PREVALENCE OF DIABETES MELLITUS AND ITS ASSOCIATION WITH HEPATITIS C VIRUS-INFECTED PAKISTANI FEMALES

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Abstract
The Hepatitis C virus is one of the leading causes of liver cirrhosis. There is a long list of associated health issues, one of which is failure in the uptake of insulin by the body’s cells, leading to insulin resistance. Both HCV and diabetes are predominant public health threats. Unfortunately, if HCV and diabetes co-exist, they become life-threatening. Therefore, we aim to evaluate the association between biochemical markers, infection with the hepatitis C virus, and diabetes of type 2 in females of Pakistan. A total of 3778 HCV-infected patients and 200 healthy controls participated in this study. Out of these 3530, 93.44% of cases were positive on anti-HCV ELISA, and 2978, HCV RNA PCR confirmed 84.36%. The PCR-positive samples were classified into three groups: HCV without diabetes, HCV with diabetes, and diabetes only. Out of 3778 HCV cases, 2490 (83.6%) were diabetic, 346 (11.61%) were HCV + diabetic, and 142 (4.7%) were diabetic only. Laboratory investigations for biochemical parameters include liver, diabetic, and lipid profiles. The procedures were carried out according to the manufacturer's instructions. The diabetic HCV group consists of the age group between 40-50 years. The levels of blood glucose and alanine aminotransferases (ALT) in diabetic patients were higher than in non-diabetic patients (p=0.001). The group of patients with HCV infection with diabetes had more cholesterol and triglyceride levels than the other two groups (p=<0.001). HCV and diabetes have some potent connections in females in Pakistan. This
connection becomes more noticeable in HCV patients with diabetes than HCV patients without diabetes.

Key words: Hepatitis C, Diabetic, Liver enzymes, Biochemical aspects, HCV infection, Lipid profile

INTRODUCTION

Hepatitis-C virus (HCV) infection epidemiology is still poorly understood in many countries. It is believed that fewer cases have been reported than the actual infected figures. HCV is recognized as a diabetogenic agent due to its involvement in several mechanisms, including autoimmune phenomena, direct cytotoxic effects on pancreatic cells, and obstruction of insulin receptors at the cellular level (Ibrahim et al., 2022). The World Health Organization (WHO) reports that over 71 million individuals worldwide are enduring chronic HCV infections, with approximately 390000 of these cases leading to HCV-related liver complications and annual fatalities (Khatun et al., 2019). It was noted that when HCV occurrence increases, the frequency of diabetes also increases. Different studies across various geographic regions and ethnicities have consistently tried to demonstrate that HCV infection and Type 2 diabetes are correlated (Attia et al., 2022). In developing countries, there is a need for ample epidemiological data, which is crucial in managing and implementing disease control strategies. However, states need accurate data and proper interpretation of the disease mechanisms to make a firm plan.

The study aimed to find a link between HCV infection and diabetes of type 2 and their influence on different biochemical markers and complications associated with females in Pakistan. We did a project on Pakistani HCV male patients in 2013 by Bashir et al. (2012), funded by HEC. This study started the project with a focus on HCV patients, diabetes and those with both diabetes and HCV, as very little data is available on female HCV patients. This study aims to investigate its relationship with HCV infection among diabetic females in Pakistan.

METHODS

Study design and data

This five-year cross-sectional study occurred between 2014 and 2019. Participants were briefly explained the nature of the study before obtaining consent. Patients with a positive immune-chromatographic (ICT) device HCV test, having abnormal liver enzymes, or presenting with any
clinical symptoms of HCV provided the serum samples. The study population was categorized based on the nature of the disease into three groups: HCV positive (without diabetes), diabetes only (without HCV), and HCV with diabetes. A hundred healthy individuals were used for control purposes. All serum samples collected in vacutainer (BD, USA) were processed for ELISA and PCR. Liver function tests, lipid profiles, and blood sugar tests have also been performed. Ethical approval was taken from Pakistan's Lahore Citi Laboratory and Research Centre.

Anti-HCV antibodies were measured using a commercially available ELISA Amgenix, USA, micro LISA HCV Antibody kit). The anti-HCV ELISA protocol (Germany's Rayto Company, Reader RT-6000) is strictly followed, which was provided with the equipment.

According to the manufacturer's protocol, HCV RNA was isolated from the patient’s blood samples (Trizol. Invitrogen, USA). PCR quantification was performed by mixing the 500 μl solution with 300 μl donor’s separated serum, and then extraction was done with chloroform and alcohol. The end product obtained was Using antisense primers and cDNA (Qiagen, Germany's Hilden). RNA viral load was detected using a suitable set of primers and five prime 5' untranslated regions (5' UTR).

Total bilirubin, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, gamma-glutamyltransferase, total protein, and albumin level are all included in the liver function profile (LFTs) were measured in patient’s serum by using clinical chemistry kit (Human, Germany). All biochemical biomarkers in the donor’s serums were analyzed on a semi-automated Biochemistry analyzer (Micro Lab 300, Merk Germany). The diabetic profile of the patient, including blood sugar level (fasting and random) and glycosylated hemoglobin (HbA1C), as well as other associated parameters such as fasting lipid profile, was also assessed, including cholesterol, triglycerides, high-density lipoproteins (HDL), and low-density lipoproteins (LDL).

**Statistical analysis**

Data were analyzed statistically with SPSS version 18. Means and S.D were used to present categorical data. The means of several groupings were compared, and independent T-tests and ANOVA were applied. P-values under 0.05 were regarded as significant.
RESULTS

A total of 3778 females suspected to be infected with HCV were tested to evaluate HCV prevalence in the Pakistani female community. Of these, 93.44% were positive on anti-HCV ELISA, and HCV RNA PCR confirmed 84.36%. The frequency of HCV +ve (without diabetes) was 83.61%, followed by diabetes only (without HCV) at 4.79% and HCV with diabetes at 11.61%. Patients were also categorized based on their marital status. HCV-affected patients were 90.6% (married) and 9.4% (unmarried), respectively (Figure 1).

The age of respondents of Hepatitis C virus patients was also studied. The highest percentage of HCV positivity was found among the age group 30+, followed by 40+. HCV-infected females with diabetes had shown a significant difference ($p \leq 0.001$) for blood glucose fasting, blood glucose random, and HbA1C (Table 2) reveals ($p < 0.005$).

LFTs were evaluated and showed raised ALT levels in HCV-infected females and diabetic females infected with HCV compared to patients with diabetes only (non-infected with HCV). Compared to individuals just infected with HCV, diabetic patients with HCV infection had a higher ALT titer, measuring $36.21 \pm 3.12 \mu$/L. ALT and AST were significantly crucial for diabetic patients infected with HCV ($p \leq 0.001$) and notable for the HCV-only group ($p < 0.005$). However, variables were found not significant ($p > 0.005$) for diabetes-only patients. ALP, $\gamma$-GT, total protein, albumin, and bilirubin did not produce any notable results in all three groups (Table 2).

Figure 1: Sample categorisation based on marital status and disease type.
**Table 1: Diabetes and HCV impact on several serum diabetic markers in female patients.**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>HCV only</th>
<th>HCV + Diabetes</th>
<th>Diabetes only</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Married (n = 2256)</td>
<td>Unmarried (n = 234)</td>
<td>p-value</td>
</tr>
<tr>
<td>HbA1C</td>
<td>4.35 ± 0.21</td>
<td>4.15 ± 0.14</td>
<td>0.435</td>
</tr>
<tr>
<td>BGF</td>
<td>91.23 ± 0.46</td>
<td>90.21 ± 0.14</td>
<td>0.391</td>
</tr>
<tr>
<td>BGR</td>
<td>161.23 ± 4.15</td>
<td>159.64 ± 1.53</td>
<td>0.535</td>
</tr>
</tbody>
</table>

Standard values: Blood glucose fasting (BGF): 70-110 mg/dl and blood glucose random (BGR): 80-160 mg/dl. Glycosylated haemoglobin (HbA1C): 4–6%.

**Highly significant p < 0.001, *Significant p < 0.005.**

**Table 2: Impact of Diabetes and HCV on female patients' liver function metrics.**

<table>
<thead>
<tr>
<th>Liver function parameter</th>
<th>HCV only</th>
<th>HCV + Diabetes</th>
<th>Diabetes only</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Married (n = 2256)</td>
<td>Unmarried (n = 234)</td>
<td>p-value</td>
</tr>
<tr>
<td>ALT</td>
<td>123.83 ± 10.75</td>
<td>118.45 ± 7.56</td>
<td>0.003*</td>
</tr>
<tr>
<td>AST</td>
<td>115.31 ± 8.64</td>
<td>102.23 ± 5.36</td>
<td>0.004*</td>
</tr>
<tr>
<td>ALP</td>
<td>410.87 ± 1.36</td>
<td>407.39 ± 9.56</td>
<td>0.0314</td>
</tr>
<tr>
<td>Gamma GT</td>
<td>89.54 ± 4.36</td>
<td>75.87 ± 3.84</td>
<td>0.218</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>1.09 ± 0.06</td>
<td>1.13 ± 0.04</td>
<td>0.089</td>
</tr>
<tr>
<td>T.Protein</td>
<td>8.63 ± 0.13</td>
<td>8.59 ± 0.09</td>
<td>0.518</td>
</tr>
<tr>
<td>Albumin</td>
<td>3.21 ± 0.05</td>
<td>3.17 ± 0.02</td>
<td>0.213</td>
</tr>
</tbody>
</table>

Diabetic females infected with HCV significantly increased triglyceride and cholesterol titers. In addition to that, titers were higher in married females. Statistically, regarding cholesterol, triglycerides, and LDL, the HCV (+) diabetic group showed a highly significant difference (p < 0.001), whereas the other two groups had a substantial difference (p less than 0.005). HDL was considered insignificant for all female patients (p > 0.005) (Table 3).

**Table 3: Impact of diabetes and HCV on parameters of serum lipid in female patients.**

<table>
<thead>
<tr>
<th>Parameters for Lipid</th>
<th>HCV</th>
<th>HCV and diabetes (Both)</th>
<th>Diabetes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Married (n = 2256)</td>
<td>Unmarried (n = 234)</td>
<td>p-value</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>234.16±3.17</td>
<td>225.45± .10</td>
<td>0.002*</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>241.65± .26</td>
<td>205.23± .18</td>
<td>0.004*</td>
</tr>
<tr>
<td>HDL</td>
<td>33.21± 1.04</td>
<td>33.56 ± 0.07</td>
<td>0.358</td>
</tr>
</tbody>
</table>

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DISCUSSION

HCV is transmitted usually by infected blood, contact with contaminated blood or body fluids, blood transfusion from HCV-infected donors, unsafe therapeutic injections, sexual intercourse, and inadequate medical instrument sterilization, like needles and syringes during clinical practice in health centers (Moosavy et al., 2017). Sexually and infected mother-to-child transmission of HCV is less common (Paiva et al., 2018). Females are more prone to HCV infection because of major aspect accidents, using blood and blood products, unsterilized instruments and syringes, blood transfusion, surgical operations, and ear-piercing during pregnancy and labor (Abdel-Gawad et al., 2023). During a previous study, we found that males were more infected with HCV than females Cusi (2014).

A satisfying relationship between age and HCV infection was observed. The findings of this study suggested that the younger age (<30 years) favors getting easily victimized by HCV. An observation abandoned by previous studies. However, various studies indicated that the percentage of clinically apparent HCV patients increased with age. Still, the rise in HCV cannot be explained exclusively by the effect of ageing in the general population. It was found that diabetic HCV-infected female patients were in the older age group. Infection of HCV has been considered a significant risk element for developing insulin resistance. Here, the close relationship between diabetes and HCV was noticed. Current study results showed that diabetes
was found in 11.61% of the patients infected with HCV, and most were married. These findings were similar to those reported by other authors (Li et al., 2019). While working on HCV patients, other researchers reported a higher frequency of diabetics; they found a prevalence of diabetes among HCV-positive patients (Chen et al., 2020). Old age is one of the significant threats to extrahepatic demonstration of age and HCV (Feutseu et al., 2023).

The highest proportion of diabetes was found in the age group greater than 40 (Figure 1). This observed inconsistency among different studies may be credited to study population size, geographical differences, differences in the parameters used to diagnose DM, genetic susceptibility, diet, and case details. Chakrborty reported that HbA1C, BGR, and BGF levels were enormously increased in diabetic patients with HCV infection than in diabetic patients without HCV. These results were the following data of current research. They documented weakly controlled BGF, BGR, and HbA1C by the diabetic HCV group, adversely influencing diabetes prognosis. Abnormal glucose tolerance was found in chronic liver disease patients (Chakraborty, et al., 2022). Researchers exposed that insulin resistance increases with HCV, which decreases diabetes prevalence (Popescu et al., 2022). This study is based on the association of HCV and diabetes, focusing on biochemical marker’s laboratory value. Liver cirrhosis is a late stage of liver scarring, and it takes time to develop. Patients did not undergo an ultrasound examination or liver biopsy to confirm the presence of cirrhosis.

The level of ALT is average among 40% of chronic HCV patients, even when tested at varying times. HCV diabetic patients reported abnormal LFTs. The levels of ALT and AST were slightly increased in HCV diabetic patients than only the group of HCV patients. Similarly, levels of transaminases were remarkably raised in diabetic HCV, as reported (Thong et al., 2021). Except for liver cirrhosis, HCV does not directly influence gamma-glutamyl transpeptidase and alkaline phosphatase levels. The protein level is high, while albumin is low in both HCV without diabetes and HCV diabetic groups, which relates to the results of Bacon et al (2011). A similar relationship was documented by a meta-analysis on type 2 diabetes (Lee et al., 2019). It was described that serum ALT levels increased by 73.7% in diabetic HCV patients than in diabetic patients without HCV infection by 18.5%. Transaminase levels rise to 20 times but usually less than five times to the upper limit of normal. The levels of ALT usually are high as compared to AST, but this may become the opposite in the case of cirrhosis-affected individuals (Li et al., 2019)
The binding of HCV with plasma lipoprotein and growing evidence of hypo-beta-lipoprotein and HCV connection was reported by different authors (Sidorkiewicz et al., 2021; Vieyres et al., 2023). Lower concentrations of total cholesterol and triglyceride have been noted in HCV-infected patients as compared to diabetes patients without HCV infection (Casas-Deza et al., 2023). All these results indicate the influence of HCV on the lipids metabolism in the body. Displacement in lipid profile shows more swelling or changed caloric metabolism, which causes difficulty to survive (Chaudhari et al., 2021).

It has been concluded from the current community-based study that HCV infection and diabetes are the high worldwide prevalence of chronic diseases of epidemic dimensions. The association between these two has been substantiated by various studies and depicted a two-way relationship. On one way, diabetes is activated by HCV infection. On the other side, diabetes intensifies the effects of hepatitis C and increases the development of HCC and cirrhosis, specifically in females. This study demonstrated the association between diabetes, HCV, and biochemical markers. Furthermore, it would be suggested to conduct more such studies to explore the clinical significance of different biomarkers' critical patterns, diabetes, and Hepatitis C more deeply. Therefore, it is necessary to figure out the scale of the problem to optimize the treatment and management of HCV.

**Ethical approval and consent to participate**

Ethical Review Board of Citi Lab and Research Centre approved this work under Ref # 26-17/ERB/CLRC/ 27th.

**Conflict of interests**

None to declare.

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**Authors contribution**

SR and MFB designed the study and collected data /samples from participants. MFB and MAR worked on the experiment and wrote the draft manuscript. MS and SR participated in questionnaire designing, statistical analysis, and manuscript finalization. SR, MAR, and MFB helped in sample collection from res. All authors critically reviewed the manuscript and approved the final version.

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REFERENCES


