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# Impact of Direct-Acting Antiviral Therapy on Hepatitis C Patients of Pakistan

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***Abstract:*** *HCV (Hepatitis C virus) is the 7th most deadly disease globally with over 150 million people infected worldwide. Pakistan has the second-highest rank of hepatitis C in the world with approximately 10 million HCV infected individuals. Only 1% of HCV infected people are taking treatment in Pakistan. The aim of the study is to determine the efficiency of ribavirin and sofosbuvir on HCV patients in Pakistan. Study was conducted at Department of Molecular Biology, Gulab Devi Educational Complex Lahore from August 2018-April 2019 which included a total of 204 patients with chronic hepatitis C. According to guidelines of the Asia Pacific Association for the Study of Liver (APASL), FDA approved drugs sofosbuvir plus ribavirin were given to these patients for 24 weeks to calculate the early virological response (EVR) and end treatment response (ETR). Peripheral blood samples were collected and routine investigation was performed including serum urea & creatinine, hemoglobin, total leukocyte count, and liver function tests (Serum Bilirubin, ALT, AST & ALP). The study involved 90 male (44.12%) and 114 female (55.88%) HCV patients for DAAs (Direct-Acting Antiviral) therapy. Out of 204 individuals 199 (97.54%) showed ETR (end treatment response) and recovered, while 5 (2.48%) showed NTR (no treatment response) and did not recover. Sofosbuvir plus ribavirin treatment is observed to be an economical and efficient treatment with extra-ordinary end treatment response (97.54%) in HCV patients. This association has high viral eradication rate and improves the liver function as well. In conclusion these drugs are highly effective for HCV recovering patients and could be the best possible treatment for HCV.*

**Keywords:** Hepatitis C Virus; Direct-Acting Antiviral Therapy; Sofosbuvir; Ribavirin; Pakistan

## INTRODUCTION

Hepatitis C virus (HCV) a single stranded RNA virus, first isolated in 1989, affects up to 150 million people worldwide with 3.9 million in USA only (1, 2). It can cause a severe infectious disease called hepatitis C (3). The virus belongs to Flaviviridae family of viruses, and has six different genotypes numbered 1 to 6 with subtypes a, b and c (4). HCV chronic infection produce an inflammatory progression in about 20-30% of patients, that affects the liver which leads to liver cirrhosis, liver cancer and even death (5). Pakistan has the second highest rank of HCV globally with approximately 5% people infected. In a general population survey Punjab has been reported with the highest HCV burden in Pakistan (6). Out of the 6 genotypes of HCV, the major genotype involved in spreading the HCV infection in Pakistan is 3a (69.1%), then genotypes 1 (7.1%), 2 (4.2%), and 4 (2.2%) (7).

HCV chronic infection has replication rate of approximately $10^{10}$ to $10^{12}$ virion per 24 hour (8), due to higher replication rate and lack of RNA dependent RNA polymerase the HC virus show great genetic variability which makes it impossible for the immune system to recognize the mutant form in the body (9). So, mostly HCV infected individuals are asymptomatic and do not show any sign and symptoms during early stage of the infection which makes it difficult to diagnose the infection (10). Symptoms of HCV infection usually develops in 7th to 8th week of exposure (11) which includes weakness, discomfort, jaundice and anorexia. HCV can be transmitted through intravenous drug usage, blood transfusion, needle stick injuries, parental routes of transmission, hemodialysis, and multiple sex partners (12). Other risk factors includes cocaine user (Intranasal), skin piercing, body tattoos, sharing of shaving equipment, sexual activity, transfer to patients from health worker (13).

The most commonly used procedures for the diagnosis of HCV is enzyme immunoassay (EIA) or chemiluminescent immunoassay (CLIA) and alanine aminotransferase (ALT) which can additionally assess of the effectiveness of anti-HCV therapy (14). Other tests includes ribonucleic acid test or pre confirmatory HCV core antigen test and immunoblot assay (recombinant immunoblot assay or Western blot assay) (15). The GOLD standards for the diagnosis of active HCV infection is nucleic acid test (NAT) for HCV RNA (16). Sometimes there is a long seronegative period during hepatitis C infection before the detection of an antibody against the HCV in the serum. The inadequate antibody response reported in the large number of patients is due to the immunosuppression (17).

The basic treatment in chronic HCV infected patient is elimination of HCV ribonucleic acid (RNA), which is assumed by accomplishment of sustained virologic response at 12 weeks (SVR12). Sustained virologic response (SVR) is termed as assurance of not detectable HCV ribonucleic acid levels at 12 weeks after completion of treatment (18).

The only therapy used for HCV infection since 1991 was interferon (IFN) which was replaced by pegylated interferon with ribavirin in 2001 (19). The SVR achieved after using these treatments is only 40-45% against genotype 1, up to 80% against genotype 2, and

only 50% against genotype 3a (20).

In the last decade the hepatitis C treatment increased due to the entry of interferon free oral therapy known as direct acting antiviral agents (DAAs). Theses DAAs effect the nonstructural protein of HCV, have high tolerance and low side effects with success rate of about 90%. DAAs has same effect on all people with respect to race, gender, age and liver fibrosis (21). The treatment for genotype one, two, three and four, compensated liver cirrhosis patients, hepatocellular carcinoma (HCC) patients and naïve patients sofsobuvir NS5B inhibitor approved for all that mention above (22, 23).

The baseline therapy in Pakistan for hepatitis C infection is revising to the new DAAs (Direct Acting Anti-viral Agents).The only registered and widely available DAAs in Pakistan are sofsobuvir (SOF) and ribavirin (RBV) since 2014. Sofsobuvir has been chosen due to its less side effects as compared to IFN-based therapy (19).

The objective of this study is to check the efficacy of ribavirin and sofsobuvir in treatment of hepatitis C patients in Pakistan.

## Methodology

### Subjects

This is a cross sectional study. A total of 204 subjects both male and females were included in the study. Patient's samples were randomly collected from hepatitis clinic of THQ Hospital Noor Pur Thal District Khushab. The collected samples were anti HCV positive and processed at Department of Molecular Biology, Gulab Devi Educational Complex Lahore during August 2019- April 2020. A written informed consent form was signed from all participants of the study. History of blood transfusion, body piercing and surgical procedures was taken. The peripheral blood samples were collected and routine investigation was performed including renal function test (Serum Urea & Creatinine), hemoglobin and total leukocyte count, liver function tests (Serum Bilirubin, ALT, AST & ALP) using kit Innoline by Martin Dow Marker Specialties (Pvt.) Ltd. PCR was run before and results were recorded carefully.

### Detection of HCV

RNA extraction was done by using DNA Technology, Research & Production kit following the kit literature. HCV RNA isolation and amplification were performed on Cobas® 6800/8800, a fully integrated and automated system that use Cobas® 6800/8800 HCV 96T CE-IVD kits. Cobas® HCV is an in vitro nucleic acid amplification test for both detection and quantitation of hepatitis C RNA in both human EDTA plasma and serum sample. Cobas® 6800 HCV test is specific for all 6 genotypes of Hepatitis C virus. A total of 20µL eluted purified RNA sample was used for PCR along with the lyophilized reagents. The tubes were then closed and put into the Real Time PCR instrument.

### Statistics

The statistical analysis of the data was done by using SPSS version 21.0. Student's t test was applied for comparative analysis. The qualitative variables were described using the frequency distributions and reported as percentages while the quantitative data were summarized as mean and standard deviation.

## Results

In current study, a total of 204 samples were studied, the study population includes 90 (44.12%) male and 114 (55.88%) female participants. With reference to gender distribution female ratio was observed high as compared to the male ratio.

The results showed that 73 (35.78%) participants have a history of blood transfusion in case of anemia or during any surgical treatment while 131 (64.22%) do not have any previous transfusion history. Surgical procedure history was found in 82 (40.20%) individuals and 122 (59.80%) did not gone through any surgical procedure. The percentage of individuals having body piercing history was 55.88% (114 individuals) as compared to the other 90 persons (44.12%) with no history of body piercing. The different qualitative variables like blood transfusion, surgical procedure and body piercing history data were represented in graphical form in Fig. 1.

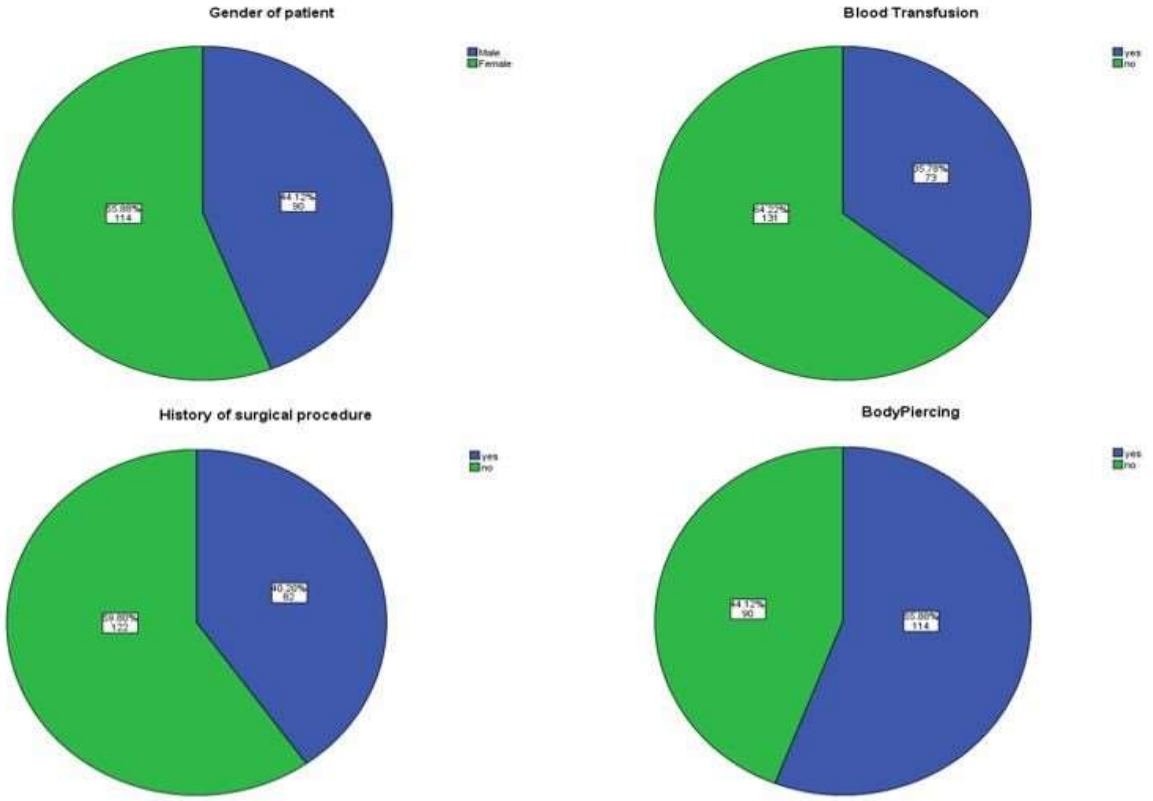


**Fig. 1.** *Frequency Distribution of Gender, Blood Transfusion, History of Surgical Procedures and Body Piercing*

The quantitative variables like age, hemoglobin (Hb) level, total leucocyte count (TLC), serum creatinine, serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and blood urea level was also measured and recorded in tabulated form in Table 1.

**Table No. 1: Statistical description of all tested clinical parameters**

| Sr. No. | Parameters | N = 204 | | Mean | Standard Deviation (S. D) |
|---|---|---|---|---|---|
| | | Minimum | Maximum | | |
| **1.** | **Age (years)** | 11 | 83 | 42.09 | 14.101 |
| **2.** | **Hemoglobin (gm/dL)** | 8.1 | 14.9 | 11.367 | 1.5064 |
| **3.** | **Total Leukocyte Count ($10^9$/L)** | 4.5 | 12.0 | 7.991 | 1.307 |
| **4.** | **Blood Urea (mg/dL)** | 17 | 60 | 35.87 | 7.181 |
| **5.** | **Serum Creatinine (mg/dL)** | 0.40 | 1.40 | 0.906 | 0.161 |
| **6.** | **Total Bilirubin (mg/dL)** | 0.40 | 3.40 | 0.978 | 0.406 |
| **7.** | **Serum ALT (IU/L)** | 25 | 212 | 53.20 | 20.952 |
| **8.** | **Serum AST (IU/L)** | 20 | 195 | 43.96 | 16.993 |
| **9.** | **Serum ALP (IU/L)** | 173 | 495 | 331.10 | 59.790 |

The mean age difference of study population was 42.09 ± 14.101 years. The minimum Hb level of the participants was observed to

be 8.1 g/dl while maximum was 14.9 g/dl with a mean Hb level of 11.367 ± 1.5064 g/dl.

The TLC count of the participating individuals remain between $4.5x10^9$/L and $12.0x10^9$/L with a mean value of $7.991x10^9$/L ± $1.307x10^9$/L. The mean serum creatinine level was 0.906 ± 0.161 mg/dl. Similarly the minimum and maximum blood urea level was measured to be 17 mg/dl and 60 mg/dl respectively with 35.87 ± 7.181 mg/dl mean value.

Liver function is important for evaluation of hepatitis in current study liver function test parameters were also evaluated the total bilirubin lowest value was 0.4mg/dl while the highest value was 3.40mg/dl with a mean value 0.978 ± 0.406 mg/dl.

The level of ALT was in the range of 25 IU/L and 212 IU/L. The mean level of serum ALT was 53.20 IU/L with a statistical difference of ± 20.952 IU/L. Similarly the mean difference of serum AST and serum ALP were 53.20 ± 20.952 IU/L and 331.10 ± 59.790 IU/L respectively.

With the increase of viral load in patients the virus put extra burden on liver that increases its enzyme level (Fig. 2). The scatter diagram shows a positive relationship between quantity of virus and serum ALT, as quantity of virus increase serum ALT increase.

A comparative analysis of viral load before start and after the treatment completion was performed by applying paired sample T test which is summarized in Table 2. Here P value is 0.001 that is highly significant. The end treatment response achieved in 199 (97.54%) patients out of the 204 patients, while 05 (2.48%) patients show viral load at the end of treatment.

## DISCUSSION

Pakistan has been facing the second biggest HCV load in the world, its transmission is associated with multiple risk factors such as barbering, ear and nose piercing, and other health related practices (blood transfusion, injectable medicines and drugs) (24).

The current study showed that 40% patients were gone through surgical procedures while 35% of the individuals have blood transfusion and 55% had body piercing history similar to some previous studies by Gaeta et al. The surgical procedures include both minor and major surgeries. So the screening of every patient is mandatory before any surgical procedure and sterilization of all the instruments is essential by following proper protocols so the risk of spread of disease can be minimized (25).

In this study, the mean Hb level was 11.367 ± 1.5064. Majority of the patients that had low Hb levels were females. Females are more prone to anemia as compared to males due to menstrual cycle, pregnancy etc. Moreover the women of the concerned area do not have a healthy and nutritional diet which could be a possible reason for low Hb levels (26).

Total leukocyte count (TLC) was between the range of $4.5x10^9$/L and $12.0x10^9$/L. The mean level of TLC calculated was $7.991 \pm 1.307x10^9$. The level of blood urea was observed to be 17 mg/dl to 60 mg/dl as reported by related studies (27).

Our research findings revealed the association of HCV with increase prevalence of renal insufficiency which is defined by serum creatinine level ≥1.40 mg/dl. HCV is identified as a potential risk factor for renal impairments and prompt the need of future population based studies regarding the HCV infection and renal diseases (28).

This research work includes observation of pathological elevation of different liver function test (total bilirubin, ALT, AST, and ALP) parameters. This was expected to happen due to active infection of hepatitis C virus. Kwo et al. has also reported the abnormality of liver function test due to HCV similar to our resultrs. However upon elimination of viral infection liver function parameters goes to normal side (29).

A scatter diagram shows a positive relationship between quantity of virus and serum ALT, as the quantity of virus increase serum ALT increase. This might be due to increase quantity of virus (30) which creates pathological disturbance in the liver that deranges its enzymes so the serum ALT increases in higher viral load patients shown in Fig No 2.

In the current research the effect of drugs ribavirin and sofsobuvir on HCV patients was observed. The study showed that use of ribavirin and sofsobuvir drugs in lowering the viral load and recovering the patients from Hepatitis C infection was significantly high with a P value 0.001. Overall recovering rate for ribavirin and sofsobuvir users was 97.54% (Table No 2). Our results correlate with the studies of Butt et al in which they reported the ETR to be 96.6% (31).

The study conducted by Iqbal et al concluded that effectiveness of ribavirin and sofsobuvir is 99.34%, these results also correlates with our study (32). Another multicenter study with n=573, by Azam et al noted that ribavirin and sofsobuvir is 98.2% effective (33). Another study performed in Karachi reported 98.5% effectiveness of sofsobuvir and ribavirin (34). In Lahore a study perform by Sarwar and Khan et al that reported SVR at 12 week is 89.2% (35).

The data regarding SVR in genotype 3 of HCV different population show different behavior in different pathological conditions. The SVR against genotype 3 after the treatment of 12 weeks attain the rate 56% in naïve patients (36). A 100% SVR gain after 24 weeks by using ribavirin and sofsobuvir in genotype 2 and 3 patients without liver cirrhosis was reported (37).

It is reported by different studies that treatment of HCV patients with ribavirin and sofsobuvir for twelve weeks and without PEG-IFN resulted in an appreciable reduction in the viral RNA, it maintain a SVR 92% for genotype 2 and 3 of HCV after treatment of 24 weeks (22, 38).

Our research provides evidence that support the consideration of HCV treatment in clinical practice for all age groups with greater SVR rates and less adverse effects are becoming the standard-of-care for HCV infection.

## CONCLUSION

This study concluded that the SOF and Ribavirin are the highly effective medicine for the patient recovering from the hepatitis C infection. This association has high viral eradication rates and also improves the liver biochemical function. As the end treatment response is 97.54% it is the best treatment for HCV than all previous medication used. Major Risk factor for the spread of disease include blood transfusion, body tattooing, surgical procedures etc. SOF+RBV now should be recommended for the treatment of

HCV until the availability of newer and better than this regimen.

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