

Prevalence of HCV induced diabetes among HCV patients in Punjab Pakistan, A cross-sectional study

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ABSTRACT

Background: With a high disease burden, hepatitis C virus (HCV) disorders are one of Pakistan's top public health concerns. Despite the country's access to efficient antiviral medications, the overall population's disease burden has not decreased. This may be due to the infection's asymptomatic character, which makes it difficult to diagnose until late in the course of symptoms. A population-based analysis is required for an efficient control of the virus in order to more precisely figure out and identify HCV infections in the nation.

Methods: In our study, 2000 HCV samples were collected from different regions of Punjab and tested for HCV by Bioline™ HCV kit for early detection and Fasting blood glucose level was determined by glucometer to check diabetes. Then all HCV positive samples sent to Genome center for molecular based diagnostics & research (GCMD) Lahore, where samples were confirmed by PCR and diabetes was confirmed by HbA1c test for HCV induced diabetes confirmation. The objective of this study is to find out the overall prevalence of HCV induced diabetic samples among HCV positive patients. Viral load, Hemoglobin levels and platelets count were other factors analyzed in this research contributing to the effective prevention, control and treatment to control HCV induced diabetes prevalence in the province of Punjab Pakistan.

Results: Overall, 80% of the samples were detected as HCV positive and HCV induced diabetic samples were 9% that were identified in this study. A significant difference in prevalence of HCV induced diabetes was observed in different age groups among (male and female) with 57% male and 43% female patients.

Conclusion: This study represents the prevalence of HCV induced diabetes among HCV infected patients of about 9% (age 22-72 years) from different regions of Punjab.

Keywords: Chronic Hepatitis C, Enzyme Linked Immunosorbent Assay, Hepatitis C Virus, Polymerase Chain Reaction, Type 2 Diabetes Mellitus

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Introduction

Hepatitis C virus (HCV) represents a serious threat to world's health due to its serious complications like cirrhosis, hepatocellular carcinoma, and chronic liver disease (1). This infection had spread over the world since the initial identification of HCV in 1989, despite intensive study to understand different

elements of the virus and the disease (2). With a significant risk of developing hepatocellular carcinoma, it is estimated that 71 million people worldwide have chronic hepatitis C (3, 4). Over 3 million new HCV infections occur each year, and the accompanying mortality rate is 0.4 million (5-8). With 10-17 million people affected (9), or around 5% of the population, Pakistan has one of the highest rates of HCV prevalence in the world (8, 10, 11). The high rates of HCV infection in Pakistan are due to a number of factors, including unsafe medical procedures like inadequate sterilization of medical and dental equipment, over usage of injections, filthy barbershops, drug users sharing needles, and unsafe blood transfusions. The general public's lack of knowledge regarding the components involved in viral transmission also aids in the spread of the HCV infection. The development of effective strategies for the prevention, control, and treatment of HCV relies on a comprehensive understanding of the epidemiology of the disease, including its prevalence, transmission routes, and associated risk factors.

Understanding the geographic distribution of HCV is essential for identifying risky groups and places, allowing for resource allocation and focused interventions. There are 71 million people who are thought to have chronic hepatitis C worldwide (3, 4), while there are differences between and within nations. In comparison to other locations, like Europe and North America, some have a higher incidence than others, especially across sub-Saharan Africa and Asia.

Chronic HCV infection is now viewed as a systemic illness even though it affects more than just the liver. Nearly 75 percent of patients also experience extrahepatic symptoms, which are already present when

chronic HCV infection is suspected (12). One of the most prevalent extrahepatic symptoms of persistent infection with HCV is type 2 diabetes (T2DM) (13).

Because the hepatitis C virus affects the hepatocyte insulin signaling pathway in numerous ways, including (i) increased production of tumor necrosis factor-, (ii) phosphorylation of the receptors for insulin, and (iii) overexpression of the insulin receptors, chronic HCV infection can result in enhanced insulin resistance SOC-3, (iv) activation of SOC7, and (v) suppressant of cytokines (SOC-3) (14-16). Not only is insulin sensitivity compromised in the liver, but also throughout the body, even in HCV-infected patients without metabolic syndrome. Higher insulin resistance is responsible for the occurrence of T2DM in patients with persistent HCV disorder (13, 17). It is believed that mediators produced by an HCV-infected liver promote insulin resistance at extra hepatic locations, primarily in skeletal muscles.

HCV prevalence was described by recent epidemiological studies on HCV that were carried out in Pakistan. These studies, however, were only conducted on a small number of people or only on high-risk populations (IDUs, blood donors, and healthcare workers) a in relatively small geographic areas. To do an analysis of HCV infections in the general population of the major cities in the Punjab province, which is home to 53% of Pakistan's population, we evaluated the seroprevalence of HCV induced diabetes in the current study. In this study, we collected the HCV samples from the different places of Punjab Pakistan in 2021-23 to check the prevalence of HCV induced diabetes.

Methods

In this cross-sectional study 2000 serum samples were collected from various regions of Punjab with the help of Genome center lab along with patient's signed consent form for this research from 2021-23. Consent form included their full name, complete address, phone number, and demographic information including their age, weight and approximate time of HCV infection which was taken more than one year. This research project was reviewed and approved by institutional review board of department of Biotechnology, University of Okara (Ref# UO-biotech 100-102) dated 04-01-2021.

Initial screening for HCV samples was done by Bioline™ HCV kit for HCV detection and fasting blood glucose level was determined by glucometer. Then samples subjected to the qualitative detection of HCV RNA described earlier by Idrees et al (18) through PCR. The identification of HCV RNA was carried out using reverse transcription PCR (RT-PCR). Using the Quigen RNA extraction kit and following the product's instructions, RNA was isolated from 100 l of the patient's serum. Using Taq DNA polymerase enzyme (Fermentas Technologies USA) in a 20 µl reaction mix volume, nested PCR was carried out. Using the "Uvitec" gel documentation system, the nested PCR products were observed on a 2% agarose gel while being

illuminated by "UV" light (19). All HCV positive samples were evaluated for HCV induced diabetes by HbA1c test for confirmation.

A glucose meter was used to check the patient's fasting blood sugar levels to evaluate the body's glucose level. A normal fasting plasma glucose level was considered to be less than 100 mg/dL (5.6 mmol/L). Levels of 126 mg/dL (7 mmol/L) were considered higher. Blood samples were also sent to the Genome center, Lahore, lab for measuring the patient's glycated hemoglobin (HbA1c) levels to get an idea of their blood sugar levels on average during the last few months. For non-diabetics, a normal HbA1c level was below 5.7%, while a level between 5.7% and 6.4% indicates prediabetes and 6.5% or higher indicates diabetes.

Results

Out of the 2000 samples 1600 were HCV infected after PCR confirmation from lab for HCV positive samples that was almost 80% of the samples. The remaining 20% samples i.e. 400 were non-HCV samples as shown in figure 1. So, our data is showing high prevalence of HCV infection as shown in pie chart. These blood samples were treated by the standard protocol for the identification of the HCV as described above in the materials and methods section.

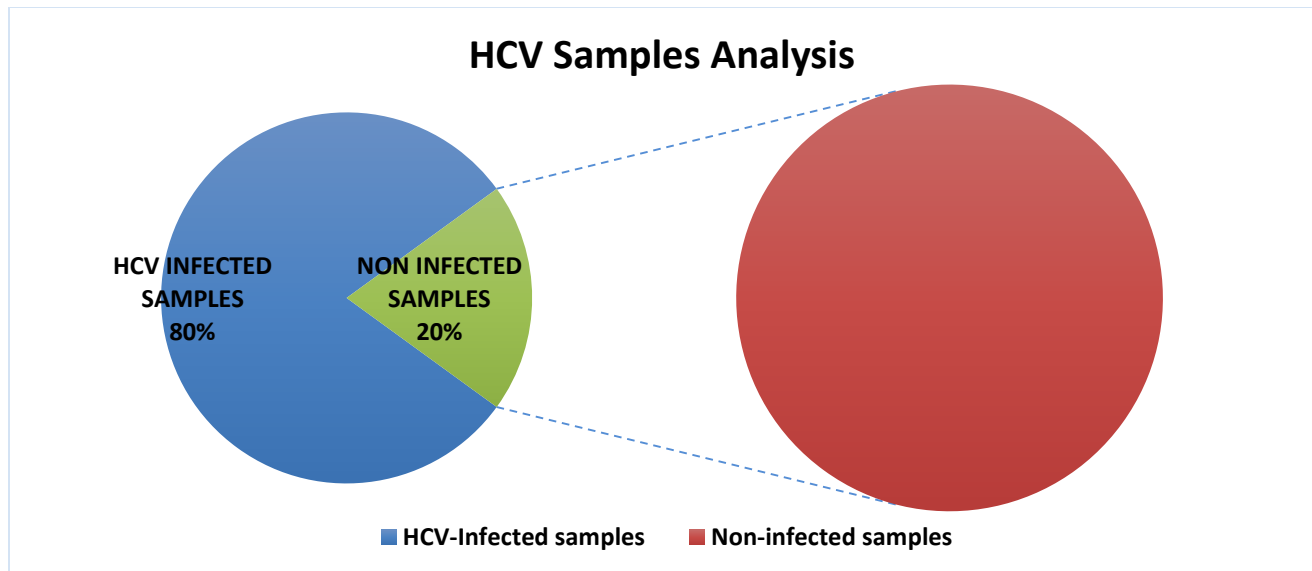


Figure 1: HCV Samples Analysis from the total samples

Initial screening of HCV induced diabetes was done on fasting higher blood glucose level checked by glucometer. Additional confirmation was done from history of patients. All the patients who were detected as HCV positive (either treated or non-treated) and having fasting higher blood glucose level were included in the study and all other patients with low blood glucose level with HCV infection were excluded from study. After confirmation with PCR, all HCV positive samples were tested by HbA1c test

for confirmation of diabetes in those patients. Total 145 samples were HCV induced diabetic samples out of 1600 as shown in pie chart in figure 2. On the basis of gender wise analysis HCV induced diabetes was higher in the males as compared to the females i.e. 82 males out of 145(57%) HCV induced diabetic samples as compared to 63 samples from female patients out of 145 (43%). This is demonstrated in the form of bar graph shown in figure 3.

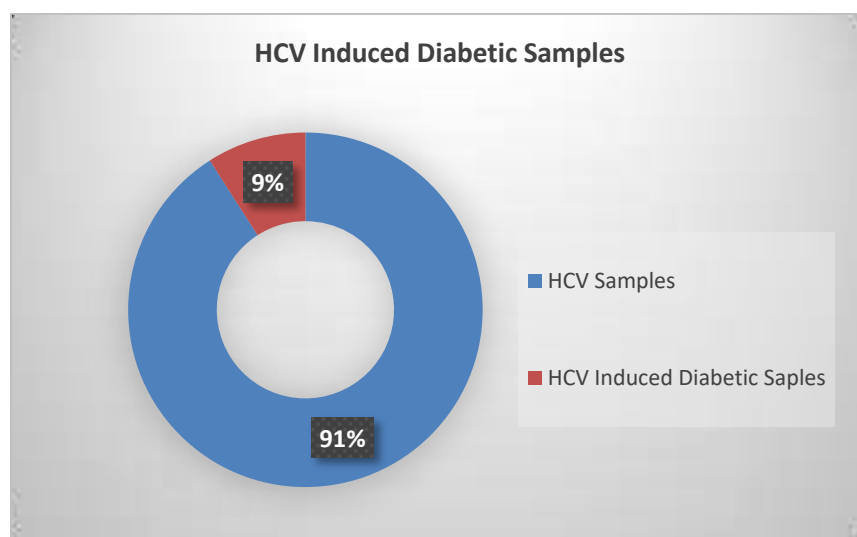


Figure 2: HCV Induced Diabetic Samples from the total HCV samples

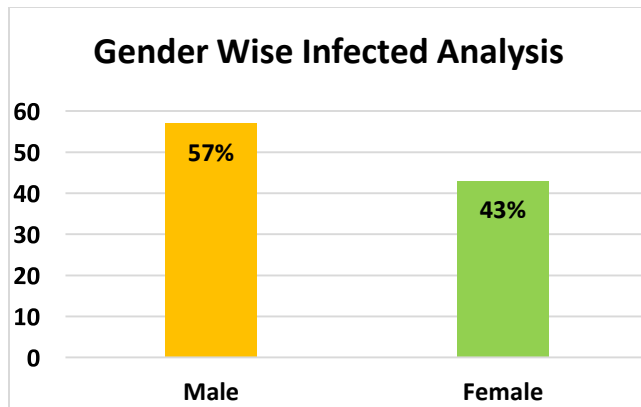


Figure 3: Gender Wise HCV induced diabetic samples Analysis

All HCV positive samples were either treated with interferons or direct acting antiviral drugs DAA (sofosbuvir and ribavirin) were included in the study as HCV might be a prominent factor in inducing

diabetes in HCV positive patients. Out of 145 HCV induced diabetic samples 41 samples were interferon treated and 14 samples were treated by sofosbuvir and ribavirin (within 4 weeks of treatment). All those were presented in a graph that showed the prevalence of HCV induced patients either treated or untreated as in figure 4. The age group used in this study is random and falls in 22-72 years range as mentioned in table 1. The HCV viral loads in these samples were also checked that ranges from 20983 I_μ/mL to 9392856541 I_μ/mL. The Hb level and platelets count were also analyzed and shown in the table 1 described below.

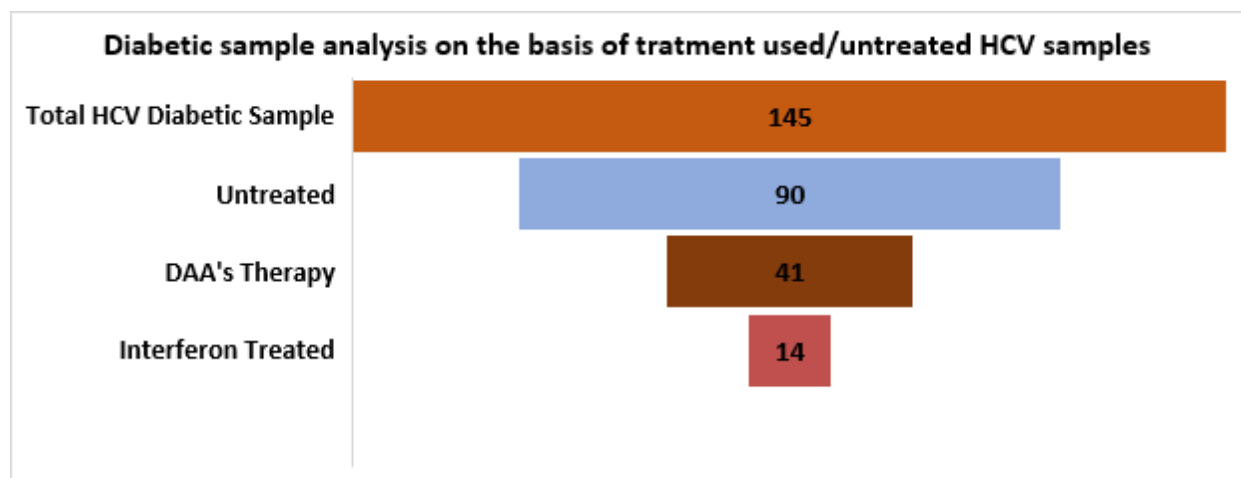


Figure 4: Diabetic sample analysis on the basis of treatment used/ untreated/ untreated HCV samples

Table 1: Several features of the samples used in the study

Feature	Minimum	Maximum
Age	22 years	72 years
Viral Load	20983 I _μ /mL	9392856541 I _μ /mL
H.B. (hemoglobin level) (Normal range is 13.5 to 18.0 g/dL for males and 12.0 to 15.0 g/dL for females)	4.5 g/dL	16 g/dL
Platelets	78 counts	670 counts

150,000 to 450,000 platelets per microliter of blood		
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Discussion

Punjab is the most populous province of Pakistan which covers almost 53% of the total population of Pakistan. Understanding the regional distribution of HCV genotype may be crucial in explaining HCV epidemiology and evolution. It is also a helpful tool for

identifying risk groups and providing information on various transmission channels (20).

The most common manifestation of chronic HCV is progressive liver fibrosis, cirrhosis, liver failure and hepatocellular carcinoma. However, several systemic manifestations of HCV have been recognized and reported in the literature. The important pathogenic agent that induces insulin resistance is HCV. Hepatic fibrosis progression, type 2 diabetes mellitus, metabolic syndrome, extra hepatic symptoms, and the emergence of hepatocellular cancer are all associated with this form of HCV-induced insulin resistance (21). The mechanisms through which hepatitis C induces T2DM involve direct viral effects, insulin resistance, pro-inflammatory cytokines and other immune-mediated processes. (13)

Diabetes mellitus and the hepatitis C virus are two devastating global health problems. According to our study, around 9% of HCV-infected patients from various Punjab regions have diabetes which is quite low as compared to a study in which diabetes was observed in 21% of HCV-infected patients. Gray et al., was the first to show a higher prevalence of HCV infection in T2DM patients with a prevalence of 8% among Asian patients.(22) The variation in these occurrence rates can be ascribed to exposure to various risk factors which are capable of enhancing the spread and transmission of this virus among individuals (23) Overall prevalence of HCV infection among diabetic patients according to another study was 13.3% out of which 8(11.3%) was obtained from the male subjects compared to 16 (14.7%) seropositivity recorded among the females. (24) In our study HCV induced diabetes was higher in the males as compared to the females i.e. 82 out of

145(57%) in males as compared to 63 out of 145 (43%). This finding disagrees with the result obtained from a similar work where the incidence of anti-HCV among diabetic female were higher compared to the male subjects. (25,26)

Prolonged HCV infection is strongly linked to an elevated prevalence of type-2 diabetes mellitus and is a major pathogenic cause of insulin resistance. T2DM is characterized by insulin resistance, which has a major impact on two insulin signaling pathways: the Ras/Raf/MAPK route and IRS1/PI3K/PKB pathway. (21) As proved by many studies that HCV is a contributing factor for insulin resistance that leads to diabetes. One study has shown that serine 473 phosphorylation is upregulated by NS5A of HCV genotype 3a, which implies that this gene disrupts the normal insulin AKT/PKB signaling pathway, which results in Type 2 diabetes mellitus and insulin resistance. (27) As proved by many studies that HCV is a contributing factor for insulin resistance that leads to diabetes.

Conclusion

According to this study, around 9% of HCV-infected patients from various Punjab regions have diabetes. So, it is possible that HCV infection may serve as an additional risk factor for the development of diabetes, beyond that attributable to chronic liver disease alone.

Recommendations

Patients with liver disease are known to have a higher prevalence of glucose intolerance, although prevalence in our study is 9%, more studies are required to establish an association between hepatitis C infection and diabetes so as to do regular testing of blood sugar and then HbA1C in hepatitis C patients establish any role of antivirals in treating diabetes in these patients.

Conflict of Interest: Nil

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- A. Conception/Study/Designing/Planning
- B. Active Participation in Active Methodology
- C. Interpretation/ Analysis and Discussion