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Estimating the level of amino acids in patients with liver disease by amino acid analyzer

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Abstract---Background: Amino acid identification can be a reliable approach to noninvasive diagnosis of liver disease. This is because liver disease can be a cause of impaired amino acid metabolism. Therefore, a method for determining amino acids in blood that is applicable for clinical purposes is essential. Objectives: The aim of this study was to find differences in amino acid levels between patients with liver disease and a control group. Material and Methods: Samples of peripheral venous blood were obtained from a group of patients with liver disease (n = 40 women at an average age of 37-70 years and 40 men at an average age of 35-70 years) and control group (n = 40 women at an average age of 35- 70 years and 40 men at an average age of 35 - 70 years). Before separation, The amino acids were derived using orthophalenealdehyde (OPA). For separation, a reverse phase shaft was used. The flow was monitored with a fluorescence detector. Results. There were statistically significant differences in the concentrations of some amino acids between patients and the control group, as well as between women and men. Relationships between some amino acids, markers of liver blood tests, and lipid metabolism were observed. Conclusions. A simple, relatively fast and selective amino acid analysis method with fluorescence detection for specificity. Amino acids have been developed in blood serum.

Keywords---amino acids, amino acid analyzer, fluorescence detection, liver disease.

Introduction

Amino acids (AA) have different functions in the body. Amino acids in the blood are determined practically by metabolism of liver and skeletal muscle [1,2] . In several studies, the levels of amino acids, particularly the branched-chain amino acids and aromatic amino acids, differed between patients with different liver diseases and the control group [3]. Rapid and accurate identification of amino acids in serum or plasma of patients with liver disease is essential for effective diagnosis and treatment monitoring [4] . Techniques used to determine levels of amino acids in serum or plasma include high performance liquid chromatography (HPLC)[5,6].

Amino acid analyzer, and although tandem mass spectrometry is very sensitive and specific, not all laboratories are equipped with such an expensive piece of equipment [7]. New methods for determining amino acids in serum or plasma are the amino acid analyzer using a pre-column or post-column derivation using o-phthalaldehyde (OPA) [8,9]. The aim of this study was to find differences in the levels of selected amino acids between patients with different liver diseases and a control group.

Material and Methods

Sample collection

Patients

Patient groups consist of (80) samples from man and women with liver disease , divided into two groups. First groups (A) consist of (n=40) sample women liver disease while second groups (B) were (n=40) sample man with liver disease. The age of man and women was ranged from (37 - 70) years . All samples were collected from Center of Alyarmok Hospital in Baghdad City, Based on the history: patients with positive family history of liver disease , smokers, received hormonal therapy, chronic disease (e.g. diabetes mellitus, rheumatoid arthritis) were excluded from the study.

Controls

Control groups (C) consist of (n= 80) samples of healthy man and women . They were collected from medical staff who were free from signs and symptoms of liver disease , age ranged from (35 - 70) years, all of them were non-smokers , free from DM, hypertension and no family history of liver disease .

Samples Collection

Fasting blood samples were collected without using any anticoagulant and were subjected to centrifugation at 3,000 rpm for 10 min at 4°C to obtain serum. Serum were stored at (-20°C) immediately after separation in multiple Eppendorf till analysis.

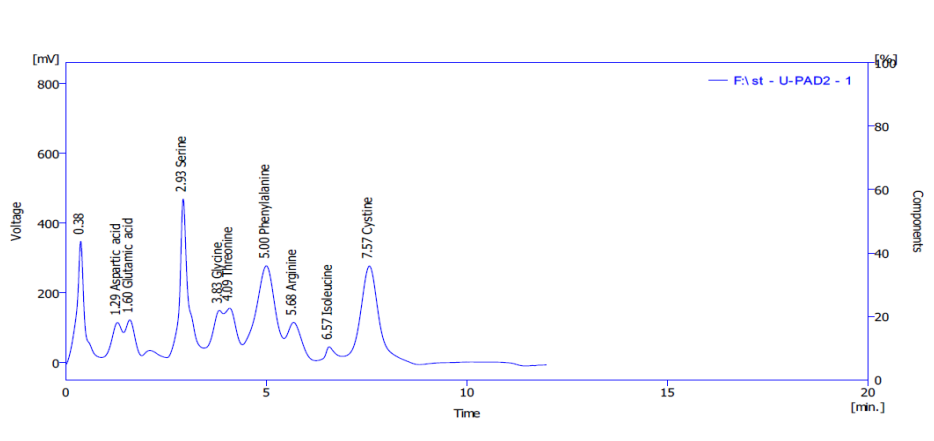
Sample preparation

The plasma proteins were precipitated by adding 10% trichloroacetic acid (TCA). After centrifugation at 10000 rpm for 5 minutes, the supernatant was removed and filtration was done using syringe filter with a 0.45 μm pore size. The materials used were obtained at high purity grade. The samples were prepared by adding (250 μl) of sample and methanol (500 μl). After thorough mixing, incubation was done at laboratory temperature for 5 minutes. After centrifugation at 5000 rpm for 5 minutes, 250 μl of the supernatant was taken and mixed with 100 μl of borate buffer. For derivatization of amino acids, 50 μl of OPA solution was added to the above solution and then incubation was performed for two minutes at room temperature [10,11]

Amino acid analyzer condition

Amino acids standard were obtained from Sigma Chemical Company (USA), and HPLC gradient grade methanol, ethanol and acetonitrile from Merck (Germany). All the others chemicals were of analytical grade. The analyses was in the Ministry of Science and Technology / Department of Environment and Water Laboratories. Using The amino acid analyzer, model Young Lin (Korea). the mobile phase consists of acetonitrile : methanol : formic acid (60 : 20 : 20) (v/v) , at a Flow rate (1 ml / min) and Column separation was ZORBAX Eclipse-AAA; 3.5 μm ; L x i.d.=150 x 4.6 mm . The Detector = Florescence Ex =365 nm , Em = 445 nm . the injection program, including derivatization steps with OPA [10,11]

Result and Dissociation



The results obtained by Mean ($\mu\text{mol} / \text{L}$) \pm SD of the amino acid analyzer showed significant differences in the concentration of amino acids between patients with liver disease and the control group. Where a significant decrease in the concentration of amino acids was observed in patients with liver disease when compared with the control group [12] . As shown in the table (1) .

Table (1): Mean \pm SD of level amino acid between patient and control groups

Group	No of sample	Mean ($\mu\text{mol} / \text{L}$) \pm S D	P - Value
Control	80	17.25 \pm 0.48	p<0.001
Patient	80	2.55 \pm 2.88	

In addition, there are slight differences between men and women with liver disease in the concentration of amino acids[13]. as shown in the table (2,3).

Table (2): Mean \pm SD of level amino acid between patient men and control men groups

Group	No of sample	Mean ($\mu\text{mol} / \text{L}$) \pm S D	P - Value
Control men	40	13.14 \pm 0.34	p<0.001
Patient men	40	1.35 \pm 1.66	

Table (3): Mean \pm SD of level amino acid between patient women and control women groups

Group	No of sample	Mean ($\mu\text{mol} / \text{L}$) \pm S D	P - Value
Control women	40	8.18 \pm 0.22	p<0.001
Patient women	40	0.35 \pm 1.43	

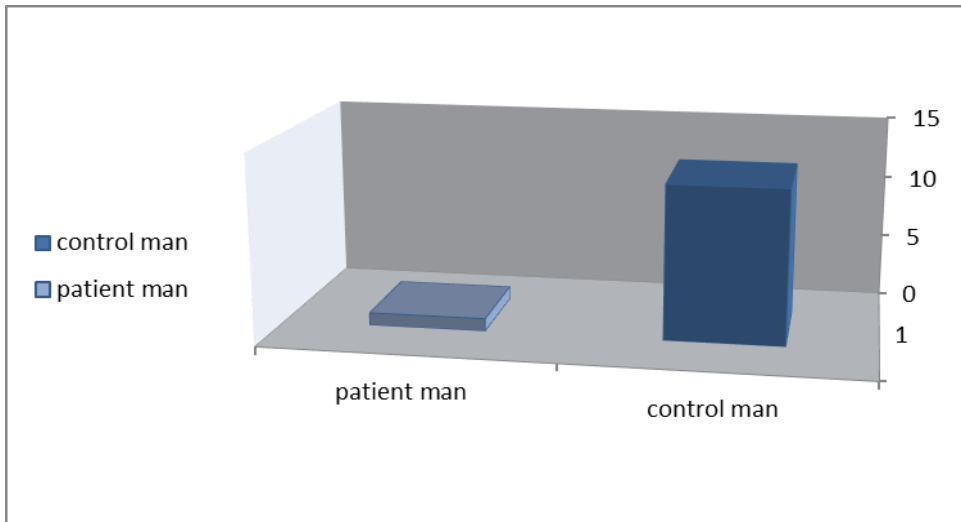


Figure (1) amino acid Levels in man with liver disease and Healthy man as Mean

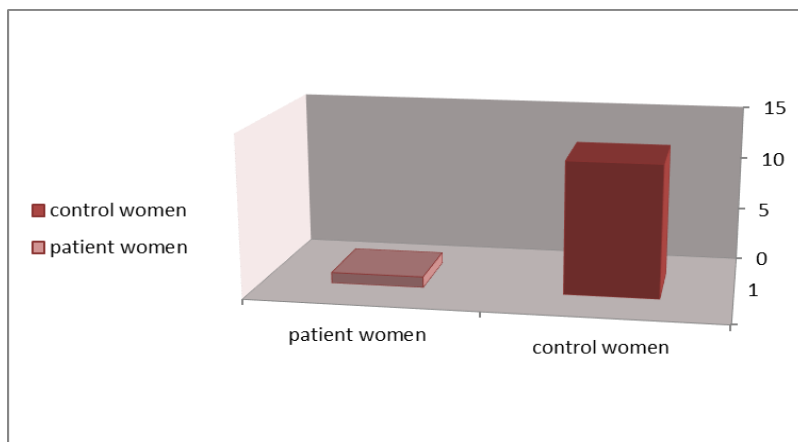


Figure (2) amino acid Levels in women with liver disease and Healthy women as Mean

This method was developed for the determination of selected amino acids in serum in patients with liver disease and indicates the presence of disturbances in amino acid metabolism in patients with imperceptible liver disease. Amino acid levels decreased statistically significantly[14]. Statistically significant differences were found in amino acid levels between women and men. For this reason, it is necessary to compare amino acid levels between female patients and female controls as well as between male patients and male controls[15]. Most of the publications report an amino acid imbalance in patients with various liver diseases. Often, there are untreated patients with different types and degrees of liver disease. We only dealt with patients who were treated in the liver clinic. These patients had liver blood test results (ALT, AST, ALP, GMT, total bilirubin) and lipid metabolism (total cholesterol, triglycerides) normal or slightly increased. Plasma amino acid levels are decreased in people with liver disease[14,16].

These changes occur due to increased catabolism in skeletal muscles, while catabolism of amino acids decreases in a failed liver. Statistically significantly. Statistically significant differences were found in amino acid concentrations between women and men, and between patients and the control group. In liver transplantation, plasma AA levels were normalized, and plasma AA levels remained below normal even though they were higher than they were before liver transplantation. Amino acid metabolism is only partially normalized after liver transplantation. After liver transplantation, insulin production remains higher and thus insulin may contribute to a continual change in AA muscle metabolism. Found low serum AA levels in patients with liver disease can be explained by the use of AA as a result of decreased immunity in the liver [14-16].

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