

## Original article

# Prevalence of diabetes mellitus type II in patients with hepatitis C and association with other risk factors

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### Abstract

A higher prevalence of diabetes mellitus (DM) has been documented in patients with hepatitis C virus (HCV) infection. The aim of this study was to evaluate the prevalence of coinfection of HCV infection with diabetes mellitus with hepatitis B virus (HBV) infection and hypertension. The study was conducted at Chemical Pathology Laboratory at Malik Haider Hospital (MHH) and Aziz Bhatti Shaheed Hospital (District Head Quarter) Gujrat. For this purpose a serum of 250 patients with chronic viral hepatitis was screened. Liver function test (LFT) profile viz., alkaline phosphatase (AP). Alanine amino transferase (ALT) and bilirubin levels were also determined. Further information like age, sex, marital status, smoker, non-smoker, hypertension, hepatitis B (HBV) co-infection and diabetes were recorded on prescribed performa. HCV was found to be more prevalent in women (60.4%) than in men (39.6%) and the highest incidence of HCV was recorded in age group 24-34 years (28%). Prevalence of diabetes was calculated to be 34.80%, prevalence in women was calculated to be 31.20% and in males 40.40%. Co-relation of HCV and DM was found to be 0.77. The percentage of co-infection with HBV was calculated to be 12.80% and with hypertension was found to be 36.40%. All these factors contribute to the development of diabetes but only one of them may be responsible for its major cause.

**Key words:** Diabetes mellitus, hepatitis, Liver function test, hypertension, risk factors, prevalence.

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## INTRODUCTION

Type II diabetes mellitus (DM) is one of the invasive diseases worldwide, and it is estimated that by year 2030 there will be 366 million people affected globally (Wild *et al.*, 2000). In the year 2000, global estimation of deaths due to diabetes was 2.9 million (Roglic *et al.*, 2005). In Pakistan, an estimated 6.3 million persons have an age adjusted, 7.9% prevalence of diabetes (PWD) among adults a 20 years or older. Pakistan in 2030 will have an estimated 11.4 million persons with PWD and prevalence of 8.9% in the absence of major interventions (Whiting *et al.*, 2011). Diabetic patients have a higher risk of suffering from Hepatitis C virus (HCV) infection in view of nature of the disease and its inherent complications. HCV infections itself play a part in the development of

diabetes mellitus (DM). There are no epidemiological evidences of this association in Pakistan and its exact biological mechanisms are not known (Ali *et al.*, 2007).

Most obvious symptoms regarding effect of HCV on liver are inflammation, slowly progressive fibrosis, leading to cirrhosis and carcinoma. HCV infection is a systemic disease involving lipid metabolism, mitochondrial function, gene expression and signaling pathways (Noto and Raskin, 2006). Chronic hyperglycemia, a major pathological feature of DM, is associated with high mortality and morbidity due to cardiac and renal complications (Lind *et al.*, 2012). Tumor necrosis factor alpha (NTF Alpha) has been identified as a mediator of insulin resistant and is induced by HCV (Fraser *et al.*, 1996). Prevalence of HCV infection worldwide is estimated to be about 3 percent

with 170 million people affected (Sy and Jamal, 2006). Increase of HCV prevalence in patients with DM suggested a link between and possibility of patient to patient HCV transmission in diabetes units. HCV infected cirrhotic patients may suffer type II DM more frequently than patients with cirrhosis of other origins (Allison *et al.*, 1994). A link between HCV infection and DM suggested by both studies in which diabetes detected among persons with chronic HCV infection (Fraser *et al.*, 1996; Grimbert *et al.*, 1996; Ozyilkan and Arslan, 1996) and studies in which the occurrence of HCV infection was examined among persons with DM (Simo *et al.*, 1996; Mason *et al.*, 1999). There is a huge data regarding association between liver disease and diabetes mellitus (DM), the overall prevalence being significantly higher than that expected by a chance association of two very common diseases. The induction of a rapid decline in residual liver function; an effect which is influenced by treatment strategies and by tumor and/or cirrhosis related factors has been the most recent evidence for an association between DM and liver disease (Huo *et al.*, 2003). The objective of the current study was to ascertain the prevalence of DM in patients with HCV infections, the association of HCV and DM with other risk factors and the co-infection of HCV with HBV in Pakistani population.

## MATERIALS AND METHODS

This study was conducted in Chemical Pathology Laboratory at Malik Haider Hospital (MHH) and Aziz Bhatti Shaheed Hospital (District Head Quarter) Gujrat. During this study a total of 250 blood samples of HCV positive patients were collected irrespective of age and sex and screened for liver function tests viz., alanine aminotransferase (ALT), bilirubin, and alkaline phosphatase (AP), along with blood sugar test. The detailed previous history of each patient was recorded on the prescribed questionnaire having the following information viz., age, sex, locality, occupation, and socioeconomic status, personal history including marital status, smoker or non-smoker, alcohol intake, stress and drug history. General physical examination was also taken, including temperature, blood pressure and weight.

### **Blood sampling**

Blood sample (10ml) was withdrawn from the branchial vein with the help of 10ml disposable syringe and dispersed in 10ml test

tube. Serum was separated with the help of centrifuge at 3000 rpm for 15 minutes. The serum was stored at 4°C for biochemical analysis to be carried later.

### **Biochemical analysis**

#### **Hepatitis C Virus**

ELISA kits (Merck) were used for *in vitro* detection of HCV antibodies in serum (Cao *et al.*, 1996). Micro plates were coated with HCV specific synthetic antigens derived from "core" and "ns" regions (core, NS3, NS4 and NS5). The solid phase was first treated with the dilution of sample and HCV Ab captured I presented, by the antigens. After first washing, in the 2<sup>nd</sup> incubation bound HCV Ab were detected following additions of anti-human IgG antibody, labeled with horseradish peroxidase (HRP).

The enzyme captured on the solid phase generates an optical signal by acting on the substrate/chromogen mixture that is proportional to the amount of anti HCV antibodies present in the sample. The cut off value is 0.200. The specimens with absorbance values less than the cut off value were considered not reactive or negative for antibodies for HCV. Those specimens were considered reactive or positive for antibodies for HCV, which have absorbance values greater than or equal to the cut off value.

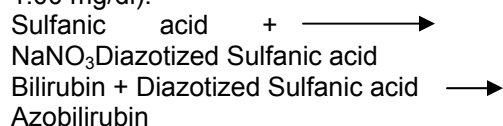
#### **Hepatitis B Virus**

ELISA kits (Merck) were used for *in vitro* detection of HBsAg in serum (Shanmugham *et al.*, 2010). The test is an immunoassay based on "sandwich" principle. Polystyrene micro titer strips wells have been coated with monoclonal anti-HBs. The test sample was incubated in the wells so that if HBsAg present in the sample will bind to solid phase antibody. Subsequently, HRP labeled guinea pig anti-HBs were added. Incubation with enzyme substrate produces a blue color in the test well, and stop reaction with sulphuric acid gives yellow color. If there are no HBsAg in the sample, the labeled antibody cannot bind specifically and only a low background color develops. The cut off value is 0.105; a negative result means no HBsAg or HBsAg below the detection limit. A positive result indicates the presence of HBsAg or non-specifically reaction factor.

#### **Bilirubin**

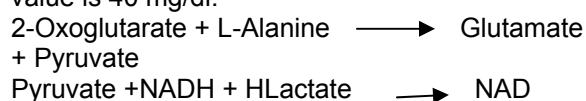
*In vitro* test for the quantitative determination of the direct bilirubin and total bilirubin in serum was done by Jendrassik-Grof

(1938) method using Merck kit. In this procedure in the presence of caffeine accelerator, a red azobilirubin dye is formed by coupling of total bilirubin with sulfanic acid. Direct bilirubin is determined without caffeine additive. Red azobilirubin dye is transformed to blue dye by the addition of alkaline titrate with absorbance maximum from 546nm to 578nm. The normal range of bilirubin as quoted by Merck kit (0.00 to 1.00 mg/dl).



### **Alanine amino transferase (ALT)**

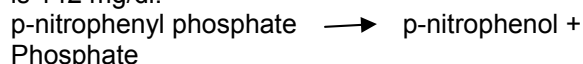
*In vitro* test for the quantitative determination of ALT in serum and plasma was done by the method recommended by the International Federation of Clinical Chemistry (IFCC) with optimized substrate concentration, using Tris buffer, simultaneous pre-incubation of serum with buffer (to avoid competing reaction with NADH), substrate start, and pyridoxal phosphate activation by Merck kit (Schumann *et al.*, 2002). The normal range for the reference value is 40 mg/dl.



The rate of NADH compensation is directly proportional to the ALT activity in the sample and is measured using photometer.

### **Alkaline phosphatase (AP)**

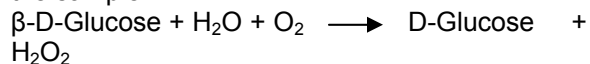
Kinetic determination of alkaline phosphate activity was done according to the method recommended by the Deutsche Gesellschaft für Klinische Chemie (DGKC) using Merck kit (Westermann *et al.*, 2004). In the presence of magnesium ion p-nitrophenyl phosphate is cleaved by phosphatase to form p-nitrophenol and phosphate. The amount of p-nitrophenol liberated is directly proportional to the alkaline phosphatase activity and measured photometrically. The normal range for reference is 142 mg/dl.



### **Glucose**

*In vitro* determination of blood glucose level was done with Glucose Oxidase (stable liquid) using Merck diagnostic kit (Holvey, 1972). In the Trinder reaction, the glucose is oxidized

and final product is a red quinoneimine dye proportional to the concentration of glucose in the sample.



The normal range of glucose as quoted by Merck diagnostic kit for random is 80 to 120 mg/dl and for fasting is 55 to 115 mg/dl.

### **Statistical Analysis**

The data were arranged in appropriate groups and analyzed statistically. Appropriate tables and graphs were constructed according to the results. Co-relation of HCV and DM was found out using Statistical Package for the Social Sciences version 16.0 (SPSS, Chicago, IL).

## **RESULTS AND DISCUSSION**

During this study, a total of 250 blood samples of patients having HCV attending Malik Haider Hospital (MHH) and Aziz Bhatti Shaheed Hospital (District Head Quarter) Gujrat were screened for the presence of alkaline phosphatase (AP), Alanine amino transferase (ALT), bilirubin, hepatitis B virus (HIV) co-infection, and diabetes. All these patients showed general signs and symptoms related to chronic liver abnormalities such as indigestion, fatigue, weakness, fever, abdominal pain, nausea, loss of appetite, dark urine and yellow eyes.

### **Prevalence of diabetes in HCV**

During this study 87 patients out of 250 patients having HCV were found to be diabetic so prevalence of diabetes was calculated to be 34.80 percent. Co-relation of HCV and DM was found to be  $r = 0.77$ . In female 47 patients having HCV were co-infected with diabetes so out of 151 HCV infected women 47 also experienced diabetes therefore, prevalence in women was calculated to be 31.12 percent. In male out of 99 HCV infected patients 40 also experienced diabetes therefore, prevalence in male was calculated to be 40.40 percent. It can be concluded that co-infection with diabetes is more prevalent in men as compared to women. These results are contrary to earlier results of Qureshiet *al.*, (2002) that 24.5 percent, in Japan diabetes was observed in 30.8 percent (Arao *et al.*, 2003), in Korea 24.0 percent (Ryu *et al.*, 2001), in Saudi Arabia 21.2 percent (Akbar *et al.*, 2002), in Spain a threefold increase in the prevalence of glucose abnormalities was

observed in HCV positive patients with chronic hepatitis in comparison in age group of 12-22 years as just one of the 23 individuals experienced diabetes irrespective of sex so its prevalence was just 4 percent. Highest prevalence of HCV co-infected with diabetes was recorded in age group 46-56 years (28.73

percent), 24-34 years (25.28 percent), 35-45 years (20.68 percent) (Table I). Less number of men is infected with diabetes in all age groups except in 24-34 years group (Figure 1). There is an increased prevalence of type II diabetes in patients with chronic HCV infection, and this is independent of cirrhosis.

**Table I: Co-infection of HCV with Diabetes, HBV and Hypertension in various age groups**

Patients	Age groups (Years)							Total	Co-infection %age
	13-23	24-34	35-45	46-56	57-67	68-78	79-89		
HCV Patients	22	70 (28%)	60	69	18	8	3	250	
HCV Patients with Diabetes	1 (01.14%)	22 (25.28%)	18 (20.68%)	25 (28.73%)	11 (12.64%)	8 (9.19%)	2 (2.29%)	87	34.80%
HCV Patients with HBV	10 (31.25%)	10 (31.25%)	3 (9.37%)	8 (25%)	0 (0%)	1 (3.12%)	0 (0%)	32	12.80%
HCV Patients with Hypertension	2 (2.19%)	23 (2.52%)	27 (29.67%)	26 (28.57)	9 (9.89%)	3 (3.29%)	1 (1.09%)	91	36.40%

### **Prevalence of HBV in HCV**

During this study only 32 patients out of 250 were found to be co-infected with hepatitis B virus (HBV). The percentage of co-infection was found to be 12.8 percent (Table 01). In female 23 patients having HCV were co-infected with HBV so out of 151 HCV infected women 23 also experienced diabetes therefore, prevalence in women was calculated to be 15.23 percent. In males out of 99 HCV infected patients 9 also experienced HBV therefore, prevalence in male was calculated to be 9.09 percent. Co-infection has been observed 25 percent in India (Sood *et al.*, 1999) and 24.1 percent in Japan (Marusawa *et al.*, 1999). Data suggested that the prevalence of HCV RNA or HBV DNA in these populations increases with the severity of hepatic injury. It has been observed that the highest prevalence of HCV co-infected with HBV was recorded in age groups 13-23 and 24-34 (31.25 percent), 46-56 years (25 percent), 35-45 years (9.37

percent) (Table 1). Less number of men were infected with HBV in all age groups (Figure 1).

### **Hypertension in HCV Patients**

#### **Prevalence of hypertension**

Less number of men are infected with HBV in all age groups except in 57-67 years group (Figure 2). During this study 91 patients out of 250 patients having HCV were found with elevated level of blood pressure than normal range 120/80 mg/dl (figure 3). Among these 91 patients 29 were male and 62 were female (figure 3). For those younger than 18, normative blood pressure have been established based on age, gender and height (Lori *et al.*, 2004). Whereas in HCV having diabetes 36 patients out of 91 patients having HCV were found to be having elevated level of blood pressure. Among these 36 patients 24 were females and 12 were males. It has been observed that the highest prevalence of HCV characterized with Hypertension was recorded in age groups 35-45

(29.67 percent), 46-56 years (28.57 percent), 57-67 years (9.89 percent) (Table 1). Hypertension is a common disease found in patients with type II diabetes, when present; it doubles the already elevated risk of future cardio-vascular events (Niskanen *et al.*, 2001). Prevalence of UDM among diabetes patients has been reported as 38.9% in Pakistan (Siddiqui *et al.*, 2014).

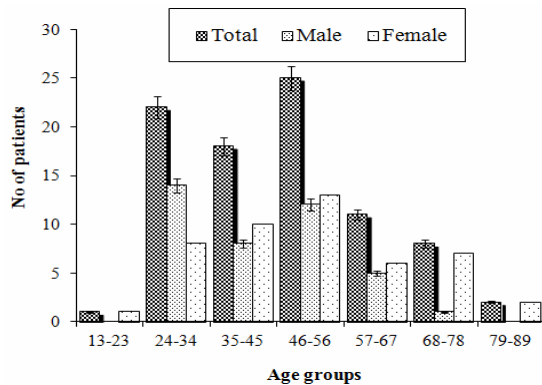


Figure 1 Age and sex specific prevalence of diabetes mellitus in HCV Patients

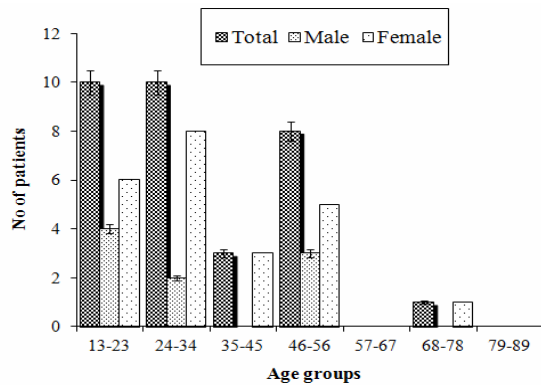


Figure 2 Age and sex specific prevalence of HBV in HCV patients.

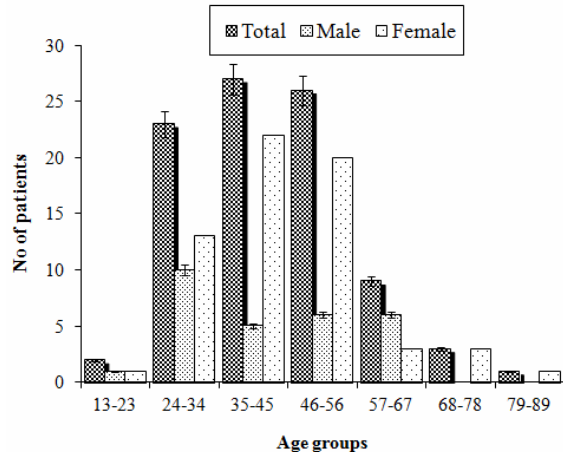


Figure 3: Age and sex specific prevalence of

**CONCLUSION AND SUGGESTIONS**

Among various types of diabetes, diabetes mellitus type II is more common in the representative population of Pakistan. Diabetes mellitus type II is more prevalent in females as compared to males of all races. Disease occurs more frequently in 20-60 years of age group. Early diagnosis of the disease and proper provision of medication is need of the hour. Epidemiological studies should be planed occasionally to check the occurrence rate of such chronic and disabling diseases. Such studies in developing countries like Pakistan have two purposes. One is to determine the prevalence and then to promote the extent of the problem. The other is to search for extrinsic causative factors by comparing the results with similarly acquired information from the developed world.

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