31-40 received RVR at 8 weeks' therapy and only 2(4%) patients and 7(12%) patients from 41-50 age and >50 age group were detected positive. SVR were also checked after 12 weeks of therapy. 44(98%) from 31-40 and 57(95%) patients from age group >50 were detected negative and Only 1(2%) from group 31-40 and 3(5%) from age group >50 were detected positive during 12 weeks' therapy. The result of our study is also similar to Aziz *et al*., 2012 showing the highest SVR and ETR rate in both 8 and 12 weeks of treatment at <30 and 31-40 age group (Aziz, Raza, Waheed, Gill, & Gill, 2012). A recent study also found that the age of a woman is a significant factor in determining responses to treatment. Women under the age of 40 have a better response to therapy than women over the age of 40 (Fried, Shiftmman, & Reddy, 2002). According to Tacke *et al*., being > 70 years old is a factor in poor SVR prognosis (73 percent, P = 0.26) (Tacke, Günther, & Buggisch, 2017). SVR rates of 92.3 percent were observed in the HCV-TARGET investigation, which had a mean patient age of 59.0 years, (Welzel, Nelson, & Morelli, 2016), and high SVR rates of 97.0 percent (Ahn, Lim, & Lee, 2016) and 93.0 percent were reported in Asian studies with average patient ages of 55 (Backus *et al*., 2015).

The effect of sofosbuvir therapy were also checked in chronic hepatitis C patients having different genotypes. Different RVR and SVR were shown by different genotypes during treatment. In total 162 patients, 126(78%) patients have hepatitis genotype 3. A high rate of RVR 99% at 8 Weeks and SVR 100% at 12 weeks were achieved by patients having hepatitis C genotype 3. A total of 30(18%) out of 162 patients have hepatitis C genotype 1 of which 28(93%) have negative of RVR and SVR at 8 and 12 weeks of therapy, while only 2(7%) have positive EVR and ETR. 3(100%) patients from genotype 4 and untypable genotype have positive RVR at 8 weeks of therapy while negative and high rate of SVR 2(67%) were achieved at 12 weeks of sofosbuvir therapy. Only

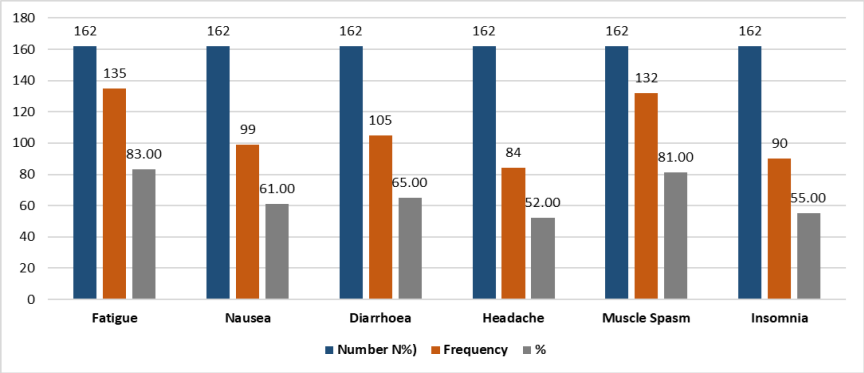

Figure 2. Adverse effect in chronic hepatitis C patient using Sofosbuvir therapy

1(33%) show positive RVR and SVR in both genotype 4 and untypable genotype. A similar study was conducted in Egypt by Doss *et al*., 2015 shows SVR 12 of HCV genotype 4 patients. In HCV genotype 2 patients treated with sofosbuvir and ribavirin in Japan, SVR12 was 90.4 % (Kanda, & Moriyama, 2017). A recent study shows the consistent result of our study which has 279 (91.8%) patients from HCV genotype 3, 23 (7.6%) patients from HCV genotype 1 and 2 (0.7%) patients from other HCV genotypes. Out of 279 HCV genotype 3 patients, 276 (98.92%) achieved both EVR and ETR. While all the 23 HCV genotype 1 patient achieved EVR and 22 patients achieved ETR. Two patients with other HCV genotypes achieved both EVR and ETR (Jamil, Waheed, Malik, & Durrani, 2018). Similar studies also show a high rate of EVR and ETR among the patients which 3a and 3b hepatitis C genotype using Sofosbuvir therapy (Wei *et al*., 2018; Huang *et al*., 2019).

Adverse effect related to hepatitis C patients during the end of sofosbuvir therapy were also observed. Patients complain of different adverse effect with 12 weeks of treatment. Out of 162 chronic hepatitis C patients, the most common adverse effect 135(83%) were fatigue, 99(61%) had Nausea, 105(65%) had diarrhea, 84(52%) had headache, 132(81) had muscle spasm and 90(55%) had Insomnia. The result of recent studies is also consistent with our findings showing adverse effects like fatigue, insomnia, headache, muscle spasm and nausea in chronic hepatitis C patients while using DAA therapy (Foster *et al*., 2015; Gayam *et al*., 2018).

5. Significance Statement

In systematic studies, modern molecular approaches should be done on the effect of Sofosbuvir therapy in chronic hepatitis C patients to know about their effect on different genotype of hepatitis C virus and its correlation with hepatitis C Viral Load. The current study suggests that shortening HCV therapy may be achievable and should be investigated further. Before using SOF therapy, it is necessary to have a clear understanding and knowledge of SOF therapy.

6. Conclusions

The current study reveals that high RVR rate of 94% were obtained by shortening the SOF therapy's treatment up to 8 weeks. Only a few patients did not receive RVR which has a high viral load and has 1, 3 genotypes. High SVR rates of 97% were achieved during 12 weeks of sofosbuvir treatment. In the current study, patients with HCV infection in Pakistan responded well to sofosbuvir treatment, irrespective of their treatment history, genotype, age, or viral level. We got a 97% response rate at the end of the treatment. It is also concluded from the current study shortening the treatment duration of sofosbuvir therapy up to 8 weeks is as impressive and possible. The current study also concludes that the treatment of HCV patients in Pakistan, the Sofosbuvir medication is very successful, safe, and cost-efficient.

The limitation of the present study is the low sample and resources. So further study in detail and of sample is needed to explore sofosbuvir effect in Pakistani population. Before using SOF treatment, patients need adequate knowledge and awareness.

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References

Ahn, S. H., Lim, Y. S., Lee, K. S. (2016). A phase 3b study of sofosbuvir plus ribavirin in treatment-naive and treatment-experienced Korean patients chronically infected with genotype 2 hepatitis C virus. *Journal Virology Hepatology*, *23*(5), 358-365.

Akbar, H., Idrees, M., Manzoor, S., Rehman, I., Butt, S., Yousaf, M. Z., . . . Aftab, M. (2009). Hepatitis C virus infection: A review of the current and future aspects and concerns in Pakistan. *Journal of General and Molecular Virology*, *1*(2), 012-018.

Akhter, T. S., Umar, M., Khaar, H. T., Aslam, F., Nisar, G., Naseer, A., . . . Osama, M. (2016). Sofosbuvir for the treatment of hepatitis C genotype 3 infected patients in Pakistan. *Journal of Ayub Medical College, Abbottabad*, *28*(4), 884-889.

Ashfaq, U. A., Javed, T., Rehman, S., Nawaz, Z., & Riazuddin, S. (2011). An overview of HCV molecular biology, replication and immune responses. *Virology journal, 8*(1), 1-10.

Aziz, H., Athar, M. A., Murtaza, S., Irfan, J., Waheed, Y., Bilal, I., & Raza, A. (2011). Predictors of response to antiviral therapy in patients with chronic hepatitis C from Pakistani population. *Chinese Medical Journal, 124*(9), 1333-1337.

Aziz, H., Raza, A., Waheed, Y., Gill, U., & Gill, M. L. (2012). Analysis of variables and interactions among variables associated with a sustained virological response to pegylated interferon alfa-2a plus ribavirin in hepatitis C virus genotype 3-infected patients. *International Journal of Infectious Diseases, 16*(8), 597-602.

Backus, L. I., Belperio, P. S., Shahoumian, T. A., Loomis, T. P., & Mole, L. A. (2015). Effectiveness of sofosbuvir-based regimens in genotype 1 and 2 hepatitis C virus infection in 4026 U.S. veterans. *Alimentary Pharmacology and Therapeutics, 42*(5), 559-573.

Borgia, SM., Hedskog, C., Parhy, B., Hyland, RH., Stamm, LM., Brainard, . . . Shafran, S. D. (2018). Identification of a novel hepatitis C virus genotype from Punjab, India: Expanding classification of hepatitis C virus into 8 genotypes. *The Journal of Infectious Diseases, 218*(11),1722-1729.

Conjeevaram, H. S., Fried, M. W., Jeffers, L. J., Terrault, N. A., Wiley–Lucas, T. E., Afdhal, N., . . . Howell, C. D. (2006). Peg interferon and ribavirin treatment in African American and Caucasian American patients with hepatitis C genotype 1. *Gastroenterology, 131* (2), 470-477.

Crotty, S., Cameron, C. E., & Andino, R. (2001). RNA virus error catastrophe: Direct molecular test by using ribavirin. *Proceedings of the National Academy of Sciences, 98*(12), 6895-6900.

Doss, W., Shiha, G., Hassany, M., Soliman, R., Fouad, R., Khairy, M., . . . Doehle, B. (2015). Sofosbuvir plus ribavirin for treating Egyptian patients with hepatitis C genotype 4. *Journal of Hepatology, 63*(3), 581-585.

Feld, J. J., Jacobson, I. M., Hézode, C., Asselah, T., Ruane, P. J., Gruener, . . . Mazzotta, F. (2015). Sofosbuvir and velpatasvir for HCV genotype 1, 2, 4, 5, and 6 infections. *New England Journal of Medicine, 373* (27), 2599-2607.

Floreani, A., Cazzagon, N., Boemo, D. G., Baldovin, T., Baldo, V., Egoue, J., . . . Minola, E. (2011). Female patients in fertile age with chronic hepatitis C, easy genotype, and persistently normal transaminases have a 100% chance to reach a sustained virological response. *European Journal of Hastroenterology and Hepatology, 23*(11), 997-1003.

Foster, G. R., Afdhal, N., Roberts, S. K., Bräu, N., Gane, E. J ., Pianko, S., . . . Towner, W. J. (2015). Sofosbuvir and velpatasvir for HCV genotype 2 and 3 infection. *New England Journal of Medicine, 373* (27), 2608-2617.

Fried, M. W., Shifmman, M. L., & Reddy, K. R. (2002). Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *New England Journal of Medicine, 347*, 975-82.

Gayam, V., Mandal, A. K., Khalid, M., Mukhtar, O., Gill, A., Garlapati, P., . . . Mansour, M. (2018). Sofosbuvir based regimens in the treatment of chronic hepatitis C with compensated liver cirrhosis in community care setting. *International Journal of Hepatology*.

Huang, R., Rao, H., Xie, Q., Gao, Z., Li, W., Jiang, D., & Wei, L. (2019). Comparison of the efficacy of sofosbuvir plus ribavirin in Chinese patients with genotype 3a or 3b HCV infection. *Journal of Medical Virology, 91*(7), 1313-1318.

Jamil, Z., Waheed, Y., Malik, M., & Durrani, A. A. (2018). Effect of Sofosbuvir plus Ribavirin therapy on hepatitis C patients in Pakistan: a retrospective study. *Peer Journal, 6*, 4853.

Kanda, T., & Moriyama, M. (2017). Direct-acting antiviral agents against hepatitis C virus and lipid metabolism. *World Journal of Gastroenterology, 23* (31), 5645.

McHutchison, J. G., Lawitz, E. J., Shiffman, M. L., Muir, A. J., Galler, G. W., McCone, J., . . . Galati, J. S. (2009). Peg interferon alfa-2b or alfa-2a with ribavirin for treatment of hepatitis C infection. *New England Journal of Medicine, 361*(6),580-593.

Morozov, V. A., & Lagaye, S. (2018). Hepatitis C virus: Morphogenesis, infection and therapy. *World Journal of Hepatology, 10*(2), 186.

Rivas-Estilla, A. M., Cordero-Pérez, P., Carmen Trujillo-Murillo, K., Ramos-Jiménez, J., Chen-López, C., Garza-Rodríguez, M. D. L., . . . Munoz-Espinosa, L. (2008). Genotyping of hepatitis C virus (HCV) in infected patients from Northeast Mexico. *Annals of Hepatology, 7*(2), 144-147.

Roberts, S. S., Miller, R. K., Jones, J. K., Lindsay, K. L., Greene, M. F., Maddrey, W. C., . . . Spiegel, R. J. (2010). The Ribavirin Pregnancy Registry: Findings after 5 years of enrollment, 2003–2009. *Birth Defects Research Part A*: *Clinical and Molecular Teratology, 88*(7), 551-559.

Roffi, L., Redaelli, A., Colloredo, G., Minola, E., Donada, C., Picciotto, A., . . . Fornaciari, G. (2001). Outcome of liver disease in a large cohort of histologically proven chronic hepatitis C: influence of HCV genotype. *European Journal of Gastroenterology and Hepatology, 13*(5), 501-506.

Saif-Al-Islam, M., Mohamed, H. S., Younis, M. A., Abdelhamid, M. Y., Ali, M. M., & Khalaf, S. (2020). Impact of gender difference on characteristics and outcome of chronic hepatitis C. *Open Journal of Gastroenterology, 10*(11), 281.

Sebastiani, M., Milazzo, L., Atzeni, F., Vacchi, C., Manfredi, A., Quartuccio, L., . . . Tavio, M. (2019). Italian consensus recommendations for the management of hepatitis C infection in patients with rheumatoid arthritis. *Modern Rheumatology*.

Smith, DB., Bukh J., Kuiken C., Muerhoff, AS., Rice, CM., Stapleton, JT., & Simmonds, P. (2014). Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: updated criteria and genotype assignment web resource. *Hepatology, 59*(1),318-327.

Tacke, F., Günther, R., & Buggisch, P. (2017). Treatment of HCV genotype 2 with sofosbuvir and ribavirin results in lower sustained virological response rates in real life than expected from clinical trials. *Liver International, 37*(2), 205-211.

Waheed, Y., Bhatti, A., & Ashraf, M. (2013). RNA dependent RNA polymerase of HCV: A potential target for the development of antiviral drugs. *Infection, Genetics and Evolution, 14*, 247-257.

Waheed, Y., Bhatti, A., & Ashraf, M. (2013). RNA dependent RNA polymerase of HCV: A potential target for the development of antiviral drugs. *Infection, Genetics and Evolution, 14*, 247-257.

Wei, L., Lim, S. G., Xie, Q., Văn, K. N., Piratvisuth, T., Huang, Y., . . . Cheng, J. (2018). Sofosbuvir-velpatasvir for treatment of chronic hepatitis C virus infection in Asia: a single-arm, open-label, phase 3 trial. *Lancet Gastroenterology Hepatology*, *18*, 30343–1.

Welzel, T. M., Nelson, D. R., & Morelli, G. (2016). HCV-TARGET study group. Effectiveness and safety of Sofosbuvir plus ribavirin for the treatment of HCV genotype 2 infection: results of the real world, clinical practice HCV-TARGET study. *Gut, 66*(10), 1844-1852.

World Health Organization. (2017). Hepatitis C. 2017. Retrieved from http://www.who.int/en/newsroom/fact- sheets/detail/hepatitis-c.

Yang, Y., Wu, F. P., Wang, W. J., Shi, J. J., Li, Y. P., Zhang, X., & Dang, S. S. (2019). Real life efficacy and safety of direct-acting antiviral therapy for treatment of patients infected with hepatitis C virus genotypes 1, 2 and 3 in northwest China. *World Journal of Gastroenterology, 25*(44), 6551.

Zein, N. N., Rakela, J., Krawitt, E. L., Reddy, K. R., Tominaga, T., & Persing, D. H. (1996). Hepatitis C virus genotypes in the United States: epidemiology, pathogenicity, and response to interferon therapy. *Annals of Internal Medicine*, *125*(8), 634-639.

Zeuzem, S., Dusheiko, G. M., Salupere, R., Mangia, A., Flisiak, R., Hyland, R. H., . . . Subramanian, G. M. (2014). Sofosbuvir and ribavirin in HCV genotypes 2 and 3. *New England Journal of Medicine*, *370* (21), 1993-2001.