Study of serum ghrelin changes and its correlation with malnutrition in liver cirrhosis in Egypt

Ahmed Elbadri, Serag Esmat, Nihal Abosaif, Ahmed Morsi, Olfat Shaker

Internal Medicine Department, Cairo University, Cairo, Egypt
Biochemistry Department, Cairo University, Cairo, Egypt
National Nutrition Institute, Cairo, Egypt

Available online 27 August 2011

Summary

Egypt has the highest prevalence of hepatitis C virus (HCV) all over the world, with an estimated 8–10 million among a population of 68 million having been exposed to the virus and 5–7 million active infections (Frank et al., 2000). It is considered the most common aetiology of chronic liver disease (CLD) in Egypt, where prevalence of antibodies to HCV (anti-HCV) is 10-fold greater than in the United States and Europe (Goldstone et al., 2002; Strickland et al., 2002).

We have studied the role of plasma ghrelin, an orexigenic hormone that was found to correlate with malnourishment in CLD depending on Child classification. Sixty patients were divided in three groups according to Child classification and were compared to normal healthy controls (20 subjects). There was a highly significant correlation of plasma ghrelin and body mass index (BMI), mid arm circumference (MAC), waist circumference (WC) and triceps skin fold thickness (TSF). Also plasma ghrelin was specific and sensitive by the ROC curve analysis to BMI, which would indicate a new marker for malnourishment and possibility of a novel therapeutic approach. © 2011 Elsevier Masson SAS. All rights reserved.

Introduction

Egypt has possibly the highest hepatitis C virus (HCV) prevalence in the world; 10%–20% of the general population is infected and HCV is the leading cause of hepatocellular carcinoma (HCC) and CLD in the country [1]. Prevalence of antibodies to HCV (anti-HCV) is 10-fold greater than in the United States and Europe [2,3]. The nutritional and metabolic consequences of cirrhosis have attracted considerable interest over the past decade because malnutrition and hypermetabolism are commonly found in cirrhotic patients. In addition, malnutrition is a well-established risk factor influencing survival in patients with cirrhosis and can modify the prognosis [4].

Malnutrition in liver cirrhosis is induced by several mechanisms, such as anorexia, disturbances in absorption and digestion of nutritional substances in the gastrointestinal tract, and impaired hepatic synthesis of energy substrates. These abnormalities gradually induce anthropometric changes and lead to hypoalbuminaemia in patients with liver cirrhosis and hence worsen the prognosis of patients with liver cirrhosis [5].
The appetite-modulating hormone ghrelin (28-amino-acid peptide produced by the oxyntic cells of the stomach) could be involved in the pathogenesis of anorexia in cirrhotic patients [6]. It stimulates appetite and food intake and plays a role in meal initiation because of its potential orexigenic effect [7–9]. On the other hand, it plays a role in insulin resistance, the key pathophysiologic abnormality in patients with non-alcoholic steatohepatitis [10].

In this study, we have evaluated serum ghrelin levels in Egyptian patients with liver cirrhosis due to HCV according to their Child-Pugh classification as previous studies showed inconsistent results [11]. Also, the impact of HCV itself on serum ghrelin has not been clarified. We aim to acquire a new marker of malnourishment in patients with CLD that can lead to a new therapeutic target.

Subjects and methods

Subjects

Sixty patients with confirmed HCV antigen positive by ELISA (HCV Ag positive) were recruited from Cairo University Kasr Alaini teaching hospital, internal medicine department and were divided into three groups according to Child-Pugh classification [12,13]; each group included 20 patients. Group 1 was considered the control group (20 subjects) and was chosen from normal subjects who attended the hospital during the study period. We excluded patients with concomitant acute complications, such as gastrointestinal haemorrhage, uncontrolled hepatic encephalopathy. Also patients with sepsis, chronic kidney disease, uncontrolled diabetes or hypertension were not included.

All subjects of this study were formally or legally consented according to the ethical committee of Cairo University. They were then fully examined and anthropometric parameters were measured which included their weight, height and body mass index (BMI) was calculated as the body weight in kilograms (kg) divided by the square of the height in meter (m²). Triceps skin fold thickness (TSF) and mid arm circumference (MAC) were used as indexes of body fat and muscle protein compartment, respectively.

TSF was measured by the same observer with a Holtain caliper at the middle point between the acromion and the olecranon of the nondominant arm [14]. MAC was measured with a tape at the same site of TSF.

Methods

Complete blood picture, liver function tests including AST, ALT, total bilirubin and direct bilirubin, serum albumin, prothrombin time (PT) and prothrombin concentration (PC), viral markers (HBS Ag, HCV antibody and HCV/PCR) were checked in all patients. All patients of the study except for the control group were HCV antibody positive. Abdominal ultrasound was performed to all patients by the same operator to measure the liver size, rule out any liver masses and assess the degree of ascites.

Specimen collection for fasting serum ghrelin was taken from venous blood samples of all patients and control group following a 12-hour overnight fasting to abolish the effect of food on ghrelin. The samples were transferred to the main lab and the plasma was separated and stored at a temperature of -20 c till assayed.

Quantisation of serum ghrelin

Serum ghrelin level was measured by using The RayBio® ghrelin enzyme immunoassay (EIA) Kit. It is an in vitro quantitative assay for detecting ghrelin peptide based on the principle of competitive enzyme immunoassay. The assay was done on a microplate, which is precoated with anti-rabbit secondary antibody. After a blocking step and incubation of the plate with anti-ghrelin antibody, both biotinylated ghrelin peptide and peptide standard or targeted peptide in samples interacts competitively with the ghrelin antibody. Uncompeted (bound) biotinylated ghrelin peptide then interacts with streptavidinhorseradish peroxidase (SA-HRP) which catalyzes a colour development reaction. The intensity of colorimetric signal is directly proportional to the amount of biotinylated peptide-SA-HRP complex and inversely proportional to the amount of ghrelin peptide in the standard or samples. This is due to the competitive binding to ghrelin antibody between biotinylated ghrelin peptide and peptides in the standard or samples. A standard curve of known concentration of ghrelin peptide was established and the concentration of ghrelin peptide in the samples was calculated accordingly.

Statistical analysis

All the statistical analyses were performed with the statistical package for the social sciences (SPSS version 19). The results of quantitative data are expressed as the mean and standard deviation (mean±SD), the results of qualitative data are expressed as numbers. Comparisons between all groups were performed by one-way Anova (analysis of variances) for multiple comparisons within and in between groups of the study. Further subanalysis was performed by Sheffe method. The relationships between plasma ghrelin levels and anthropometric and metabolic variables were examined by simple linear regression and Spearman’s correlation analyses. A linear regression analysis was used to find out the effect of different parameters on serum ghrelin level. Statistical significance was considered when P less than 0.01.

Results

Demographic, anthropometric and clinical data of all groups

Comparison of the control group (group 1) with cirrhotic patients groups including different Child Pugh classes (groups 2, 3 and 4) regarding clinical and demographic characteristics showed that there was a significant difference in the body mass index (BMI) (27.895±2.504, 24.575±3.339, 21.105±1.976, 19.69±1.234, P<0.001 respectively) among and in between groups apart from group 3 and 4 there was no significant difference. The same significance applied for the waist circumference (WC) (93.05±7.119, 98.475±5.837, 87.35±5.27,
Table 1 Characteristics of all groups of the study.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control group</th>
<th>Child A</th>
<th>Child B</th>
<th>Child C</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group 1</td>
<td>Group 2</td>
<td>Group 3</td>
<td>Group 4</td>
<td></td>
</tr>
<tr>
<td>Demographic criteria</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Age (year)</td>
<td>53.05 ± 6.51</td>
<td>52.55 ± 6.28</td>
<td>54.30 ± 8.52</td>
<td>54.75 ± 6.81</td>
<td>0.739a</td>
</tr>
<tr>
<td>Gender (males/females)</td>
<td>10/10</td>
<td>8/12</td>
<td>8/12</td>
<td>11/9</td>
<td>0.715f</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.89 ± 2.51</td>
<td>24.58 ± 3.339</td>
<td>21.10 ± 1.98</td>
<td>19.69 ± 1.234</td>
<td>&lt; 0.001a, 0.329e</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>93.05 ± 7.12</td>
<td>98.48 ± 5.837</td>
<td>87.35 ± 5.27</td>
<td>74.45 ± 4.674</td>
<td>&lt; 0.001a, 0.044c, 0.03c</td>
</tr>
<tr>
<td>Mid-arm circumference (cm)</td>
<td>26.5 ± 3.16</td>
<td>30.4 ± 3.515</td>
<td>24.05 ± 1.93</td>
<td>20.30 ± 3.10</td>
<td>&lt; 0.001a, 0.04c</td>
</tr>
<tr>
<td>Triceps skin fold thickness (mm)</td>
<td>19.35 ± 1.75</td>
<td>19.35 ± 2.60</td>
<td>16.90 ± 1.65</td>
<td>15.05 ± 2.156</td>
<td>&lt; 0.001a, 1.00b, 0.007c, 0.057d</td>
</tr>
<tr>
<td>Liver function tests</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>INR</td>
<td>0.91 ± 0.12</td>
<td>1.17 ± 0.11</td>
<td>2.045 ± 0.264</td>
<td>2.06 ± 0.21</td>
<td>&lt; 0.001a, 0.994e</td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td>0.41 ± 0.194</td>
<td>1.02 ± 0.17</td>
<td>2.58 ± 0.396</td>
<td>3.16 ± 0.47</td>
<td>&lt; 0.001a, 1.000b, 0.0088</td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>4.80 ± 0.44</td>
<td>4.255 ± 0.61</td>
<td>3.075 ± 0.253</td>
<td>2.77 ± 0.21</td>
<td>&lt; 0.001a, 0.151e</td>
</tr>
<tr>
<td>ALT (IU/l)</td>
<td>16.75 ± 2.83</td>
<td>32.68 ± 12.27</td>
<td>60.25 ± 8.039</td>
<td>63.10 ± 10.56</td>
<td>&lt; 0.001a, 0.327</td>
</tr>
<tr>
<td>AST (IU/l)</td>
<td>17.85 ± 3.422</td>
<td>34.74 ± 1.45</td>
<td>67.45 ± 10.95</td>
<td>68.30 ± 11.46</td>
<td>&lt; 0.001a, 0.038</td>
</tr>
<tr>
<td>Ghrelin (pg/ml)</td>
<td>3.01 ± 0.32</td>
<td>3.725 ± 0.401</td>
<td>6.595 ± 2.261</td>
<td>7.15 ± 2.02</td>
<td>&lt; 0.001a, 0.559b, 0.737</td>
</tr>
<tr>
<td>Clinical criteria</td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Ascites (total)</td>
<td>0/20</td>
<td>0/20</td>
<td>16/20 (80%)</td>
<td>20/20 (100%)</td>
<td>&lt; 0.001f</td>
</tr>
<tr>
<td>Controlled medically</td>
<td>0</td>
<td>0</td>
<td>9/20 (56.3%)</td>
<td>7/20 (43.8%)</td>
<td>&lt; 0.001f</td>
</tr>
<tr>
<td>Encephalopathy</td>
<td>0/20</td>
<td>0/20</td>
<td>3/20 (15%)</td>
<td>15/20 (100%)</td>
<td>&lt; 0.001f</td>
</tr>
<tr>
<td>Controlled medically</td>
<td>8 (34.8%)</td>
<td>15 (65.2%)</td>
<td></td>
<td></td>
<td>&lt; 0.001f</td>
</tr>
</tbody>
</table>

Data are presented as mean ± SD except for the clinical data that are presented as numbers and percentages. Waist circumference (94–102 cm for males, 80–88 cm for females), mid-arm circumference (20–26 cm for males and 18–24 cm for females), triceps skin fold thickness (12–18 mm for males and 12–15 mm for females). P Value is significant if less than 0.01. Albumin level (3.5–5.2 g/dl), bilirubin (0.1–1.2 mg/dl), INR (0.9–1.3), AST (1–37 U/L), ALT (1–40 U/L).

Table 2 Correlation between the fasting serum ghrelin level and different parameters of the study.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ghrelin</th>
<th>r</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI</td>
<td>−0.581</td>
<td>&lt; 0.001a</td>
<td></td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>−0.534</td>
<td>&lt; 0.001a</td>
<td></td>
</tr>
<tr>
<td>Mid-arm circumference (cm)</td>
<td>−0.488</td>
<td>&lt; 0.001a</td>
<td></td>
</tr>
<tr>
<td>Triceps skin fold thickness (mm)</td>
<td>−0.394</td>
<td>&lt; 0.001a</td>
<td></td>
</tr>
<tr>
<td>INR</td>
<td>0.66</td>
<td>&lt; 0.001a</td>
<td></td>
</tr>
<tr>
<td>Albumin (g/dl)</td>
<td>−0.686</td>
<td>&lt; 0.001a</td>
<td></td>
</tr>
<tr>
<td>Total bilirubin (mg/dl)</td>
<td>0.742</td>
<td>&lt; 0.001a</td>
<td></td>
</tr>
<tr>
<td>AST (IU/l)</td>
<td>0.670</td>
<td>&lt; 0.001a</td>
<td></td>
</tr>
<tr>
<td>ALT (IU/l)</td>
<td>0.690</td>
<td>&lt; 0.001a</td>
<td></td>
</tr>
</tbody>
</table>

a Spearman correlation coefficient significant at the 0.01 level (2-tailed).
Serum ghrelin in liver cirrhosis

Correlation of serum ghrelin and anthropometric parameters of the study

The correlation between plasma ghrelin levels and anthropometric parameters are shown in (Table 2). Plasma ghrelin levels showed a significant negative correlation with BMI ($r = -0.581$, $P < 0.001$), AMC ($r = -0.488$, $P < 0.001$) and TSF ($r = -0.394$, $P < 0.001$). These parameters were not related to the severity of liver damage.

Relationship among plasma ghrelin levels and biochemical markers

The relationships between plasma ghrelin levels and laboratory parameters in patients with liver cirrhosis are shown in Table 2. Plasma ghrelin levels showed a significant negative correlation with serum albumin ($r = -0.686$, $P < 0.001$), and showed a significant positive correlation with INR ($r = 0.66$, $P < 0.001$), total bilirubin ($r = 0.742$, $P < 0.001$), AST ($r = 0.670$, $P < 0.001$), ALT ($r = 0.690$, $P < 0.001$). Receiver operator curve (ROC) analysis of sensitivity and specificity of BMI to plasma ghrelin level was highly significant and confirmed the above findings (Fig. 2).

Discussion

The nutritional and metabolic consequences of liver cirrhosis have attracted considerable interest over the past decade because malnutrition and hypermetabolism are commonly found in cirrhotic patients. In addition, malnutrition is a well-established risk factor influencing survival in patients with cirrhosis and can modify the prognosis [4,15,16].

The pathogenesis of anorexia in cirrhotic patients is complex and the appetite-modulating hormone ghrelin could be involved. Acylated ghrelin is the biologically active form that modifies insulin sensitivity and body composition. Liver failure causes decreased protein synthesis and enhanced protein breakdown, which together with anorexia and reduced food intake can lead to severe protein energy malnutrition [17].

Malnutrition in liver cirrhosis is induced by several mechanisms, such as poor food intake, disturbances in absorption and digestion of nutritional substances in the gastrointestinal tract, and impaired hepatic synthesis of energy substrates. These abnormalities gradually induce anthropometric changes and hypoalbuminaemia in patients with liver cirrhosis. It has been shown that malnutrition affects the prognosis of patients with liver cirrhosis [5]. Ghrelin is a novel endogenous ligand for the growth hormone (GH) secretagogue receptor that has recently been isolated from both human and rat stomach [6]. Ghrelin controls energy balance, enhancing fat mass deposition and food intake through the activation of the hypothalamic nuclei and the promotion of neuropeptide Y (NPY) and agouti-related protein (AGRP) expression [18]. Ghrelin administration stimulates GH secretion independent of hypothalamic GH-releasing hormone, and also causes weight gain and adiposity by increasing food intake and reducing fat utilization in rodents.

Ghrelin also acts through antagonism to leptin which is a peptide hormone that is predominantly produced by adipocytes [19]. Leptin regulates energy balance by suppressing appetite and increasing energy expenditure [20]. It inhibits NPY and AGRP [21].

Fasting total ghrelin levels have been studied in hepatic failure patients and was found to be variable [16,22,23]. These discrepancies could be explained by different patients, control subject selection, or both. Some patients had malignancies and were not BMI-matched with control subjects [24].

In our study, we aimed to evaluate the changes in the plasma acylated ghrelin level in patients with liver cirrhosis secondary to HCV with different Child classes and to find its correlation to the nutritional status and its role in cachexia associated with this liver cirrhosis. Plasma acylated ghrelin level was significantly higher in Child C patients compared to both Child A and control group (Fig. 1). It was significantly negatively correlated to BMI, MAC, WC, TSF and serum albumin levels which denote that the level of elevation in plasma ghrelin depends on nutritional status (Table 2). In our particular group of patients with positive HCV antibody, we think...
Ghrelin can be an early marker of malnutrition and hence a prognostic factor for survival. These findings coincide with the study of Ataseven et al., 2006 [4] and Elshehaby, 2010 who found the increase of plasma ghrelin more prominent in Child C cirrhotic patients compared to Child A grade (P < 0.05) [24]. The fact that metabolic decompensation and clinical complications like malnutrition increases with Child’s classification, could explain the rise in plasma ghrelin in order to counteract these changes. That would lead to GH release, induction of hyperglycaemia, modulation of energy balance, stimulation of appetite and hence improvement in food intake.

The positive correlation between plasma ghrelin and liver enzymes that was detected in our study (Table 2) could also indicate the significance of active liver disease on plasma ghrelin that can be used as a marker to improve the dietary supplements to liver cirrhosis and help with anorexia. Tacke et al., 2003 [22] reported that plasma ghrelin levels in liver cirrhosis patients were significantly elevated when compared with healthy controls but were not correlated with liver function, although elevated levels were seen in patients having a Child’s classification of grade C and severe complications (gastrointestinal bleeding, ascites and hepatic encephalopathy).

These results were against what was found by Marchesini et al., 2004 and Kalaitzakis et al., 2007 as they showed plasma ghrelin levels were no higher than healthy controls and was not related to severity of liver damage, but mainly closely associated with food intake in disease-associated malnutrition [17,25].

These patients with poor appetite, however, appeared to be insensitive to the orexigenic actions of ghrelin. Desensitization of the hypothalamic ghrelin receptor, GHS-R, implicated in the control of food intake could explain in part the paradoxical response of increased ghrelin and decreased feeding leading to a malnutrition state. Thus plasma ghrelin can be used as a marker to diagnose malnourishment in early stages of CLD and hence improve appetite and food intake. Therapeutic use of ghrelin is limited because it is a protein with a very short half-life and must be administrated by injection. RC-1291 is an orally active ghrelin mimetic and GHS-R agonist that has promising results in cirrhotics and cancer patients. It is expected to increase appetite by virtue of its ghrelin agonist properties and produce anabolic partitioning of nutrients to increase lean body mass, thereby offering a potential new therapy for treatment of anorexia and cachexia. Results indicates that RC-1291 produces a dose-related increase in body weight with no dose limiting adverse effects, may be an effective treatment for anorexia [28].

Other possible benefits of ghrelin that were found recently by Moreno et al., that recombinant ghrelin treatment reduced the fibrogenic response, decreased liver injury and myofibroblast accumulation, and attenuated the altered gene expression profile in bile duct-ligated rats. Moreover, ghrelin reduced the fibrogenic properties of hepatic stellate cells. Ghrelin also protected rats from acute liver injury and reduced the extent of oxidative stress and inflammation [26]. It helped to protect against hepatic steatosis as was found by Gutierrez-Grobe et al. [27].

Finally, ghrelin is a very promising hormone can be used as a treatment of malnutrition in patients with liver cirrhosis and therefore improve the clinical outcome especially in our population of HCV positive patients.

**Disclosure of interest**

The authors declare that they have no conflicts of interest concerning this article.

**References**


