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Viral load in interferon treated & untreated patients of HCV

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ABSTRACT

BACKGROUND & OBJECTIVE: Hepatitis C (HC) is a chronic viral disease that chronically infects liver cells. It has got at least 6 genotypes. The virus uses cellular machinery to multiply and increase in number. Viral load is the copies of HC virus ribonucleic acid (RNA) that can be quantitatively measured on polymerase chain reaction (PCR) test. There are many treatment options available for the treatment of HCV, including standard interferon, pegylated interferon with oral anti-viral drugs. In our research, we studied the levels of viral load in Hepatitis C (HC) positive patients who were treated with interferon and those HC-positive patients who were untreated and observe the effect of interferon treatment on viral load. To measure and compare the viral load in treated and untreated patients of the Hepatitis C virus.

METHODOLOGY: After obtaining written consent, a total of 54 HC patients, both male and female, were included in our study that were further divided into two groups Group-I included 26 freshly diagnosed patients of HC and Group II of 28. All the patients were from the Hazara division in Khyber Pakhtunkhwa (KPK), Pakistan. Quantitative PCR was performed to assess the viral load. Data obtained were analyzed using IBM SPSS version 23 and was also compared within groups.

RESULTS: There was a significant difference ($p \leq 0.001$) found in the viral load of interferon-treated and untreated patients of hepatitis C, but there was no significant difference found when compared among gender-different districts of the Hazara region.

CONCLUSION: Significantly higher viral load in untreated patients of HCV when compared to interferon-treated patients of HCV suggest that interferon is still effective against HCV.

KEYWORDS: Hepatitis C virus, HCV antiviral treatment, Serology, Viral load.

INTRODUCTION

Hepatitis C (HC) is a chronic viral disease that affects liver cells [1]. HC virus enters hepatocytes and uses its machinery to multiply in number, increasing viral load [2]. HC is prevalent in about 6% of the Pakistani population [3]. HC has seven major genotypes and nearly 67 subgenotypes. Genotype 3a is the most common genotype in Pakistan [3,4].

Interferon (INF) is a cytokine normally produced by immune cells. INF was used along with Ribavirin for the treatment of HC infection. Ribavirin is replaced with a new agent Sofosbuvir that is quite effective against genotype 3a [3].

HCV is diagnosed by the detection of HCV antibodies in the blood and confirmed by the presence of HCV RNA through polymerase chain reaction (PCR) [4,5]. Viral load decreases with interferon treatment [6,7].

End of treatment response (ETR) is the levels of viral load on PCR 12 weeks after the end of treatment. Sustained viral response (SVR) is viral load at 24 weeks after the treatment ends. Both ETR & SVR are considered to be the markers of successful INF treatment [8,9]. Direct-acting antiviral agents (DAA) have a better response (ETR) as compared to INF treatment [9]. DAA may be considered in all patients with HCV infection [10].

In view of the above discussion, in the present study, the viral load in treated and untreated patients of the HC was measured before and after the treatment to assess the efficacy of interferon treatment along with Ribavirin as interferon is still in use as part of national hepatitis program treatment protocol. This study will help in rethinking the treatment protocol for Hepatitis C. This study will also help provide local data and fill the gap of knowledge on the local level.

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METHODOLOGY

Our study was a cross-sectional study. The sample size was calculated to be 54 by using 5% level of significance. A total of 54 male and female patients was selected of HCV from the Hazara division of KPK. They were further divided into two groups; Group-I included 26 freshly diagnosed patients of HC, and Group-II of 28 HC patients who had completed their interferon treatment.

This research was approved by the Ethical review committee of the University of Health Sciences (UHS), Lahore (Reference: no: UHS/CE-17/T-Exam/2265). Randomized sampling was done, and patients were included in this study after written and informed consent in the local Urdu language.

Adult Subjects aged 18-60 of both sexes were included on the basis of a positive ELISA and PCR for both groups. Group II subjects were the ones who had completed HCV INF treatment in the last 1-2 weeks. HCV patients with conditions like Hepatitis A and B, diabetics or any other known disease were excluded as they may interfere with HCV.

Sampling was carried out at the Hepatitis center at DHQ, Abbottabad. Blood samples were secured in serum separation tubes (SST) with proper labelling for extraction of serum. Serum was extracted by centrifugation for 10 minutes at 3000 rpm, and serum was transferred to aliquots, stored and kept at -8 o Celsius till transported. All the samples were transported to UHS, Lahore, in their own private vehicle within six hours, completely sealed and packed with ice packs that maintained a cold chain. Aliquots were stored at UHS, Lahore, at -80o till analyzed. Real-time quantitative PCR was done for viral load later using CFX 96 by BIORAD.

The data collected was entered and analyzed through IBM SPSS (Statistical Package for Social Sciences) version 23. Mean ±SD was used for quantitative variables. Percentages and Frequencies were given for qualitative variables. Schapiro-Wilk distribution was used in order to test the normality of data. Data was presented as Mean±SD for normally distributed data while median plus interquartile range (IQR) was used for non-normally distributed data variables. Comparison between the two groups was made by Mann Whitney U test to see any significant difference by using tests of significance as the data was not normally distributed.

RESULTS

The study population comprised of 54 subjects was divided into two sample groups of 26 untreated HCV cases (with no interferon treatment received) and 28 treated HCV cases (with interferon for six months). Mean±SD age was 47.69±11.98 years in untreated subjects and 40.36±13.90 years in treated subjects. Females were 15(57.7%) in the untreated group, whereas there were 18(64.3%) females in the treated group. The subject cases were selected from two districts of the Hazara division, i.e., Abbottabad (including Haripur) and Mansehra (including Batagram and Kohistan). The median (IQR) of viral load for untreated was 700000 (208614-1642299) and 3394 (1297-625537) in the treated group.

Table-I: Comparison of Viral load between INF-treated and untreated HCV groups.

Sample Groups	Untreated Cases n=26	Treated Cases n=28	p-value
Parameter	Median (IQR)	Median (IQR)	<0.001*
Viral load (IU/ml)	700000.00 (208614-1642299)	3394.00 (1297-625537)	

A non-parametric Mann-Whitney U Test was applied for significance between the two groups as our data for the viral load was non-normally distributed, which shows a significant difference (p≤0.001).

Table-II: Comparison of Viral load between INF-treated and untreated HCV groups among gender.

Sample Groups			Untreated Cases n=26	p-value		Treated Cases n=28	p-value
Parameters	Groups		Median (IQR)			Median (IQR)	
Gender	Male	n=11	595891 (251940-1067748) IU/ml	0.212	n=10	1652 (624-8093600)	0.547
	Female	n=15	1110211 (190000-2017500) IU/ml		n=18	5276 (1503-688191)	

Median (IQR) viral load of males in the untreated group was 595891 (251940-1067748) IU/ml and 1110211 (190000-2017500) IU/ml for females showing no significant difference (Mann-Whitney U Test; p=0.212).

The median (IQR) viral load of males in the treated group was 1652 (624-8093600) and 5276 (1503-688191.29) for females showing no significant difference (Mann-Whitney U Test; p=0.547).

DISCUSSION

HCV is a common health issue in Pakistan. HCV enters the body through different routes, most commonly blood transfusion and IVDA. Approximately 6% of the people are affected by HCV in Pakistan [3]. Genotype 3 is the most common type in Pakistan, and it has a good response when treated with interferon [3,4].

Once HCV enters the body, it attaches itself to hepatocytes and uses its machinery to multiply and the viral load increases. HC treatments target to decrease the number of viral copies in the blood, thus decreasing the viral load in the blood. Therefore, viral load will be higher in untreated patients of HC. That is why the viral load is higher in

untreated subjects in our study, too, and it should decrease with treatment, as interferon affects HCV entry and replication inside the hepatocytes, thus decreasing viral load [6,7]. In our study, Mean±SD viral load in untreated subjects was 1,477,439±2239088 IU/ml. Kazi et al., reported a mean viral load of 6,918,309.7 IU/ml [11].

In our study, the Median (IQR) for the untreated group was 700000 (208614-1642299) IU/ml and 3394 (1297-625537) IU/ml in the treated group. This was a significant difference between untreated and treated patients of HCV (Mann-Whitney U Test; $p \leq 0.001$). This suggests that interferon is still an effective antiviral treatment against the hepatitis C virus.

A study by Ali et al., in the same population of the hazara division, supports our result, which states that untreated HCV patients had a significantly higher viral load ($p=0.014$), whereas there was no significant difference between the viral load when compared within gender [12]. Hepatitis C antiviral treatment is equally effective in both males and females as there was no significant difference of gender in viral load on treatment with interferon. In our study, no significance was found in viral load when compared between male and female patients of HCV.

The difference was again no significance found when a comparison was made between the Abbottabad and Mansehra regions of KPK, Pakistan. Another study also reported a non-significant difference in HCV viral load within gender [13]. There is no difference in viral load in different regions as all the patients share the same genetics, and genotype 3 is mostly responsible for hepatitis C in Pakistan as well as in the Hazara region [14].

CONCLUSION

Significant differences in viral load between untreated and interferon-treated groups of HCV patients were found in comparison, but there was no significant difference found when levels of viral load were compared within gender and different districts of the Hazara region.

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Authors' Contribution:

Alamzeb Jadoon: Substantial contributions to the conception and design of the work

Ayesha Gohier: Drafting the work and reviewing it critically for important intellectual content.

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