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ORIGINAL ARTICLE

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

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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 tricuspid 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.

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].

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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 pathophysiological 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 (m^2). 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 $\pm$ 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,