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Incidence of helicobacter pylori, diabetes and various biochemical markers in hepatitis c virus-infected patients in Mardan, Pakistan

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Abstract
Hepatitis C Virus (HCV) infection can lead to cirrhosis and hepatocellular carcinoma. It is still considered as one of the major health issue globally. HCV infected person experiences several biochemical abnormalities. In this study, we investigated to correlate the HCV infection with few other diseases like *Helicobacter pylori* and diabetes and determine the fluctuations in various biochemical markers i.e. ALT, PLT, Hb, and RA-factor. In this cross-sectional and observational study, 191 (139 male and 52 female) HCV-positive patients were included. The HCV-RNA was detected by qualitative-PCR. All the HCV-positive samples were subjected to various clinical diagnostic assays to determine the abnormalities in different biochemical markers occurred due to HCV infection. In the results, 45 (32.3%) of the male patients and 19 (36.5%) of the female patients showed a low level of Hb. 18 (12.9%) of male patients and 12 (23%) of the female patients showed a decrease in the count of the platelet. 59 (42.4%) of the male patients and 21 (40.3%) of the female patients were found positive for having an elevated level of ALT. 32 (23%) of the male patients and 32 (34.6%) of the female patients were found positive for *H. pylori* infection. 7 (5%) of the male patients and 3 (5.7%) of the female patients showed a decreased level of blood glucose. However, no significant association was found between HCV and diabetes. 16 (11.5%) of the male patients and 4 (7.6%) of the female patients were detected positive for RA-factor in their blood. These factors are strongly associated with disease progression and defining therapy response. However, they are less significant in case of detecting early HCV infection because they can also be increased or decreased in case of other liver diseases.

Keywords: HCV, *H. pylori*, PLT, ALT, HB

1. Introduction
HCV is a global health issue affecting around 2.5% (177.5 million people) of the total population. Developing nations of the world are highly affected e.g. Pakistan in which approximately 10 million people are affected by this life-threatening disease [1]. HCV infection is recognized as the main cause of cirrhosis, liver fibrosis and hepatocellular carcinoma (HCC) in a significant count of patients [2]. Genotypes, viral load and other various clinical findings in patients infected with chronic HCV are the significant predictors for determining the effective antiviral therapy and the possible therapeutic outcomes [3, 4]. So far, with the current therapeutic strategies genotyping of HCV infection is not broadly recommended by Pakistan's society of Gastroenterology (PSG) because of the high frequency of genotype 3a [5]. Further genotyping of HCV is not accessible to everyone because of poverty and the high cost of the genotyping test. However, determining of HCV genotype is not only required for the purpose of knowing the HCV variant but also crucial for determining the disease severity and bringing improvement in the current therapeutic strategies for enhanced treatment response [6]. Based on the clinical findings and therapeutic outcomes evaluation of the disease development and its progression is still very critical for the patients infected with HCV. Previously, several researchers have focused on investigating the correlation between host and viral biochemical factors, i.e. viral load, genotype, ALT, AST, bilirubin and several other factors [7-11].
Studies have shown the association of HCV infection with other disorders, i.e. lymphoproliferative disease, autoimmune hemolytic anemia and autoimmune thrombocytopenia purpura in which some of the disorders exhibit autoimmune mechanisms while some does not exhibit such mechanisms [4, 12, 13].

The association of thrombocytopenia with hepatitis A, B and C were reported in several studies [3, 5, 14]. While some studies have reported the detection of *H. pylori* and *H. pullorum* DNA in the liver of HCV patients [6]. As these pathogens are responsible for particular diseases and are also associated with the HCV infection by worsening the health of infected patients. HCV infection can give rise to cancer in different organs of the body. According to one report by the International agency for research on cancer in 1994, various parasites e.g. schistosomes, liver flukes and bacteria like *H. pylori* were recognized as type 1 carcinogen [9]. This shows that patients co-infected with HCV and these pathogens are at higher risk of developing cancer in various organs. *H. pylori* is a common pathogen responsible for upper GIT diseases such as acute and chronic gastritis, duodenal ulcers, gastric ulcers and gastric adenocarcinoma [7-9]. Studies have shown the induction of hepatotoxicity by *H. pylori* [10]. The association between HCV infection and ALT level in the body was determined by few studies in which it was concluded that anomaly in ALT level results in the liver necrosis [15]. Nonetheless, glucose intolerance was observed in patients infected with HCV when compared with controls. This further suggested that HCV infection can be responsible for the development of diabetes mellitus in some cases [16, 17]. The aim of the current study was to determine the prevalence of *Helicobacter Pylori* infection and diabetes in HCV infected patients. In the present study, we also investigated the correlation of various clinical findings like Hb, ALT, Complete blood count (CBC) and RA-factor with HCV infection.

2. Materials and Methods

2.1 Sample collection

A total of 191 Anti-HCV positive blood samples were received from Mardan, KP. Serum was extracted from blood. The study was conducted from August 2017 to November 2017. These HCV-positive samples were subjected to various clinical assays for the evaluation and determination of other infections occurring simultaneously with HCV.

2.2 Qualitative and quantitative analysis of HCV

The extracted serum was subjected to qualitative PCR for the detection of HCV-RNA. The RNA detection was based on specific primers designed for targeting the particular conserved regions of HCV-RNA. Qualitative detection of HCV-RNA was carried out by using Biogen RNA detection kit. The amplification of HCV-RNA was done by Roter-gene 3000 series.

Quantitative analysis of HCV infection was done by RT-PCR to determine the viral load. The viral load <500 IU/ml was considered as negative. HCV-positive samples usually exhibited viral load from moderate to high i.e. 500-5.0 × 10^8 IU/ml. After qualitative and quantitative analysis, the HCV positive serum was subjected to other assays to determine the prevalence of various biochemical markers.

2.3 ALT level

ALT level in the HCV infected sera was quantified by Merck kit. From the kit, reagent 1 was taken 800µl and reagent 2 was taken 200µl and was added to microfuge tubes. 100µl from each HCV-positive serum was taken and added to each tube. The tubes were then subjected to pulse vortexing for proper mixing. The mixture was then analyzed by Microlab 300 semi-automated chemical chemistry analyzer.

2.4 Detection of *H. pylori*

H. pylori infection was detected by using ICT strips. 30µl of the HCV infected sera was added to the tubes followed by addition of 40µl buffer. The strips were then placed at room temperature for 15min. For *H. pylori* positive infection the 'T' region of the ICT strip developed a line. The positive and negative infection can be distinguished by the development of the red line in the 'T' region just like developed in the 'C' region in case of positive infection. While in case of negative infection the line only appeared in the 'C' region.

2.5 Blood glucose level measurement

Measurement of the blood glucose level in the HCV infected sera was done by Merck kit. 10µl of the infected sera was added to fresh microfuge tubes along with specific reagents. The blood glucose level in the sera was analyzed by Microlab 300 semi-automated chemical chemistry analyzer.

2.6 Complete blood profile

The complete blood profile was determined for the detection of thrombocytopenia. Blood samples from the HCV infected patients were collected in EDTA tubes. The complete blood count was done by ALERE-380 blood cell counter machine.

2.7 Detection of RA-factor

The RA-factor detection was done by Latex coagulation kit. 50µl of the HCV infected sera was taken and added to the slide followed by addition of 50µl latex reagent. The slides were placed at room temperature for 5-10min. After incubation, the serum samples were coagulated. Coagulation of the samples shows the positivity for RA-factor while the negative samples were not coagulated.

3. Results

A total of 191 HCV-positive samples were received from Mardan, KP. The studied population included 139 male and 52 female patients. Serum was isolated from all HCV patients and was subjected to various diagnostic assays, including qualitative and quantitative PCR for HCV infection, ALT level measurement, blood glucose level measurement, complete blood count, *H. pylori* and RA-factor detection. The diagnostic assay results were categorized for male and female patients separately as shown in (Fig 1).

3.1 Low Hb level

Out of 139 HCV-positive male patients, 45 were having a low Hb level while the remaining patient's Hb level was in the normal range. It was concluded from the results that 32.3% of the male patients were having Hb levels below than normal range. While out of 52 female HCV-positive patients, 19(36.5%) were having a low Hb level. For comparison of the Hb level of HCV-positive patients, 40 HCV-negative control samples were randomly collected from the general population and were all having Hb level in the normal range.

3.2 Low PLT level

Out of 139 HCV-positive male patients, 18 (12.9%) were having PLT level below than normal range. While out of 52 HCV-positive female patients 12 (23%) were found positive having low PLT level. To compare the results of HCV infected patients, 40 HCV-negative control samples were included in the study and they were all found normal.

3.3 High ALT level

Out of 139 HCV-positive male patients, 59 (42.4%) were having an elevated ALT level than the normal range. While

out of 52 HCV-positive female patients, 21 (40.3%) were found positive having an elevated level of ALT. The results were compared with 40 HCV-negative control samples and they all were having a normal level of ALT.

3.4 *H. pylori*

Out of 139 HCV-positive male patients, 32 (23%) were found positive for *H. pylori* infection while the remaining patients were detected negative. While out of 52 HCV-positive female patients, 32 (34.6%) were detected positive for *H. pylori* infection. The results were compared with 40 HCV-negative control samples and they were all found negative.

3.5 Blood glucose level

Out of 139 HCV-positive male patients, 7 (5%) were having an elevated level of blood glucose. While out of 52 HCV-positive female patients, 3 (5.7%) were found positive having increased blood glucose level, which was much higher when compared with 40 HCV-negative control samples.

3.6 RA factor

Out of 139 HCV-positive male patients, 16 (11.5%) were found positive for RA-factor. While out of 52 HCV-positive female patients 4 (7.6%) were detected positive for RA-factor. These results were compared with the results of 40 HCV-negative control samples from the general population, which were having no RA-factor in their blood.

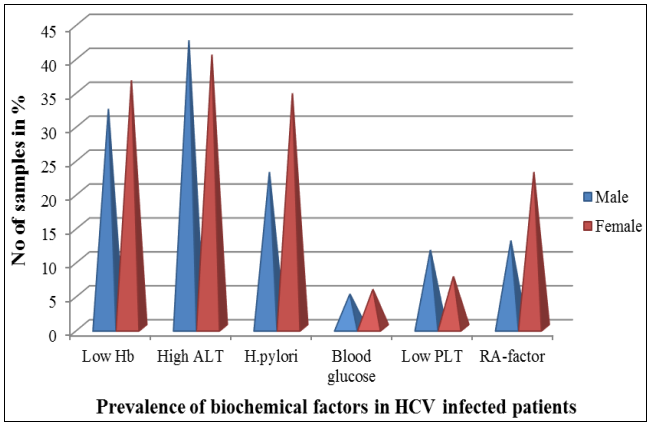

Fig 1: The graph shows the prevalence of different biochemical factors in HCV infected patients.

4. Discussion

The aim of the current study was to determine the association between HCV infection and the abnormalities in the biochemical factors. These factors are primarily linked to the proper functioning of the liver, which is abnormally increased or decreased during the infection. These biochemical factors are linked to several other infections, but in this study, the focus was toward HCV infection. We concluded in our results that 32.3% of the male patients and 36.5% of the female patients were having low Hb level than the normal range. This frequency was much higher in case of HCV infection as compared to the control samples from the general population. In a previous investigation, the correlation between HCV infection and peripheral blood count was established in which a very small portion of the studied population i.e. 5% displayed low Hb level. However, no significant difference was found in Hb level between HCV-positive and HCV-negative participants [18]. Another similar study established a relationship between HCV infection and higher Hb level in HD patients [19].

As from the results of complete blood count (CBC), we concluded that 12.9% of the male patients and 23% of the female patients were having low platelet count in HCV infected patients. In a relevant study, the neutrophils and platelet count were reduced in the HCV infected patients. It was determined that 13% of the HCV patients were having platelet count below than normal [18]. Several others studies have established the strong correlation of thrombopoietin levels and platelet counts with abnormal liver function and also the severity of hepatic fibrosis in HCV infection [20, 21]. Other studies have related the increase in the platelet counts and thrombopoietin levels, with post-IFN therapy for HCV infection [22].

High level of ALT was also observed in the HCV infected patients in which 42.4% male patients and 40.3% female patients revealed an abnormal increase in the ALT level. In a similar study, it was concluded that elevation in ALT level is the strong indicators of liver cirrhosis and which commonly occurs in HCV infection. However, elevation in the ALT level varies with infection of various HCV genotypes [23]. Another related study has concluded the same results in which AST/ALT ratio was typically high in cirrhotic patients than in non-cirrhotic patients [24].

In this study, 23% of the male patients and 34.6% of the female patients were found positive for *H. pylori* infection. The prevalence of *H. pylori* infection was much higher in HCV patients as compared to the general population included in this study as a control group. These results were in accordance with a previous study, determining the prevalence of *H. pylori* infection in cirrhotic patients. Approximately 89% of the cirrhotic patients were found positive for *H. pylori* infection as compared to the 59% prevalence in the control group [11].

HCV infection can also be positively linked to increased prevalence of type 2 Diabetes mellitus. In the current study, 5% of the male patients and 5.7% of the female patients were suffering from one or other form of diabetes, which was concluded from the high blood glucose level. These results were in accordance with a previous related study, in which 21% to 50% of the HCV infected patients were suffering from DM. This prevalence was much higher as compared to the general population or among the patients with other forms of liver abnormalities [25].

The patients included in this study also showed an increased level of RA-factor among the HCV infected patients. It was concluded from our study that 11.5% of the male patients and 7.6% of the female patients were found positive for RA-factor and all the participants from the control group were found negative.

5. Conclusion

HCV infection is predominantly associated with various clinical conditions. Significant relations have been established by several investigators between HCV infection and various biochemical markers. In this study, the association of HCV with other diseases like *H. pylori*, diabetes and various biochemical markers i.e. ALT, PLT, Hb, and RA-factor have been determined. The Hb level was low in HCV infected patients when compared with the participant from the general population. The decrease in the platelet count was also obtained, however, the patients showed a significant elevation in the ALT level. *H. pylori* infection was also very prominent in HCV infected patients. This study also focused on establishing the correlation between diabetes and HCV infection; however, the results were not significant. The RA-

factor was more prevalent in HCV patients as compared to HCV-negative participants. These biochemical markers were used for the early detection of HCV infection and describing the therapeutic strategy. However, because of less significant and inefficient results, these factors were not considered as appropriate targets for disease detection. The focus for disease diagnosis is shifted toward more sophisticated diagnostics and therapeutic strategies. These factors still define the disease severity and therapeutic response.

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7. Declaration of interest
None of the authors of this paper had any personal or financial conflicts of interest.

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