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BRIEF ARTICLE

Metabolic investigations in patients with hepatitis B and C

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Abstract

AIM: To investigate the similarities and dissimilarities in patients with hepatitis B and hepatitis C, clinically and metabolically.

METHODS: Fifty patients with hepatitis B virus and hepatitis C virus infection were included in this study, along with fifty healthy controls for comparison purposes. Intravenous blood (10 mL) samples from patients and healthy subjects were collected and made to clot before serum was separated and immediately levels of the enzymes, alkaline phosphatase (ALK), creatinine phosphokinase (CPK), lactate dehydrogenase (LDH), serum glutamate oxaloacetate transaminase (s-GOT) and serum glutamate pyruvate transaminase (s-GPT) were determined by a kit method. For total content of each metal the serum samples were analyzed using atomic absorption spectrophotometry. Levels of cholesterol, triglycerides, urea, creatinine and uric acid were determined using a kit method on Microlab 300.

RESULTS: Serum magnesium and copper levels remained unchanged, whereas the concentration of zinc decreased and iron increased significantly in both groups of patients. Total antioxidant activity was significantly decreased in both hepatitis B and C. Among the enzymes analyzed, ALK, s-GPT, LDH and s-GOT were all significantly increased in both patients with hepatitis B and C whereas CPK was significantly decreased in patients with hepatitis B and remained unchanged in patients with hepatitis C.

CONCLUSION: The information accumulated by this study will help provide a better understanding of involved metabolic processes in order to design appropriate therapeutic approaches for treating these patients, so they can recover and lead normal lives.

Key words: Hepatitis B virus; Hepatitis C virus; Antioxidant activity; Metal content; Enzyme activity

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INTRODUCTION

The liver is one of the most important organs in energy

metabolism. Most plasma apolipoproteins, endogenous lipids and lipoproteins are synthesized in the liver. This depends on the integrity of liver cellular function, which ensures homeostasis of lipid and lipoprotein metabolism. Hepatitis B virus (HBV) infection, a major world health problem, is hyper-endemic in South-East Asia and sub-Saharan Africa. Being a major cause of morbidity and mortality, prophylaxis using the highly efficacious hepatitis B vaccine is recommended for those at risk[1]. 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[2]. HCV is one of the leading causes of chronic liver disease worldwide, affecting 3% of the world's population. Diagnosis and treatment of HCV-related autoimmune features has become a clinical challenge in HCV-infected patients, in whom chronic liver disease associated with severe autoimmune features may contribute to a very poor prognosis[3,4].

Enzymes are biocatalysts and catalyze relevant bio-reactions. Metals such as magnesium (Mg), copper (Cu), zinc (Zn) and iron (Fe) are the cofactors of enzymes. Enzymes are released into the blood by injured tissue along with the metals and each affected tissue can be identified by evaluation of the variation in enzyme activity and metals in blood serum. Metals are present in bones, tissues and body fluids and are built into key ingredients in enzymes and hormones. They also assist in every aspect of life from production of hormones, vitamins and energy, digestion, neurotransmission and muscle contraction to regulation of pH, metabolism, cholesterol and blood sugar. Our physical wellbeing is more dependent upon the minerals we take into our system than upon calories or vitamins or carbohydrates and proteins.

In the present study, the estimation of serum enzyme activity of alkaline phosphatase (ALK), glutamate pyruvate transaminase, (SGPT or ALT), lactate dehydrogenase (LDH) and serum glutamic oxaloacetic transaminase (SGOT or AST) along with levels of metals (Cu, Fe, Mg, and Zn) are evaluated in patients with hepatitis B and C to study similarities and dissimilarities in these hepatitis groups, with the aim of defining biochemical mechanisms to help in selecting or designing chemotherapy suitable for these patients.

MATERIALS AND METHODS

Third-generation micro-ELISA assays were used for detection of hepatitis B surface antigen, antibody to hepatitis B core and surface antibody, secretory form of hepatitis B envelop antigen (HBeAg), antibody to secretory form of HBeAg, and ELISA for antibody to HCV. Clinical and laboratory features are helpful, but liver biopsy is essential for definitive diagnosis. Once the diagnosis was confirmed, 50 patients in each group of HBV and HCV were included in this study. Fifty healthy controls were included for comparison purposes. The clinical data regarding patient and healthy control groups are shown in Table 1.

Table 1 Clinical data of patients with HBV and HCV (mean ± SE)

	Controls	Hepatitis B	Hepatitis C
Age (yr)	40.5 ± 1.5	43.5 ± 1.8	42.8 ± 1.3
Sex (M/F)	35/15	33/17	36/14
Albumin (g/dL)	3.68 ± 0.083	3.20 ± 0.079[c]	3.07 ± 0.090[c]
Total protein (g/dL)	8.40 ± 1.23	6.84 ± 0.80[b]	6.74 ± 0.82[c]
Glucose (F) (mg/dL)	81.56 ± 1.47	77.94 ± 1.27[a]	76.56 ± 1.43[a]

[a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$ *vs* controls. HBV: Hepatitis B virus; HCV: Hepatitis C virus.

Table 2 Metabolic parameters measured in plasma of HBV/HCV patients and healthy controls (mean ± SE, mg/dL)

	Controls	Hepatitis B	Hepatitis C
Urea	28.84 ± 0.89	24.28 ± 0.779[c]	22.06 ± 0.832[c]
Creatinine	0.73 ± 0.021	0.59 ± 0.015[a]	0.57 ± 0.013[c]
Uric acid	3.68 ± 0.069	5.13 ± 0.22[b]	5.02 ± 0.169[c]
Cholesterol	172.44 ± 3.03	127.56 ± 1.70[c]	124.24 ± 1.77[c]
HDL	30.42 ± 0.57	27.96 ± 0.36[b]	27.44 ± 0.385[c]
LDL	112.34 ± 2.98	97.88 ± 1.87[c]	95.16 ± 1.81[c]
Triglycerides	96.00 ± 4.33	95.30 ± 3.76[a]	84.12 ± 1.51[b]
Mg	19.76 ± 0.59	20.49 ± 0.70	20.94 ± 0.82
Fe	2.93 ± 0.14	3.14 ± 0.05[b]	3.22 ± 0.07[b]
Zn	4.16 ± 0.12	3.26 ± 0.11[c]	3.38 ± 0.13[b]
Cu	2.03 ± 0.05	2.08 ± 0.02[a]	2.11 ± 0.03
Antioxidant activity	1.52 ± 0.05	0.37 ± 0.05[c]	0.43 ± 0.05[c]

[a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$ *vs* controls.

Intravenous blood (10 mL) samples from hepatitis B/C patients and healthy subjects were collected and made to clot before serum was separated by centrifuging at 5000 r/min for 20 min and immediately the enzymes ALK, creatinine phosphokinase (CPK), LDH, serum glutamate oxaloacetate transaminase (s-GOT) and serum glutamate pyruvate transaminase (s-GPT) were determined by a kit method. For total content of each metal the serum samples were analyzed using a Hitachi atomic absorption spectrophotometer (Tokyo, Japan). Cholesterol, triglycerides, urea, creatinine and uric acid were determined using a kit method on Microlab 300.

Statistical analysis

All values are expressed as mean ± SE. For comparison between the patients *vs* healthy controls, Student's *t* test, non-parametric Mann-Whitney test and SPSS 15 were used. A *P* value less than 0.05 was considered statistically significant.

RESULTS

Table 2 shows plasma levels of urea, creatinine and uric acid in B and C hepatitis patients and healthy controls. Among the biochemical parameters associated with kidney function, the levels of urea and creatinine were significantly decreased in both HBV and HCV patients,

Table 3 Enzyme activity in blood serum of patients with Hepatitis B/C and healthy controls

	Controls	Hepatitis B	Hepatitis C
ALK (IU/L)	115.64 ± 3.98	145.82 ± 8.47[b]	120.34 ± 5.84
ALT or s-GPT (mU/mL)	22.34 ± 1.67	59.24 ± 3.28[c]	89.60 ± 6.41[c]
CPK (IU/L)	106.56 ± 6.52	86.10 ± 3.34[a]	101.88 ± 3.99
LDH (U/L)	331.06 ± 9.13	353.72 ± 9.37[a]	395.48 ± 11.16[c]
AST or s-GOT (IU/L)	18.86 ± 0.71	40.88 ± 1.44[c]	45.80 ± 1.95[c]
ALT/AST ratio	1.13 ± 0.071	1.56 ± 0.083[c]	1.94 ± 0.11[c]

[a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$ *vs* controls.

however, uric acid was increased significantly in both patient groups. With regard to plasma cholesterol levels (total, HDL and LDL) and triglycerides in hepatitis groups (HBV and HCV) and control subjects; cholesterol level was decreased in both patient groups whereas triglyceride levels decreased significantly in HCV and remained unchanged in HBV group. Table 2 also shows the serum levels of Mg, Fe, Zn and Cu. There was no significant difference in Mg and Cu levels for both HBV and HCV groups. Serum levels of Fe increased significantly whereas Zn decreased significantly in both patient groups. Total antioxidant activity showed significant decreases in both groups of patients.

Table 3 shows activity of the enzymes ALK, ALT, AST, CPK and LDH. ALK was only increased significantly in HBV patients and remained unchanged in HCV patients. ALT increased significantly in both patient groups but markedly in HCV patients. CPK was decreased in HBV but remained unchanged in HCV patients. LDH increased significantly in both group of patients and markedly in HCV patients. AST increased in both groups of patients. The ratio between ALT and AST was increased significantly in both patient groups.

DISCUSSION

Hepatitis C infection is associated with diabetes mellitus and insulin resistance and it is suggested that metabolic syndrome is common in patients with hepatitis C. Microalbuminuria is common in patients with diabetes and metabolic syndrome[5].

A decade ago, various authors described the association of chronic HCV infection with a heterogeneous group of non-hepatic conditions, such as pulmonary fibrosis, cutaneous vasculitis, glomerulonephritis, Mooren's ulcer, porphyria cutanea tarda and lichen planus[6]. The drug regimens available for treating chronic HCV infection are monotherapy with interferon α (IFN-α) and combined therapy with IFN-α and ribavirin[7]. With progressive liver disease serum albumin levels fall, reflecting its decreased synthesis. We found a significant decrease in albumin levels in patients with both hepatitis B and C.

HCV viremia appears to be associated with lower serum cholesterol and triglyceride levels which implies that HCV itself might play a significant role in serum lipid profiles of patients with chronic HCV infection[8]. In our study, low levels of cholesterol (total, HDL and LDL) were found in both groups of hepatitis patients whereas significant decrease in triglycerides was only observed in 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. The liver is the primary target organ for glucagon action, where it promotes glycogenolysis, gluconeogenesis, and ketogenesis[9].

Among the metals, the central importance of Fe in the pathophysiology of disease is derived from the ease with which Fe is reversibly oxidized and reduced. This property, while essential for its metabolic functions, makes Fe potentially hazardous because of its ability to participate in the generation of powerful oxidant species such as the hydroxyl radical[8]. It is now well established that oxidants can cause the release of catalytic Fe[7]; thus, a vicious cycle is initiated that leads to the formation of more reactive oxygen species.

In our study, the serum level of Fe was elevated significantly in both HBV and HCV patients. These data on the relevance of Fe as a prognostic factor prompted us to ascertain whether HCV- and HBV-related liver damage is mediated by Fe accumulation. We have also observed decreased total oxidative activity in both patient groups and hence increases in Fe generate reactive oxygen species which may exceed the capacity of the antioxidant system and perpetuate oxidative stress to cells. Oxidative stress with the attendant low-grade inflammation is implicated in a number of pathological conditions, including aging, atherosclerosis, and diabetes[6]. In a study of patients with unexplained hepatic Fe overload, most were found to be insulin-resistant, which suggests a common etiologic link between hepatic Fe, hepatic dysfunction, and insulin resistance[10]. Insulin deficiency due to iron deposition in the interstitial pancreatic cells, with resultant excess collagen deposition and defective microcirculation[10] and insulin resistance[11], are the likely mechanisms for type 2 diabetes. It has been shown that treatment with intravenous or oral chelation improves glucose tolerance in up to one-third of these patients, suggesting a causal role for Fe[12,13]. In one study it was shown that Fe overload may be responsible for insulin resistance, or *vice versa*[1]. Other metals such as Cu and Zn are essential trace elements for several metabolic processes. Regarding Zn, patients with chronic hepatic encephalopathy have been shown to have low serum Zn levels. Moreover, in a controlled study, significant improvement was seen in those patients on oral Zn supplementation[14]. In our patients with HBV and HCV, both groups showed significantly decreased Zn levels. Various studies have shown both Cu and Mg levels in serum remained unchanged in HBV and HCV patients, though content of Zn and selenium in plasma and erythrocytes were significantly lower in hepatitis C

and B patients[15,16]. Our results show a similar tendency in HBV and HCV patients.

Disturbances in the antioxidant system could play a role in the pathogenesis of chronic liver disease. During the course of chronic liver disease we may observe slight irregularities in iron status relating to both the serum and store pool of this element. The most significant disturbances are seen in patients with alcoholic cirrhosis of the liver[17]. These findings suggest that disturbances in antioxidant parameters in the blood of patients with chronic liver disease may be the cause of the peroxidative damage of cells. In both HBV and HCV, antioxidant activity is significantly decreased. The release of oxidative free radicals, deficiency in antioxidant enzymes and the expression of bcl-2 protein might play a role in the pathogenesis of viral hepatitis. The ability to measure bcl-2 protein in the serum could be useful as a prognostic marker in cancer patients[18].

The availability of serum blood chemistry tests for screening both symptomatic and asymptomatic patients has resulted in a marked increase in the number of abnormal liver chemistry tests that must be interpreted by physicians. Usually the first step in the evaluation of a patient with elevated liver enzymes is to repeat the test to confirm the result. There is a hypothesis that enzymes conventionally associated with liver dysfunction, AST, ALT and γ-glutamyltransferase (GGT), may predict diabetes. However, the role of enzymes such as ALK, s-GPT, LDH, CPK and s-GOT or AST is controversial in many studies[15]. In our study, significantly increased levels of ALK, SGPT (or ALT), LDH and SGOT (or AST) were found, whereas, CPK activity decreased in both groups of hepatitis patients. A recent study showed that one-third of the hospitalized patients with liver cirrhosis are infected with HBV or HCV infection, with raised ALT/AST and ALK being more common with superadded viral infection[19]. Another study showed that there were also significant correlations between Fe status, as indicated by transferrin saturation or serum ferritin levels, and SGOT, SGPT, and GGT levels. Moreover, abnormal liver function, as represented by elevated levels of SGOT, SGPT, GGT and serum ALK, was observed more frequently in patients with Fe overload than in patients with a lower degree of Fe burden[16].

AST is present in cytosolic and mitochondrial isoenzymes and is found in the liver, cardiac muscle, skeletal muscle, kidneys, brain, pancreas, lungs, leucocytes, and red cells[18]. ALT, a cytosolic enzyme, is found in its highest concentrations in the liver and is more specific to the liver[20,21]. The most common causes of elevated AST levels are chronic hepatitis B and C, autoimmune hepatitis, non-alcoholic steatohepatitis, hemochromatosis, Wilson's disease, celiac sprue, muscle damage and myocardial infarction[21,22].

Among the liver enzymes, 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. Our study showed that this enzyme increased significantly in both HCV and HBV patients. 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) an elevated ALT to AST ratio often falls to values of 1 or less[23]. Indeed, in our study high ALT to AST ratios were observed in HCV and HBV patients.

From the results of our study, we conclude that parameters such as enzyme activity and the levels of metals such as Fe, Zn, Mg and Cu, along with total antioxidant activity, in patients with hepatitis B and C can be used to measure the status of the disease. We recommend further research in the area of antioxidant therapy.

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COMMENTS

Background

Patients with hepatitis B and C are at risk for poor nutritional status, poor response to bacterial and viral infections, stomach ulcers, kidney disorders, gallstones, liver cancer, and diabetes mellitus. All such events lead to life threatening complications and act as poor prognostic factors.

Research frontiers

Disturbances in liver biochemistry, serum metal levels and dyslipidemia are usually observed in patients with chronic viral hepatitis. There has been no study to differentiate between the two hepatitis viruses in terms of metabolic derangement as compared with healthy subjects. This study is designed with the objective of investigating the similarities and dissimilarities in patients with hepatitis B and hepatitis C, clinically and metabolically.

Innovations and breakthroughs

It was identified that serum magnesium (Mg) and copper (Cu) levels remained unchanged, whereas the concentration of zinc (Zn) decreased and iron (Fe) increased significantly in both groups of patients. Total antioxidant activity was significantly decreased in both hepatitis B and C. Among the enzymes analyzed, ALK, s-GPT, LDH and s-GOT were all significantly increased in both patients with hepatitis B and C, whereas CPK was significantly decreased in patients with hepatitis B and remained unchanged in patients with hepatitis C.

Applications

By understanding the liver enzyme profile, serum biochemistry and metal levels in relation to hepatitis B and C virus, one can assess the exact health status of such patients and plan the specific and necessary parameters to control such life-threatening biochemical modifications. For the development of future therapeutic strategies, this study may need to be continued in a more advanced and extensive manner at different health care centers.

Peer review

This study shows the comparison of serum metal content of healthy individuals to HCV or HBV infected persons. As most of the parameters have already been known for HBV and HCV infected individuals, peer reviewer think it should be accepted as a brief report after the consolidation of the discussion part.

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