

Original Research Article

Association of Biochemical Markers, Hepatitis C Virus and Diabetes Mellitus in Pakistani Males

Muhammad Faisal Bashir^{1, 2}, Muhammad Saleem Haider^{3*}, Naeem Rashid¹ and Saba Riaz^{2,4}

¹School of Biological Sciences, University of the Punjab, Lahore 54590, ²Division of Molecular Pathology, Citi lab and Research Centre, 525 A Faisal Town, Lahore, ³Institute of Agricultural Sciences, ⁴Department of Microbiology and Molecular Genetics, University of the Punjab, Lahore 54590, Pakistan.

*For correspondence: **Email:** haider65us@yahoo.com, sabbb1502@yahoo.com; **Tel:** +9242 99231846

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Abstract

Purpose: To investigate the association between Hepatitis C Virus (HCV) infection and diabetes mellitus (DM), and the effects of these pathological conditions on some biochemical markers in Pakistanis.

Methods: A total number of 4717 chronic HCV patients were enrolled in this study out of which 4250 were positive with the enzyme linked immunosorbant assay (ELISA). Out of this, HCV was detected in 3513 samples by qualitative polymerase chain reaction (PCR). PCR positive samples were divided into: HCV without diabetes (n = 3136) and HCV with diabetes (n = 377) groups; 130 patients with diabetes only (negative for HCV ELISA) were also included in the study. Biochemical tests of all three groups were performed to determine liver, diabetic and lipid profiles.

Results: There was increased prevalence of HCV alone and HCV + diabetes patients in the 4th decade of life. Alanine aminotransferase (ALT) titers were higher in HCV patients with diabetes than in HCV patients without diabetes ($p \leq 0.001$). Fasting blood glucose was greater in HCV patients with diabetes than in diabetes only patients. Total cholesterol and triglyceride were moderately lower in non-diabetic HCV patients than in diabetes only patients ($p \leq 0.005$). However, total cholesterol and triglyceride levels were significantly higher in HCV patients with diabetes than in the other two groups ($p \leq 0.001$).

Conclusion: There is positive correlation of HCV with diabetes in the population studied. This association is more pronounced (where there are elevated levels of triglyceride and fasting blood glucose) in HCV patients with diabetes than diabetes patients without HCV infection.

Keywords: Hepatitis C, Diabetes mellitus, Biochemical markers, Lipid profile, Glycosylated haemoglobin

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INTRODUCTION

Hepatitis C virus (HCV) infection is a major public health problem and is the leading cause of hepatic cancer. There are about 170 million chronic HCV carriers throughout the world [1]. In Pakistan, the ratio of HCV carriers and infected patients is high (30 %) and is more than 20 % in major Asian countries [2]. HCV mainly infects

the liver but other tissues outside the liver can be involved, resulting in a wide spectrum of extra-hepatic manifestations [3]. The onset of HCV infection is usually silent, and usually penetrates the other tissues of the body without obvious symptoms [4]. Extra-hepatic manifestations in patients with HCV infections include rheumatologic, dermatologic, diabetes and renal impairment [1].

It is believed that diabetes is one of the extra-hepatic conditions attributable to HCV infection. Schlimovich *et al* [5] have argued that patients with diabetes have an increased risk of exposure to HCV; the authors found abnormalities in glucose tolerance in HCV patients. History of HCV infection and advanced liver disease, such as cirrhosis and hepatoma are two important factors in diabetes mellitus and HCV association. Insulin resistance and glucose intolerance may also result from liver cirrhosis [6]. Diabetes mellitus may give rise to non-alcoholic fatty liver disease which could progress to cirrhosis and hepatocellular carcinoma. The severity of liver disease associated with HCV differs widely, as does the rate of progression towards the cirrhotic stage. The cirrhotic stage may depend on several, cofactors, such as age, gender, level of alcohol consumption, obesity, immune status and co-infections [7]. Diabetes changes the course of hepatitis C infection. It is possible that patients may develop insulin resistance independently, but clinical and experimental data suggest that HCV may contribute to its pathogenesis [8]. Treatment of diabetes in the cirrhotic patient is much more difficult because of liver damage and the hepatotoxicity of oral hypoglycemic drugs [9].

This study was designed to determine, if any, the association between HCV and diabetes.

EXPERIMENTAL

Sample collection

HCV infected serum samples were collected from patients already positive with HCV rapid test kit, or abnormal liver function test or with any clinical symptoms from various cities in Pakistan. The duration of sample collection was from January 2007 to December 2010. Serum samples (using vacutainer, BD Becton, Dickinson and Company, USA) of HCV alone, HCV + diabetes, diabetes alone and control were analyzed by HCV enzyme linked immunosorbant assay (ELISA) and HCV polymerase chain reaction (PCR), and for liver function tests, as well as for diabetic and lipid profiles. The study was approved by the Ethics Committee of Citi Lab and Research Centre, Lahore, Pakistan (ref no..CLRC-131/07) and followed international guidelines for human studies [10]. Patients were duly informed about the study and freely consented to participate in the study.

ELISA test for anti-HCV antibody

Test for anti-HCV antibody was performed by ELISA using a commercial kit (Micro LISA HCV Ab. Amgenix, USA). Ninety-six well plates were

coated with antigen. Patient's blood serum was isolated and added into the wells before incubation. The plates were subsequently washed 5 times with phosphate buffered saline (PBS) and then horseradish peroxidase labeled mono-antibody was added. After incubation, the manufacturer's instructions were strictly followed; the plates were washed and the absorbance of the developed color was taken using an absorbance reader (microplate reader RT-6000 Rayto Company, Germany) at 450 nm.

HCV RNA isolation, cDNA synthesis and HCV PCR qualitative detection

RNA from donor's blood sample was prepared according to the manufacturer's protocol with minor alteration in the phase separation step, viz, centrifugation time was reduced to 12 min from 15 min (Trizol. Invitrogen, USA). The isolated serum (300 μ l) was mixed with 500 μ l of TRIzol reagent and extracted with chloroform and alcohol. After quantification, it was reverse transcribed into cDNA using antisense primers (Qaigen, Hilden Germany). Qualitative PCR of the five prime untranslated region (5' UTR) with appropriate primers was performed.

Assessment of biochemical markers

Clinical chemistry kits (Human, Germany) and automated biochemistry analyzer (Micro Lab 300, Merk Germany) were used to analyze biochemical markers in serum. Diabetic profile, including fasting blood glucose (FBG), random blood glucose (RBG) and glycosylated haemoglobin (HbA1C), as well as lipid profile - including cholesterol, triglycerides, high density lipoproteins (HDL) and low density lipoproteins (LDL) were among the biochemical markers analyzed. Liver profile, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), gamma-glutamyltransferase (γ -GT), total bilirubin, total protein and albumin level were also evaluated.

Statistical analysis

Data was analysed using SPSS 18 version, Quantitative data was presented in form of mean \pm S.D, Independent Sample t-test was applied to compare means of two groups and ANOVA was used to compare the means of more than 2 groups: $P \leq 0.05$ was considered as significant, $P \leq 0.001$ was highly significant and $P > 0.05$ was non-significant.

RESULTS

A total number of 4717 suspected HCV males were enrolled in the study, out of which 4250 and 3513 were positive based on ELSIA and PCR, respectively. The results showed that participants with HCV only (without diabetes) were 3136 (86.08%), HCV + Diabetes 377(10.36 %) and diabetes only patients (negative for HCV ELISA) 130 (3.56%). One hundred healthy participants were used as control.

The patients were grouped according to marital status. Married male participants accounted for 78.2, 83.2 and 90 % for HCV only, HCV + diabetes and diabetes only groups, respectively, while 21.8, 16.7 and 10 % unmarried patients were in HCV only, HCV + diabetes and diabetes only groups, respectively (Table 1). The patients were grouped according to age to see the prevalence of HCV only, HCV + diabetes and diabetes only patients in different decades of life of patients. The age groups, 30+, 40+ and 50+, accounted for HCV only (29.9 %), HCV + diabetes (29.44 %) and diabetes only (33.88 %) patients, respectively (Figure 1). HCV qualitative PCR test was used to confirm HCV infection (Figure 2).

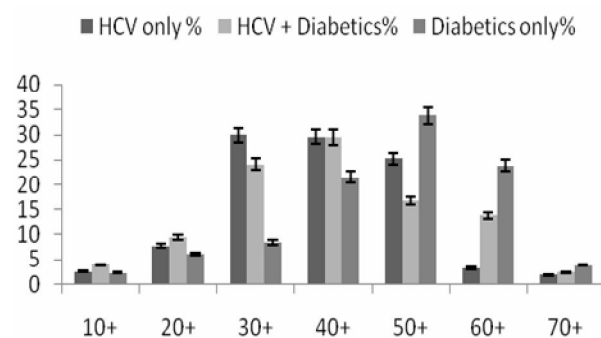


Figure 1: Sample groups according to the age of patients

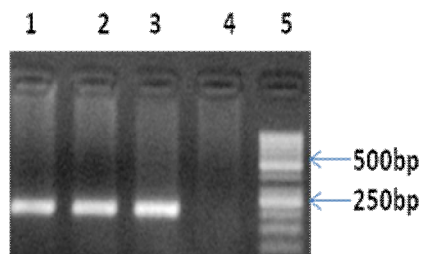


Figure 2: HCV qualitative PCR. **Key:** Lane 1 = positive patient A, Lane 2 = positive patient B, Lane 3 = positive control, Lane 4 = negative control, Lane 5 = marker

When liver function tests (LFTs) were analyzed ALT was raised in HCV alone and HCV + diabetes patients as compare to diabetes only patients. It was observed that ALT titer was more raised in HCV + diabetes patients than HCV only patients. Patients of HCV + diabetes group showed 26.36 ± 0.36 U/L higher ALT levels than HCV only patients. ALT, AST and ALP were highly significant for HCV+ diabetes group ($P \leq 0.001$) and significant for HCV only group ($P \leq 0.005$) whereas these parameters were found nonsignificant ($P > 0.005$) for diabetes only patients. γ -GT was significant for HCV+ diabetes patients only ($P \leq 0.005$). Albumin, bilirubin and total protein have not shown statistically any significant result in all three groups (Table 2).

Glycosylated haemoglobin (HbA1C) levels in married and unmarried HCV+DM patients were identical. HCV + diabetes patients have shown more elevated levels of HbA1C, BGF and BGR than diabetes only patients. BGF and BGR showed highly significant difference for HCV + diabetes group ($P \leq 0.001$) and significant difference for diabetes only group ($P \leq 0.005$). HbA1C showed significant difference for HCV + diabetes and diabetes only patients ($P \leq 0.005$) (Table 3).

Among three groups, HCV+ diabetes group showed a marked increase in serum cholesterol and triglyceride level than other two groups. It was observed that married patients had more raised levels of serum cholesterol and triglyceride levels than unmarried ones in all three groups. Cholesterol and triglyceride had shown highly significant difference for HCV + DM group ($P \leq 0.001$) and significant for other two groups ($P \leq 0.005$). On the other hand LDL was slightly higher than normal in HCV only group, relatively more raised in DM only groups while markedly raised in HCV + DM group. Whereas LDL have shown significant difference for HCV + DM and DM only group ($P \leq 0.005$). HDL was observed insignificant for all three groups of patients ($P > 0.005$) (Table 4).

Table 1: Sample groups categorized according to marital status of patients

	HCV only (n = 3136)		HCV + diabetes (n = 377)		Diabetes only (n = 130)	
	Married	Unmarried	Married	Unmarried	Married	Unmarried
No.	2451	685	314	63	117	13
%	78.15	21.84	83.28	16.71	90	10

Table 2: Effect of HCV and diabetes on liver function parameters

Liver function parameter	HCV only			HCV+Diabetics			Diabetics only		
	Married (n = 2451)	Unmarried (n = 685)	p value	Married (n = 314)	Unmarried (n = 63)	p value	Married (n = 117)	Unmarried (n = 13)	p value
ALT	117.21 ± 2.85	114.35 ± 4.75	0.004*	143.56 ± 3.21	139.21 ± 2.26	0.001**	19.34 ± 0.47	21.65 ± 0.42	0.235
AST	102.63 ± 3.52	93.56 ± 5.31	0.003*	122.51 ± 2.31	116.21 ± 1.62	0.001**	18.21 ± 0.54	20.25 ± 0.35	0.326
ALP	417.64 ± 11.32	472.42 ± 15.92	0.002*	419.25 ± 2.13	481.65 ± 5.61	0.001**	251.35 ± 8.56	269.68 ± 7.34	0.015
Gamma GT	107.52 ± 4.51	101.32 ± 4.35	0.009	115.32 ± 1.25	97.84 ± 6.23	0.002	16.23 ± 1.25	14.56 ± 1.87	0.318
Bilirubin	1.23 ± .08	1.15 ± .06	0.023	1.29 ± 0.07	1.17 ± 0.03	0.010	0.54 ± 0.09	0.43 ± 0.07	0.542
T.Protein	8.81 ± 0.18	8.67 ± 0.57	0.321	9.43 ± 0.59	9.08 ± 0.41	0.112	6.52 ± 0.19	6.78 ± 0.35	0.634
Albumin	3.22 ± 0.13	3.18 ± 0.11	0.152	3.20 ± 0.09	3.12 ± 0.07	0.161	4.52 ± 0.45	4.47 ± 0.41	0.453

Normal values: alanine aminotransferase (ALT) < 40U/L, aspartate aminotransferase (AST) < 40U/L, alkaline phosphatase (ALP) < 306 U/L, gamma-glutamyltransferase (Gamma GT) < 40U/L, Bilirubin < 1mg/dl, T.Protein: 6-8.5 g/dl, Albumin: 3.4-4.8 g/dl, **Highly significant P ≤ 0.001, *Significant P ≤ 0.005

Table 3: Effect of HCV and diabetes on some serum parameters

Parameter	HCV only			HCV + diabetes			Diabetes only		
	Married (n = 2451)	Unmarried (n = 685)	p value	Married (n = 314)	Unmarried (n = 63)	p value	Married (n = 117)	Unmarried (n = 13)	p value
HbA1C	4.71 ± 0.56	4.45 ± 0.43	0.563	8.53 ± 0.84	8.04 ± 0.63	0.003*	7.51 ± 0.42	7.03 ± 0.61	0.004*
BGF	95.46 ± 1.52	93.69 ± 2.89	0.264	195.63 ± 3.21	179.12 ± 2.65	0.001**	180.39 ± 4.35	175.64 ± 3.21	0.003*
BGR	169.23 ± 2.13	165.15 ± 1.56	0.342	305.62 ± 5.72	283.21 ± 4.62	0.001**	279.65 ± 4.96	254.23 ± 5.34	0.004*

Normal values: Glycosylated hemoglobin (HbA1C): 4 - 6%, Blood glucose fasting (BGF): 70-110 mg/dl, Blood glucose random (BGR): 80-160 mg/dl, **Highly significant P ≤ 0.001, *Significant P ≤ 0.005

Table 4: Effect of HCV and diabetes on serum lipid parameters

Lipid parameter	HCV only			HCV + diabetes			Diabetes only		
	Married (n = 2451)	Unmarried (n = 685)	p value	Married (n = 314)	Unmarried (n = 63)	p value	Married (n = 117)	Unmarried (n = 13)	p value
Cholesterol	221.52 ± 1.87	215.24 ± 1.21	0.004*	276.35 ± 4.62	245.63 ± 6.23	0.001**	245.32 ± 6.58	220.98 ± 4.32	0.003*
Triglycerides	207.21 ± 2.45	198.31 ± 1.32	0.004*	307.58 ± 8.36	235.19 ± 4.37	0.001**	287.65 ± 5.36	215.63 ± 4.21	0.002*
HDL	34.06 ± 0.09	34.59 ± 0.08	0.546	33.12 ± 0.09	33.59 ± 0.12	0.521	35.42 ± 7.32	36.12 ± 4.16	0.421
LDL	146.02 ± 1.36	141.01 ± 1.21	0.423	181.72 ± 2.34	165.01 ± 3.45	0.004*	153.37 ± 3.12	141.74 ± 2.93	0.003*

Normal values: cholesterol < 200 mg/dl, triglycerides 80 - 150 mg/dl, high density lipoproteins (HDL) 35 - 55 mg/dl, low density lipoproteins (LDL) 10 - 140 mg/dl; **highly significant (p ≤ 0.001), *significant (p ≤ 0.005)

DISCUSSION

In this study, male Pakistanis were more infected with HCV, as they were more exposed to risk factors such as blood transfusion, accidents, surgical operations, and shaving of beards with unsterilized instruments at barber salons [3]. Other risk factors such as multiple sexual relationships is also an important risk factor for HCV infections. Here, close association between HCV and diabetes was found in married males. Generally, diabetic HCV-infected males were in the 4th and 5th decades of their lives. This age group is highly exposed to risk factors of HCV and other extra-hepatic manifestations. Mason et al [11] documented that age and HCV infection were independent predictors for diabetes mellitus. Fraser et al [12] also suggested that both HCV infection and increasing age were independent risk factors for diabetes. It was

reported that advance age is one of the most frequent risk factor for extra hepatic manifestation of HCV [13]. We observed higher prevalence of HCV parallels the higher incidence rates of diabetes. It was reported earlier that there are great chances of HCV patients to get diabetes [14].

It was observed that ALT and AST was relatively more raised in HCV and diabetic group than HCV alone group. Similarly, significantly elevated levels of ALT and AST in diabetic HCV patients than non-diabetic HCV patients were reported elsewhere [15]. Serum ALT was reported raised in 73.7% diabetic HCV patients than 18.5% diabetic patients without HCV infection [16]. Abnormal liver function tests have been reported by HCV diabetic patients [3]. 32% of HCV diabetic patients have shown raised level of ALT than 5% of diabetic patients without HCV

infection [17]. Married patients of HCV only and HCV+DM groups showed raised AST levels. ALT and AST levels increase up to 20 times but usually less than 5 times to upper limit of normal. Up to 40% of people with chronic HCV infection have normal ALT levels, even when tested on multiple occasions. ALT levels are usually higher than AST levels, but this may be reversed in patients with cirrhosis [18].

Alkaline phosphatase was high in both study groups. Generally, alkaline phosphatase and gamma glutamyl transpeptidase levels are usually not affected directly by HCV infections but there is a significant rise in gamma GT in liver cirrhosis. Total protein was higher while albumin was in both HCV alone and HCV diabetic groups and this is agreement with the results of Bacon *et al* [19]. It has been documented that HCV patients who develop diabetes show more severe liver disease based on biochemical markers and biopsy findings [20]. Mansour *et al* [15] reported that BGF, BGR and HbA1C were significantly higher in HCV diabetic patients than in non-diabetic HCV patients and these results are in agreement with our findings. Hence, HCV infection may adversely influence diabetes prognosis. A consequence of adverse prognosis, is poor renal functions [21]. HCV infection has a link with insulin resistance and diabetes. Abnormal glucose tolerance was 28 % in chronic liver disease patients [16].

Researchers have revealed that HCV eradication increases insulin sensitivity which reduces incidence of diabetes [18]. A possible mechanism has been suggested for HCV and diabetes association. HCV core protein induces changes in signaling in the pathway of insulin. This change affects β -cells and hence there is decrease in the secretion of insulin and this causes a rise in blood glucose level [8]. Lower total cholesterol and triglyceride have been reported to be higher in HCV patients than in diabetes patients without HCV infection [15] and this has been buttressed by the findings of the current study. HCV diabetic patients have been reported to show elevated triglyceride levels [22] and this is in agreement with the findings of our study but was different from that of Casqueiro *et al* [18]. This may be due to changes in the cellular pathways of triglyceride metabolism in HCV patients with diabetes. Disturbance in lipid profile indicates increased level of inflammation or altered caloric metabolism, resulting in difficulty in survival [23].

CONCLUSION

There is significant correlation between hepatitis C virus infection and diabetes. Both aggravate each other. Serum ALT and triglyceride are important parameters in this correlation. Further studies should throw more light that would help to improve the clinical management of diabetes patients with HCV infection.

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