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# Anti-Oxidant Activity and Metabolic Disturbances in Hepatitis C Patients with or Without Diabet Mellitus

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**Abstract:** The liver plays a crucial role in the regulation of carbohydrate metabolism. Its normal functioning is essential for the maintenance of blood glucose levels and of a continued supply to organs that require a glucose energy source. This central role for the liver in glucose homeostasis offers a clue to the pathogenesis of glucose intolerance in liver diseases not much is known. To test the hypothesis that enzymes conventionally associated with liver dysfunction (aspartate aminotransferase, alanine aminotransferase, creatinine phosphokinase, lactate dehydrogenase and alkaline phosphatase) may predict diabetes, we have studied these enzyme activity profile, kidney function main parameters such as creatinine, urea, uric acid levels, cholesterol and the role of magnesium, zinc, copper and iron along with anti-oxidant activity in patients with hepatitis C with or without diabetic mellitus. Higher alanine aminotransferase and aspartate aminotransferase were significantly elevated in hepatitis C patients with and without diabetes mellitus. Regarding metals content, magnesium decreased significantly in hepatitis patients with diabetes mellitus where as it remains unchanged in hepatitis patients. Zinc is decreased where as iron is increased significantly in both groups of the patients but copper remained unchanged in both groups. There is a significant decrease in anti-oxidation activity in hepatitis C patient either with or without diabetes mellitis. From these data, it is concluded that metabolic dysfunctions are mainly associated with hepatitis C than diabetes mellitis.

**Key words:** Hepatitis C virus · Metal content · Enzyme activity · Antioxidant activity
**Abbreviations:** ALP = alkaline phosphatase, ALT= alanine aminotransferase, AST= creatinine phosphokinase, CPK = lactate dehydrogenase, LDH = magnesium, Mg = zinc, Zn = copper, Cu = iron, Fe.

## INTRODUCTION

Chronic infection with the hepatitis C virus affects over 170 million individuals worldwide and 20% of patients develop cirrhosis after 20 years. The hepatitis C virus (HCV) is a linear, single-stranded RNA virus of the Flaviviridae family that was identified in 1989 and is recognized as the major causal agent of non-A, non-B hepatitis [1]. This virus can be transmitted by narcotics use, transfusion of blood products and exposure of medical personnel to infected patients. HCV inflicts most of its damage by latching onto molecules of iron and generating free-radical damage to liver cells. These free radicals can induce liver inflammation, cirrhosis and primary liver cancer via oxidative attacks on liver cells. Increased iron stores and hepatic iron content have been suggested to be important in fibrosis progression. Up to 70 percent of chronic carriers will go on to develop some other form of chronic liver disease, from mild liver enzyme abnormalities to cirrhosis and liver cancer. Evidence suggests that chronic HCV is associated with increased risk of development of diabetes mellitus (DM), irrespective of the presence of cirrhosis [2]. Several cross-sectional studies have found a higher prevalence of HCV antibodies in type 2 diabetic patients than expected in the general population [3-6]. In addition, all studies in which a control group of nondiabetic subjects had been included found a significantly higher prevalence of HCV antibodies in type 2 diabetic patients [6-8]. Ageing, obesity, family history of diabetes, African-American origin and HIV coinfection are recognized influencing factors associated with diabetes development among HCV infected patients [9-10].

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While there is no vaccine for HCV, the current optimal treatment is combination therapy with peginterferon alfa (an immune stimulant) and ribavirin (an inhibitor of viral replication). Insulin resistance in the setting of chronic HCV infection could be related etiologically to viral factors but is also often seen with concomitant nonalcoholic fatty liver disease, the hepatic manifestation of the metabolic syndrome. Insulin resistance decreases the likelihood of response to interferon-based therapies and may be an independent risk factor for the progression of HCV-related liver disease [11].

DM and advanced liver disease are associated with each other more frequently than expected by chance and such an association carries a significant risk of morbidity and mortality. A metabolic pathway leading to HCV resulting into DM is unknown and the role of enzymes associate with liver, kidney and heart are not studied. Though the role of iron is only studied in detail but no study has shown association of zinc, copper and magnesium in HCV patients with or without DM. In this study, we have investigated the association between hepatitis with diabetes to reveal largely unidentified mechanisms.

## MATERIALS AND METHODS

The study was case-control one, that conducted on patients with chronic hepatitis C who presented the department of Medicine, Liaquat University Hospital, Jamshoro, Pakistan with elevated ALT. Those patients who were found to have hepatitis C virus positive by PCR were included in this study. Anti-HCV was performed by AXSYM Systems (Abbott Lab. Chicago, IL) and HCV RNA was performed by Amplicor Version 1 (Roche Diagnostics, Switzerland). All patients either with DM or without underwent blood glucose determination and were categorized as diabetic if fasting plasma glucose levels were more than 126 mg/dl on more than one occasion, in absence of specialized diet or parenteral nutrition, which is in accordance with new WHO criteria [12].

The study included 50 patients with HCV (positive for serum anti-HCV using a third-generation enzyme immunoassay and for HCV RNA using a quantitative or a qualitative HCV RNA assay) and 50 patients with chronic hepatitis C having diabetes mellitus with elevated serum transaminase levels for at least 6 months. 50 age matched healthy subjects were also included for comparison.

The exclusion criteria were: prior antiviral treatment, established diabetes, concurrent hepatitis B and C virus infection, autoimmune hepatitis, primary biliary cirrhosis, sclerosing cholangitis, hemochromatosis, a1-antitrypsin deficiency. Patients with intake of any narcotic and family history of diabetes were excluded.

After an overnight fast, venous blood samples were collected from all participants in 10 ml tube and the samples were left for clotting. It was centrifuged at 5000 rpm for 15 min within 1 hour of collection and serum was stored at -80°C till analysis. Serum levels of triglycerides, total cholesterol, all enzymes, HDL cholesterol and glucose were measured using enzymatic methods. For total content of each metal, serum samples collected were deprotonized using sulphosalisylic acid and centrifuged at 5000rpm the supernant was separated for the analysis using Microlab 300 (Merck, Darmstad, Germany).

This study was approved by the local ethics committee and conducted in concordance with the Declaration of Helsinki. Patients were assigned into the following three groups: chronic hepatitis C group, chronic hepatitis C group with diabetes mellitus and healthy controls. Student's t test, Mann-Whitney U, SPSS-15 were used for comparisons between groups. A p value less than 0.05 was considered statistically significant. The clinical data are presented in Table 1.

## RESULTS

The Table 2 shows the serum levels of Urea, creatinine and uric acid in HCV patients with or without DM and healthy controls. Urea and creatinine levels are significantly decreased in HCV patients and remained unchanged in HCV patients with DM. Uric acid level is significantly increased in both patient groups showing its dependency on HCV and DM simultaneously.

Table 3 shows serum levels of Total, HDL- and LDL- Cholesterol and triglyceride in HCV patients. The results show that Cholesterol levels (total, HDL and LDL) are significantly decreased in HCV patients but remained unchanged in diabetic HCV patients. Triglyceride level decreased significantly in HCV patient where as it increased significantly in diabetic HCV patients.

Table 4 shows the enzyme activity in HCV patients. ALK is significantly increased in diabetic HCV patients but remained unchanged in HCV patients. ALT is significantly increased in both groups of HCV patients. CPK remained unchanged in both HCV patients. LDH is only increased in HCV patients but remained unchanged in diabetic HCV patients. The ratio between ALT to AST is significantly increased in HCV patients, however, it remained unchanged in HCV patients with DM.

Table 1: Clinical data on HCV patients with or without diabetes

| | Control | Hepatitis C | Diabetics with HCV |
|---|---|---|---|
| Age | 40.46±1.55 | 42.82 ±1.25 | 40.44±1.25 |
| Sex (M/F) | 35/15 | 37/13 | 34/16 |
| Albumin | 3.68±0.83 | 3.07±0.90*** | 3.76±0.065 |
| Protein | 8.40±1.23 | 6.74±0.082*** | 7.29±0.074** |
| Fasting Glucose | 81.56±1.47 | 76.56±1.43* | 192.44±20.17*** |
| Calcium | 8.74±0.09 | 8.37±0.111* | 8.69±0.082 |

* P < 0.05, **P < 0.01, *** P < 0.001

Table 2: Serum levels of Urea, creatinine and uric acid in diabetic and non-diabetic HCV patients: The kidney function parameters

| | Control | Hepatitis C | Diabetics with HCV |
|---|---|---|---|
| Urea | 28.84±0.89 | 22.06±0.832*** | 30.50±0.89 |
| Creatinine | 0.73±0.021 | 0.57±0.013*** | 0.75±0.024 |
| Uric acid | 3.68±0.069 | 5.02±0.169*** | 4.18±0.115** |

* P < 0.05, **P < 0.01, *** P < 0.001

Table 3: Serum levels of lipid of profile in cases Vs control

| | Control | Hepatitis C | Diabetics with HCV |
|---|---|---|---|
| Cholesterol | 172.44±3.03 | 124.24±1.77*** | 173.58±2.87 |
| HDL-Cholesterol | 30.42±0.57 | 27.44±0.385*** | 30.48±0.53 |
| LDL-Cholesterol | 112.34±2.98 | 95.16±1.81*** | 118.70±2.37 |
| Triglyceride | 96.00±4.33 | 84.12±1.51* | 123.44±6.33** |

* P < 0.05, **P < 0.01, *** P < 0.001

Table 4: Serum levels of liver enzymes among HCV cases Vs controls

| | Control | Hepatitis C | Diabetics with HCV |
|---|---|---|---|
| ALK | 115.64±3.98 | 120.34±5.84 | 282.16±15.44*** |
| ALT | 22.34±1.67 | 89.60±6.41*** | 29.44±2.03* |
| CPK | 106.56±6.52 | 101.88±3.99 | 103.04±4.70 |
| LDH | 331.06±9.13 | 395.48±11.16*** | 341.20±9.61 |
| AST | 18.86±0.71 | 45.80±1.95*** | 23.78±0.99** |
| ALT: AST ratio | 1.13 ±0.071 | 1.94±.11*** | 1.16±.091 |

*P < 0.05, **P < 0.01, *** P < 0.001

Table: 5 Serum levels of trace elements among patients Vs controls (serum metal levels)

| | Control | Hepatitis C | Diabetics with HCV |
|---|---|---|---|
| Magnesium | 19.76±0.59 | 20.94±0.822 | 17.90±0.526* |
| Iron | 2.93±0.137 | 3.22±0.067*** | 3.42±0.126*** |
| Zinc | 4.16±0.120 | 3.38±0.13** | 3.93±0.097** |
| Copper | 2.03±0.052 | 2.11±0.027 | 2.04±0.032 |
| Anti-oxidant activity | 1.52±0.05 | 0.43±0.05*** | 1.01±0.03*** |

* P < 0.05, P < 0.01, *** P < 0.001

Table: 5 shows serum metal levels in HCV patients. Magnesium is only significantly decreased in HCV patients with DM and remained unchanged in HCV patients. Iron is increased significantly in both groups of patients. Zinc is decreased significantly in both groups of HCV patients, however copper remained unchanged. Total anti-oxidant activity is significantly decreased in both groups of HCV patients.

## DISCUSSION

It is documented that hepatitis C virus (HCV) targets the liver and can induce diseases of many organs. Recently, much attention is drawn to metabolic disorders in HCV infection. First, hepatic steatosis and derangement in lipid metabolism have been found characteristic of HCV infection and later on, a correlation was noted between

HCV infection and diabetes mellitus (DM) as well as insulin resistance [13]. Generally, HCV is considered as a hepatotropic virus [14], however, it has also been identified in extrahepatic tissues, including kidney, lung, testis, peripheral blood mononuclear cells and also in the pancreas [15-18]. Laskus et al. [16] documented the presence of HCV-RNA in the pancreas acinar cells and in the epithelial cells of the pancreatic duct. Recently, Masini et al. [18] detected virus-like particles in pancreatic ß-cells from HCV-positive donors associated with morphological changes and a reduced in vitro glucose-stimulated insulin release.

Most of the kidney biochemical parameter such as urea and creatinine show decreasing tendency in HCV patients, but these parameters are unchanged in diabetic HCV patients, however, Uric acid shows increasing tendency in both groups (Table 2). Several factors, including metabolic profile, are predictive of response to standard antiviral therapy in HCV patients. In a recent retrospective study [19], the prognostic role of serum uric acid level =5.8 mg/dl was predictive of poor response to HCV treatment.

Recently, in a large scale community study [20], HCV viremia appears to be associated with lower serum cholesterol and triglyceride levels which implies that HCV itself might play a significant role on serum lipid profile of patients with chronic HCV infection. It was also shown in a single population that chronic HCV infection is associated with glucose intolerance and, despite that, a favorable lipid pattern, consisting of a reduction in total cholesterol, LDL cholesterol and triglycerides [21]. These results are similar to our study where decrease in serum cholesterol levels in HCV patients is observed, however, there is no change in serum cholesterol except significant increase of triglyceride in diabetic HCV patients. It is known that about 50% of insulin secreted by the pancreas is removed by first-pass extraction in the liver. Insulin promotes glycogen synthesis (glycogenesis) in the liver and inhibits its breakdown (glycogenolysis). It promotes protein, cholesterol and triglyceride synthesis and stimulates formation of very-low-density lipoprotein cholesterol. It also inhibits hepatic gluconeogenesis, stimulates glycolysis and inhibits ketogenesis. The liver is the primary target organ for glucagon action, where it promotes glycogenolysis, gluconeogenesis and ketogenesis [22].

Among the enzymes, the ALT is both sensitive and specific for liver disease of a hepatocellular injury type. Elevations in ALT levels should be interpreted as indicative of liver disease with only rare exceptions: severe rhabdomyolysis or systemic myopathies. This enzyme is increased significantly in both HCV patients; however the increase is 4 times in nondiabetic HCV patients. The AST enzyme is less sensitive and specific for liver disease but still should be employed as a screening test because the ALT to AST ratio can often be used to suggest the cause and/or extent of liver disease. ALT to AST ratios greater than 1 are typically found in patients with viral hepatitis, drug-induced liver disease, autoimmune disorders, etc, whereas ratios less than 1 are more often associated with alcohol-induced liver disease, ischemic forms of liver disease (passive congestion or under perfusion), biliary tract obstruction and certain disorders that tend to result in a predominantly mitochondrial form of cell injury such as fatty liver of pregnancy, tetracycline toxicity, Reye's syndrome, etc. The use of this ratio can also be helpful when assessing the severity of liver disease because once liver disease has progressed to cirrhosis (regardless of the underlying etiology) a previously elevated ALT to AST ratio often falls to values of 1 or less [23]. In our study indeed high ALT to AST ratio are observed in HCV patients where as no change was observed in diabetic HCV patients.

ALK increases only in diabetic HCV patients where as it remains unchanged in HCV patients. There is no change of CPK in both patient groups, however LDH is significantly increased in only HCV patients and has no relationship with DM.

Calcium ($Ca^{+2}$) is decreased in only HCV patients with diabetes, but remained unchanged in diabetic HCV patients (Table 1). $Ca^{+2}$ deficiency may be related to poor vitamin D status, poor nutrition or malabsorption; correcting the underlying abnormality may restore calcium balance. Magnesium ($Mg^{+2}$) deficiency may occur due to inadequate dietary intake, but develops most often in patients taking diuretics to treat fluid retention. Symptoms include muscle cramps, fatigue, weakness, nausea and vomiting. Our results showed significant decrease in $Mg^{+2}$ concentration in diabetic HCV patients, however, remained unchanged in HCV patients.

The liver is the primary storage organ for iron and it is well documented that patients with chronic hepatitis C frequently show serum and hepatic iron overload, but the mechanism is unknown. Recently identified hepcidin, exclusively synthesized in the liver, is thought to be a key regulator for iron homeostasis and is induced by infection and inflammation [24,25]. We have observed increase in iron in both groups of HCV patients and hence this may cause hepatic iron deposition which may be responsible for insulin resistance by interfering with the ability of

insulin to suppress hepatic glucose production [26]. Iron overload is also associated with hepatocellular carcinoma in patients with end-stage liver disease, suggesting a possible carcinogenic or cocarcinogenic role for iron in chronic liver disease [27, 28]. Increase in serum iron is believed to play vital role in oxidative stress and our study shows significance decrease in anti-oxidant activity in both HCV patient groups Further more, it is increasingly recognized that iron influences glucose metabolism, even in the absence of significant iron overload. In the general population, body Iron stores are positively associated with the development of glucose intolerance, type 2 diabetes and gestational diabetes. In fact, the initial and most common abnormality seen in iron overload conditions is liver insulin resistance [29]. There is some evidence that iron overload also affects skeletal muscle [30], the main effector of insulin action. Iron participates, through the Fenton reaction, in the formation of highly toxic free radicals, such as hydroxide and the superoxide anion, which are capable of inducing lipid peroxidation. For iron to act as a prooxidant agent, it must be in its free form. Iron can be released from ferritin by the action of reducing agents that convert Ferric ($Fe^{3+}$) into Ferrous ($Fe^{2+}$)[31]. Glycation of transferrin decreases its ability to bind ferrous iron [32] and, by increasing the pool of free iron, stimulates ferritin synthesis. Glycated holotransferrin is additionally known to facilitate the production of free oxygen radicals, such as hydroxide, that further amplify the oxidative effects of iron [33]. Elevations in serum transferrin-iron saturation and ferritin are common in patients with chronic HCV infection, especially if they have concomitant elevations in serum AST and ALT [34].

Among the other metals, copper shows no change in its level in both HCV patients with or without DM, where as zinc is significantly reduced in both groups of HCV patients. It was reported that [24] the incidence of DM in adults with HCV and HBV (25% and 22.5%, respectively) is four times higher than that in the general population. Patients with chronic hepatitis have impaired glucose metabolism with hyperinsulinemia and insulin resistance. This hyperinsulinemia has been shown to be due to decreased insulin catabolism rather than increased pancreatic insulin secretion. [35] Up to 60%-80% of patients with cirrhosis have glucose intolerance and about 20% eventually develop frank diabetes mellitus. [36,37]. The mechanisms through which HCV infection increases the risk of diabetes are not very clear, but considerable evidence suggests that the effects of viral proteins on cellular processes involved in hepatic lipid metabolisms, early defects in insulin signaling pathways, hepatic steatosis, insulin resistance and impaired insulin secretion might be associated with the development of diabetes [38-39]. A direct involvement of the virus in the development of insulin resistance has been proposed and â-cell dysfunction in HCV-positive patients has been observed in some cases. The insulin secretion functional defects of islets from HCV-positive donors might contribute to the development of diabetes in predisposed subjects. In conclusion, the present study proposes that HCV can infect human pancreatic ß-cells due to large iron level creating free radicals resulting into low anti-oxidant activity which is one of the risk factor in ß-cell dysfunction resulting into diabetes from HCV.

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