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Investigation the Role of Some Biochemical Variables in Liver Cirrhosis

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Abstract
Objectives: The objective of the current study is to assess nitric oxide levels as well as a few other biochemical parameters in individuals with hepatic Cirrhosis disease.
Methods: The study included 55 age-matched controls and 57 individuals with cirrhosis disease that were both clinically and biochemically identified. Adenosine deaminase (ADA), albumin, aspartate transaminase (AST), alanine transaminase (ALT), -glutamyl transpeptidase (GGT), and alkaline phosphatase were measured as part of the biochemical analyses (ALP).
Results: The study group's serum nitric oxide (NO) levels were substantially (*P* 0.001) greater than those of the control group's. Additionally, the results showed that the activities of the enzymes ADA, GGT, ALP, AST, and ALT were significantly higher in liver cirrhotic patients than in the control group.
Conclusion: Nitric oxide concentration elevation may serve as a potential diagnostic indicator for liver cirrhosis.
Keywords: Liver Cirrhosis, Nitric oxide, liver enzymes

Introduction

Chronic liver diseases may possibly classify into liver cirrhosis and chronic active hepatitis. Liver illnesses it could be attended by portal hypertension. Several systemic changes may occur with liver cirrhosis and portal hypertension.[1] These abnormalities changes of patients with liver cirrhosis have been stated to elevate the risk of hyperdynamic circulation like (ascites, edema, hepatorenal syndrome etc.). Furthermore, Liver cirrhosis is related to many cardiovascular irregularities such us cirrhotic cardiomyopathy, and pulmonary vascular abnormalities.[2,3] With the progression of the liver cirrhosis the metabolic impairment leads to fibrosis of the liver and change efficient disturbances of various organ systems include heart, kidneys, lungs, immune systems and other organ systems.[4] In addition, with the cirrhotic apparent, the cardiac mitochondrial functions reduce in breathing regulator ratio and rise mitochondrial inflammation.[5]

Nitric oxide (NO) is one of the most abundant products induced with the catabolism of L-arginine in various mammalian cells, by the action of enzymes that catalyze and regulate synthesis and catabolize arginine. These induced and in certain alterations in the nitric oxide synthase play main role in metabolic outcome of arginine in health and disease subject.[6,7] In the beginning, the alteration of cell NO releases was established mostly in the cardiovascular system, but nowadays the ultimate role and twin effects (low concentration and high concentration) had been recognized in various organizations of human body.[8] The objective of the recent study was purposed to determine the serum levels of Nitric Oxide in patient whom suffering with the liver cirrhosis and to exhibit their correlation with the extra considerations such us serum albumin level and Serum enzymes: ALP, ACP, GGT and ADA.

Materials and Methods

Study Subjects

A total of fifty seven cases which were diagnosed clinically and biochemically as Liver Cirrhosis and fifty five healthy subjects were participated in the study their ages ranged from 36–62 years. A total of thirty seven serum samples were obtained from individuals diagnosed with Liver Cirrhosis. Their ages ranged from 36–62 years.

Collection of Blood Samples

Approximately 5 ml of venous blood was collected from individuals. After coagulation, all samples were centrifuged at 3000 rpm for 10 minutes and sera were stored at–70˚C until analyzed.

Biochemical Analysis

Assay of Nitric Oxide Concentration

Nitric oxide concentration has been estimated by using sandwich enzyme immunoassay (ELISA) technique (BIOSOURCE, Europe S.A., Belgium, Lot No. 051501/B; 060601).

Principle of Human Nitric Oxide (NO) ELISA Kit

The double-sandwich ELISA technique was employed in this investigation, and the ELISA kit that was provided was standard. Human NO monoclonal antibody served as the precoated antibody, while polyclonal antibody with biotin label served as the detecting antibody. ELISA plate wells were filled with samples and biotin-labeling antibodies, then the mixture was removed using PBS or TBS. Following the addition of each conjugate in turn, the ELISA wells were colored using TMB substrate after the reactant had been fully removed with TBS or PBS. TMB is catalyzed by peroxidase to turn blue, and then under the influence of acid, it turns yellow. Samples' testing variables and color depth had a positive correlation.

Assay of Liver Enzymes Activities

Liver enzymes activities which included γ-glutamyl transpeptidase (GGT), alkaline phosphatase (ALP), Adenosine deaminase (ADA), aspartate transaminase AST(GPT) and alanine transaminase.

ALT(GOT) were measured spectrophotometrically in accordance with the manufacturer's instructions using kits from (RANDOX BRAZIL Ltd.). kits.

Statistical Analysis of Data

The results are given as mean SD. Comparative statistical analysis was done using (SPSS, Chicago, Il, USA). and a *P*-value of < 0.05 was regarded as sufficiently significant.

Results and Discussion

The mean serum NO in liver cirrhosis, and control subjects were 90.34 ± 8.45 mmol/L, 45.01 ± 4.27 mmol/L and respectively. Statistical evaluation showed that nitric oxide level was elevated in case groups compared with the control group ($P < 0.0001$) as shown in Figure 1.

Fibrogenesis, inflammation and necrosis are the most alteration of liver mechanism process which causes the conversion from prolonged liver disease to cirrhosis. These changes typically happen due to spreading of nodular revival bounded by thick fibrotic septa with following parenchymal cell elimination and collapse of hepatic vascular construction.[9] In addition, the disturbances of the hepatic architecture produced by cirrhotic process increase the intra-hepatic resistance against portal blood flow, which the main issue is foremost to portal hypertension. Portal hypertension is a most abundant and terrible obstacle of people suffering chronic liver illness.[10] The pathological condition resulting from a portal hypertension which has happened in consequence of destruction to the portal veins is splanchnic overcrowding and opening of arteriovenous inosculation, which that finally attribute to edema, ascites, splenomegaly etc. furthermore, concerning splanchnic arteriolar vasodilatation which will causes portal hypertension facilitated by NO and act to fill arteriolar vascular cavity, as well as making salt and water retention due to stimulating antidiuretic hormone.[2]

Our research revealed that patients with liver cirrhosis had higher serum concentrations of nitric oxide. Additionally, numerous researchers found that hepatic cirrhosis results in higher serum NO concentrations.[11–13] The presence of fibrosis and inflammation may affect serum nitrite and nitrate levels, which is the best explanation for the rising NO concentrations. Although the cause of excessive nitric oxide generation is uncertain, portal venous hypertension, which increases shear stress and controls endothelial nitric oxide synthase, definitely plays a role.[14,15] The anatomic sites of the enhanced NO production and release are unknown. The jejunum, gastric mucosa, and esophageal mucosa may all have enhanced NO synthase activity, according to some studies.[16-18]

Nitric oxide levels are higher in portal venous plasma than in peripheral venous plasma in cirrhotic people, indicating enhanced splanchnic nitric oxide production.[19] Nitric oxide synthase activity in polymorphonuclear cells and monocytes of cirrhotic patients was elevated. There is evidence that this kind of the enzyme may contribute to peripheral vasodilation in cirrhotic patients because inducible nitric oxide synthase predominates in these cells.[20]

Nitric Oxide which has been recognized as the utmost important vasodilator of hepatic vascular nature regulation is formed by endothelial cells. It is generally formed from the metabolism of guanidine group of arginine.[21, 2] In the recent study, our results found significantly higher levels of NO in the patients with liver cirrhotic group than in the control group Figure 1. The various studies all agreed that serum NO concentrations are raised in liver cirrhosis.[1,2,5] Despite the anatomic and the mechanisms of the enhanced NO releases during cirrhosis is remained unclear and difficult to analyses. Confirmation proposes that at different sites, such as esophageal mucosa, gastric mucosa, and jejunum, NO synthase activity can be uncontrolled, and the activity of erythrocyte adversely related with the serum level of NO in liver cirrhotic patients.[22]

Figure 2 showed that patients' serum concentrations of -glutamyl transpeptidase (GGT) and adenosine deaminase (ADA) were significantly higher than those of controls ($P < 0.0001$ and $P < 0.001$, respectively).

In comparison to normal healthy people of all ages, patients with liver cirrhosis have considerably higher serum ADA activity (p 0.001). The data are consistent with earlier findings.[23,24] Estimating a number of non-functional plasma enzymes specific to that tissue or organ can help in the diagnosis of organ dysfunction. The amount of enzymes generated is influenced by the degree of cellular injury, intracellular enzyme concentrations, and the mass of the afflicted tissue. The enzymes released into the blood circulation are unaffected by the damage's underlying cause, whether it be a viral infection, hypoxia, surgical, chemical, or mechanical trauma. The amount of enzymes released reflects how much harm has been done. While cytoplasmic enzymes are more likely to be released from necrotic tissue than from mild inflammatory conditions. While necrotic circumstances also produce mitochondrial enzymes, mild inflammatory situations are more likely to release cytoplasmic enzymes.

The use of appropriate normal ranges is important in assessing abnormal levels of plasma enzymes.

Figure 3 revealed significant elevation ($P < 0.0001$, $P < 0.001$) respectively in serum concentration of γ- glutamyl transpeptidase (GGT) and Adenosine deaminase (ADA) levels in patients) as compared to controls.

Figure 2 revealed significant elevation ($P < 0.0001$) in serum concentration of ALP, ALT and AST levels in patients) as compared to controls.

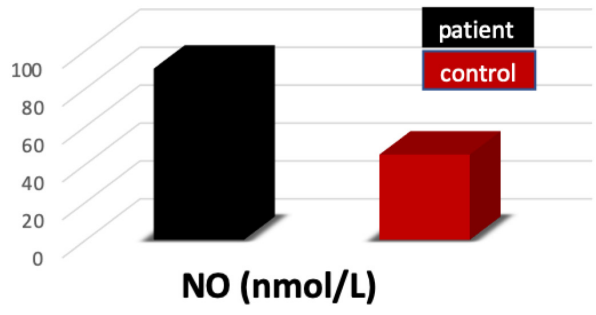

Fig. 1 **Mean values of serum NO in control and patient groups.**

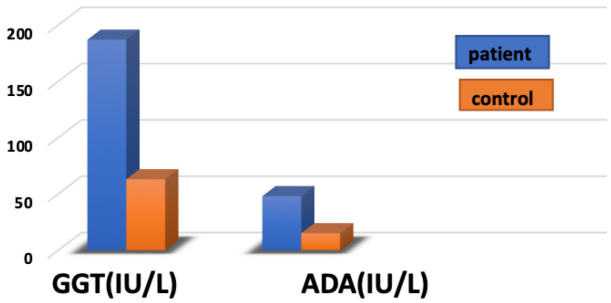

Fig. 2 **Mean values of serum GGT and ADA in control and patient groups.**

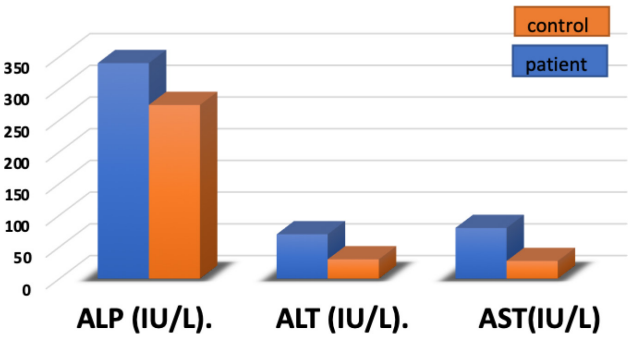

Fig. 3 **Mean values of serum ALP, ALT and AST in control and patient groups.**

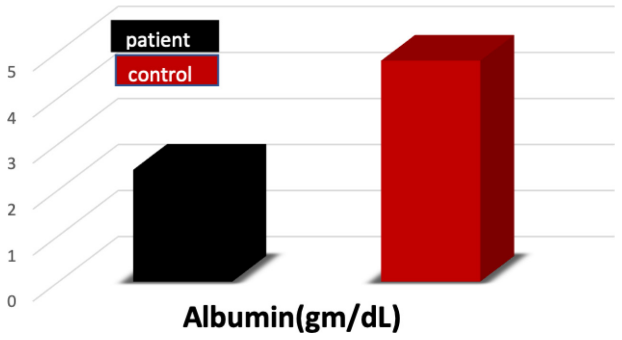

Fig. 4 **Mean values of serum Albumin in control and patient groups.**

Concerning liver injury, not liver normally functions are referred to measurements each of the aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase. These laboratory measurement parameters are extremely reproducible. Therefore, hepatic cirrhosis as a last stage of liver disease or hepatic illnesses is required to assess and potential evaluation of AST, ALT and ALP. In addition, the elevation of gamma-glutamyl transferase (GGT) or ALP fractionation may trust an elevated ALP level of hepatic origin. In spite of, AST is elevated in the liver dysfunctions but it is not considered the more specific marker of hepatocellular damage, because it is present in other organs rather than liver including heart, brain, kidney and skeletal muscle. However, ALT is a more focused indication than AST., since it is existing primarily in the liver.[25] Table 1 shows significantly elevated level of GGT, ADA, ALP, ALT and AST in hepatic cirrhosis patients as compared to control group. GGT is very sensitive and reliable enzyme to identify liver injury in both intra and extra hepatic failure, due to it is existence in the cell membranes of hepatobiliary scheme. In different kinds of liver disease such as viral hepatitis, cholestasis and alcoholic liver disease the value of GGT is increases up to 5 times to 15 time's upper than normal values.[26] However, in our study persistence elevation of GGT is changed from 63 ± 11.67 controls to 187 ± 35.09 as an indicator of cirrhosis.

Increasing activities of plasma specific enzyme like ALT, AST, ADA and ALP considered as a feature diagnostic of hepatic diseases, subsequently enzymes are secreted into the bloodstream after the worsening of the liver organ. Despite releasing these mitochondrial and cytoplasmic enzymes into serum and others like lactate dehydrogenase (LDL), cholesterol, triglycerides, serum lipid profile and lipoproteins are used to estimate the status of liver function, while an elevation of transaminase enzyme more than two times folded is considered as a marker index of liver cirrhosis and hepatotoxicity.[27] In this study, all of the ADA, ALP and alanine transaminase as well as aspartate transaminase enzymes raised from (15.07 ± 1.92, 274 ± 18.50, 30.5 ± 7.22 & 27.91 ± 5.71) to (48.01 ± 6.40, 340 ± 25.01, 70.02 ± 27.35 & 80 ± 27.12) respectively.

Figure 4 revealed significant reduction ($P < 0.05$) in serum concentration of albumin levels in patients as compared to controls.

Albumin is a family of spherical proteins produced in the liver and is closely attendant with the systems of pathophysiology in different forms of liver disease. Therefore, it has an important role in the controlling fluid spreading in the body.[28] The result, in the current study shows considerably decrease concentration of serum albumin in patient group, Table 1. This could cause by the changing qualitatively in albumin structure and not purely a quantitative decrease which is responsible for the defeat of it is physiological role that alters the basic knowledge of the processes that possibly cause significantly reduced albumin functioning in liver disease. Albumin, bilirubin, and time of prothrombin are hepatocellular function indicators that can be significantly influenced by extrahepatic factors.[29]

Conclusion

Increased levels of nitric oxide contributed to the hemodynamic changes occur in patients of liver cirrhosis. Indicate that nitric oxide has a pathophysiological role in liver cirrhosis.

Conflict of Interest

None.

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