Study on Insulin-Leptin Axis in Hepatitis C-Virus Infected Patients Using Radiometric Techniques

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ABSTRACT

Hepatitis C-virus (HCV) infection is a global health problem because of its chronicity rate, and its prevalence in Egypt is the highest worldwide. Leptin is a protein hormone produced by adipocytes having a major function on regulation of energy expenditure, food intake and weight reduction. Insulin-Leptin axis might be related to the increased energy expenditure observed in some diseases. The present study aimed at investigating the variations which may occur in some biochemical parameters with the study of insulin-leptin axis in patients infected with hepatitis C-virus. The study executed on 15 male patients suffering from HCV infection and 15 healthy volunteers in the same range. For all subjects, liver enzymes (ALT & AST), lipid profile (cholesterol & triglycerides) and estimation of insulin and leptin by RIA were done. The results revealed highly significant increase in serum ALT and AST activities, triglycerides, and insulin level in HCV-infected patients compared to their corresponding results in the controls. On the other hand, there was non-significant change in serum cholesterol concentration and leptin level in the patients compared to controls. It could be concluded that the significant increase in insulin level in HCV-infected patients make them more prone to insulin resistance and diabetes.

Keywords: HCV/ Insulin/ Leptin.

INTRODUCTION

Hepatitis is a general term meaning inflammation of the liver and can be caused by several mechanisms including infectious agents. Viral hepatitis can be caused by a variety of different viruses such as hepatitis A, B, C, D, and E. Hepatitis C virus (HCV) infection is rated by the world health organization (WHO) and international consensus conferences as a global health problem, based on its prevalence, high rate of chronicity, rate of severe complications as cirrhosis and hepatocellular carcinoma (HCC), as well as the high costs of antiviral therapy and liver transplantation (1, 2). HCV is considered the most common etiology of chronic liver disease (CLD) in Egypt, where the prevalence of antibodies to HCV (anti-HCV) is approximately 10-fold greater than in the United State and Europe (3). HCV is spread primarily by direct contact with the contaminated blood or plasma derivatives. Other modes of transmission such as social, cultural, and behavioral practices using percutaneous procedures (e.g. ear and body piercing, circumcision, tattooing) can occur if inadequately sterilized equipment is used. HCV is not spread by sneezing, hugging, coughing, food or water sharing eating utensils or causal contact (4).

Insulin is a polypeptide hormone, with a molecular weight of about 6 kDa. It is synthesized in significant quantities only in pancreatic β-cells (5). Insulin is an important regulator of energy homeostasis. It stimulates glucose, free fatty acid and amino acid uptake by tissues, and tissue anabolism (6). Leptin is a protein hormone produced by adipocytes (7). It act basically on the central hypothalamic leading to reduction of food intake and augmentation of energy expenditure and thus to
reduction of body weight (8). Insulin-leptin axis might be related to the increased energy expenditure observed in some diseases. Insulin is a potent stimulator of increased leptin expression in human adipose tissue. On the other hand, leptin inhibits insulin secretion via activation of ATP sensitive K channels (9). The aim of the present study is to investigate the serum levels of some biochemical parameters with the study of insulin-leptin axis in HCV-infected patients.

**SUBJECTS AND METHODS**

This study was executed on 15 HCV-infected males with age range from 45-55 years and 15 healthy volunteers with matched age. The patients were selected from the Medical Center of Integrated Sugar Company, El-Hawamdia, Giza, Egypt. Any patients with diabetes mellitus or congestive heart failure are excluded from the present study. None of the patients were receiving antiviral therapy. Venous blood samples were collected from all subjects and placed in plain tubes. Serum samples were separated by cooling centrifugation (HENZ Jametzki, Germany, 3000 rpm) and preserved at -20°C till analysis.

**BIOCHEMICAL AND STATISTICAL ANALYSIS**

Serum ALT and AST activities were measured according to the kinetic methods of Sherwin (10), Cholesterol by the method of Ellfson and caraway (11), while triglycerides by the method of Mgowan et al. (12). Using radioimmunoassay (RIA), insulin was determined according to Bates (13) and leptin by Zhang et al. (7).

Data were analyzed using computer programs SPSS (Statistical Package for the Social Science; SPSS Inc., Chicago, IL, USA) version 15.0 for Microsoft Windows. The statistical significance of the mean values between groups was performed using student’s t-test. The results were expressed as Means±SD and values of P≤ 0.05 were considered statistically significant.

**RESULTS**

Table 1 shows a highly significant increase in the mean value of both serum ALT and AST activities in the HCV-infected patients compared to their levels in the controls. Table 2 shows no difference in the mean value of serum cholesterol concentration in the HCV-infected patients compared to its level in the controls. A highly significant increase in the mean value of serum triglycerides concentration is also evident.

Table 3 shows highly significant increase in the mean value of serum insulin level and a non-significant difference in the mean value of serum leptin level in the patients compared to their levels in the controls. There were positive correlations between insulin and leptin (R= 0.438, P= 0.004, Fig. 1), insulin and ALT (R= 0.198, P= 0.046, Fig. 2), and insulin and AST (R= 0.234, P= 0.034, Fig. 3).

**Table 1: Liver enzymes (ALT & AST) activities in controls and HCV-infected patients**

<table>
<thead>
<tr>
<th></th>
<th>Control Mean±SD</th>
<th>HCV Mean±SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ALT (IU/L)</td>
<td>27.27±4.74</td>
<td>57.87±14.62</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>AST (IU/L)</td>
<td>23.73±4.10</td>
<td>69.87±17.10</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

- Data are expressed as Mean ± SD
- P< 0.001 (highly significant)
Table 2: Serum cholesterol and triglycerides concentrations in controls and HCV-infected patients

<table>
<thead>
<tr>
<th></th>
<th>Control Mean±SD</th>
<th>HCV Mean±SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum cholesterol (mg/dl)</td>
<td>159.33±18.89</td>
<td>171.53±28.01</td>
<td>NS</td>
</tr>
<tr>
<td>Serum triglycerides (mg/dl)</td>
<td>82.20±19.23</td>
<td>135.60±18.79</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

- Data are expressed as Mean ± SD
- P< 0.001 (highly significant)
- P> 0.05 (not-significant, NS)

Table 3: Serum insulin and leptin levels in controls and HCV-infected patients

<table>
<thead>
<tr>
<th></th>
<th>Control Mean±SD</th>
<th>HCV Mean±SD</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum insulin (g/dl)</td>
<td>16.18±3.64</td>
<td>32.13±6.18</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Serum leptin (g/dl)</td>
<td>6.50±1.90</td>
<td>7.27±2.43</td>
<td>NS</td>
</tr>
</tbody>
</table>

- Data are expressed as Mean ± SD
- P< 0.001 (highly significant)
- P> 0.05 (not-significant, NS)

Figure (1): Correlation between serum insulin and leptin in HCV-infected patients.
Figure 2: Correlation between serum insulin and ALT in HCV-infected patients.

Fig. 3: Correlation between serum insulin and AST in HCV-infected patients.
DISCUSSION

Liver enzymes are proteins that accelerate chemical reactions in the liver. Blood liver function tests are used to evaluate various liver functions (14). The most commonly used indicators of liver damage are the alanine aminotransferase (ALT) and aspartate aminotransferase (AST) enzymes measured in blood serum. These enzymes are normally found in liver cells that leak out of these cells and make their way to the peripheral blood when liver cells are injured (15). Liver transaminases (AST/ALT) are biomarkers of liver injury in a patient with some degree of intact liver function (16). In the present study, very highly significant increase in the mean value of serum liver enzymes (ALT, AST) were found in HCV-infected patients compared to their corresponding levels in the controls. These results were agreement with the results of Tungtrongchitr et al. (17) who reported that in the chronic liver disease, the enzymes activity levels were too high comparing with that of the controls. The elevations in both AST and ALT indicate liver cell damage which may be attributed to HCV infection; similar findings were reported by Mark and Ira (18).

Lipids are essential component of biological membranes, free molecules and metabolic regulators that control cellular function and homeostasis. Liver plays a vital role in lipid metabolism. It contributes in both exogenous and endogenous cycles of lipid metabolism and transport of lipids through plasma (19). In the present study, there was a highly significant increase in the mean value of serum triglycerides concentration and a non-significant increase in the mean value of serum cholesterol concentration in the HCV-infected patients compared to their levels in the controls. These results were in agreement with the results of Ben-Ari et al. (20) and Piche et al. (21), but disagreed with those of Mahboob et al. (22) and Nashaat (23) who reported that in chronic liver disease, there was a decrease in both cholesterol and triglycerides due to decreased biosynthetistic capacity of the liver.

Insulin plays an important role in carbohydrate, lipid, and protein metabolism. The hepatitis C virus (HCV) infection has been shown to have direct or indirect effect on glucose metabolism, leading to insulin resistance (IR) and, in predisposed individuals, type-2-diabetes. In the present study, insulin showed highly significant increase in HCV-infected patients. This was in harmony with several studies that reported direct interactions between HCV products and hepatocyte insulin signaling pathway (24, 25, 26). Bakir et al. (27) found that untreated chronic HCV patients demonstrated significant changes in HbA1c, and highly significant changes in random blood sugars, fasting insulin level, and homeostasis model assessment of insulin resistance (HOMA-IR) after 6 months of antiviral therapy. The latter investigators concluded that chronic hepatitis C eradication is associated with improved insulin sensitivity and they recommended close and long term monitoring of non-responder chronic HCV patients liable to increased risk of developing diabetes. Sink (28) reported that IR in patients with chronic hepatitis C, as measured by HOMA-IR, is highest in non-diabetic patients with chronic hepatitis C, but especially in diabetic ones, this can be considered as an indicator for the predisposition to diabetes in HCV infection.

Leptin is important in the regulation of appetite, food intake and energy expenditure, sexual maturation and fertility, haematopoesis and activity of the hypothalamic-pituitary-gonadal axis (29). The present study showed that there was non-significant difference in the mean value of serum leptin level in HCV-infected patient compared to its level in the controls. This result was in accordance with the results reported by Ben-Ari et al. (20), Comlekci et al. (30), and Tungtrongchitr et al. (17) indicating that, there was no significant change in the circulating leptin levels in HCV-infected patients. On the other hand, this result disagreed with the result of Testa et al. (31) who noted significantly lower absolute serum leptin levels in patients with chronic viral hepatitis, due to hepatitis C virus (HCV) and hepatitis B virus (HBV), compared to serum leptin levels in the controls. These investigators also concluded that, in patients with chronic viral liver disease, serum leptin levels tend to increase as liver function worsens. The tendency of circulating leptin concentration to increase may be interpreted as an early signal of the loss of the ability to down-regulate energy expenditure in response to anorexia.
and/or to alterations in substrate utilization as chronic viral hepatitis progresses toward liver cirrhosis. The precise mechanisms, by which chronic HCV infection causes elevated serum leptin levels, are not yet completely understood. Factors such as inflammation, abnormal fat metabolism, and hepatic steatosis in patients with chronic HCV infection, might be involved. Also, Piche et al. reported that in chronic HCV-infected patients, the levels of leptin increased according to the progression of the stage of fibrosis, and the severity of liver fibrosis was associated with high leptin levels in chronic hepatitis C. In contrast previously, Greco et al. reported that the more severe the liver damage, the lower the leptin levels. They interpreted this result on the light of the finding that in post-hepatitis cirrhosis, circulating levels of leptin closely reflect the degree of malnutrition and fat mass reduction, which is strictly related to the degree of liver insufficiency. Although two years later, Ben-Ari et al. found no correlation between serum leptin levels and the severity of liver disease.

It can be concluded that:

- The significant increase in insulin level in HCV patients shows that these patients are more prone to insulin resistance and to diabetes. Although there was no significant difference in leptin level between patients and controls yet, the insulin resistance and the increased lipid profile point to the importance of weight monitoring and dietary control in our cases.
- Weight reduction, hypocholesterolemic agents, and antiviral therapy may be helpful in decreased incidence of diabetes and hypertension in HCV patients.
- Deterioration of liver functions and the significant increase in liver enzymes in HCV patients is a life threatening condition, and it suggests an intimate relation between the severity of hepatitis as an inflammatory process and the development of insulin resistance as well as increased leptin and lipid profile.

Acknowledgement:

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REFERENCES

(2) D. Lavanchy; Liver Int. 29 (1): 74-81, (2009).