Antioxidant status and Some Biochemical parameters in cirrhotic liver patients

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Abstract

A clinical study of 21 patients with liver cirrhosis, aged (35-66) years, attending different hospitals in Mosul city .

Each cirrhotic patient was evaluated clinically and laboratory examinations have been done including peroxy nitrite as an oxidant indicator and the antioxidants like (SOD, Vitamin C, Vitamin E), Total protein, albumin, globulin, total bilirubin, Iron, Sulfate, triglycerides and cholesterol levels were evaluated .

The analysis of results showed that the levels of albumin, globulin, total protein, Vitamin E, Vitamin C, Iron and peroxy nitrite were significantly lower, while bilirubin, triglycerides and cholesterol were significantly elevated in cirrhotic patient when compared with control .

A non significant decreased in SOD activity and a non significant elevated in sulfate concentration obtained in all patients compared with normotensive .

الخلاصة

أجـريت الدراسة السريرية لـ21 مريضا بتشمع الـكبد ، تراوحت أعـمارهم (35-66) سـنة ، ممن يراجعون المستشفيات المختلفة لمدينة الموصل ، وتم إجراء التقييم السريري والمختبري لكـل مـريض بتشمع الكبد ، اذ شملت الفحوصات المختبرية قياس مستويات البيروكسي نتريت كمؤشر للأكسدة ومضـادات الاكسدة مثل SOD ، فيتامين C ، فيتامين E ، البروتين الكلي ، الالبومين ، الكلوبيولين ، البيليروبين الكلي ، الحديد ، الكبريتات ، الكليسيريدات الثلاثية والكوليستيرول .

أوضحت نتائج التحليل الإحصائي إن مستوى (الألبومين ، الكلوبيولين ، البروتين الكلي ، فيتامين E ، فيتامين C ، الحديد ، البيروكسي نتريت) قد انخفض بشكل معنـوي واضـح ، فـي حـين إن مسـتوى (البيليروبين ، الكليسيريدات الثلاثية ، الكوليستيرول) اعطى ارتفاعا معنويا عند مرضى تشمع الكبد مقارنة مع مجموعة السيطرة .

كما بينت النتائج انخفاضا غير محسوس في فعالية SOD لمرضى التشمع مقارنـة مـع مجموعـة السيطرة ، في حين إن تركيز الكبريتات قد ارتفع ارتفاعا غير معنويا عند جميع المرضى مقارنة مع مجموعة السيطرة .

Introduction

Cirrhosis represents the final common histologic pathway for a wide variety of chronic liver diseases.

The word "cirrhosis" derived from the Greek term *Scirrhus*, meaning tawny (the orange – yellow colour of the diseased liver). While the clinical entity was known before, it was Laennec who gave it the name "cirrhosis" in his 1819 work he also describes the stethoscope [1] .

Cirrhosis is a consequence of chronic liver diseases characterized by replacement of liver tissue by fibrosis, scar tissue and regenerative nodules (lumps that occur as a result of a process in which damaged tissue is regenerated) [2,3,4], leading to loss of liver function. Cirrhosis is most commonly caused by alcoholism, hepatitis B and C, and fatty liver disease but it has many other possible causes [5] .

Cirrhosis and chronic liver disease were the 10th leading cause of death for men and the 12th for women in the United States [6] . The risk of death due to all causes is increased twelve fold ; if one excludes the direct consequences of the liver disease, there is still a five fold increased risk of death in all disease categories [7] .

Cirrhosis is generally irreversible, and treatment usually focuses on preventing progression and complications [5] .

Cirrhosis can cause immune system dysfunction, leading to infection, bruising and bleeding due to decreased production of coagulation factors, jaundice, sensitivity to medication due to decreased metabolism of active compounds [8] .It can also lead to other problems like kidney failure [9] . The balance between oxidants and antioxidants can play an important role in the initiation and development of liver diseases[10]. Evidence of enhanced production of free radicals or significant decrease of antioxidant defense have been reported in all types of liver damage.

A large number of studies have focused on the pathogentic significance of oxidative stress in liver injury as well as on therapeutic interventions with antioxidant and metabolic scavengers [11,12,13,14]. Free radicals are implicated in the pathogensis of liver damage due to viral hepatitis , Wilson`s disease , haemochromatosis [15] and ischaemic reperfusion injury e.g. : following transplantation [16] . Cellular damage arises from oxidative stress i.e. : an imbalance between reactive oxygen species generating and scavenging systems (antioxidant enzymes) [17] . With this view in mind, it was thought pertinent to evaluate the role of oxidative stress in liver injury, specifically cirrhosis .

Materials and Methods

A clinical study of 21 male cirrhotic patients aged(35-66) attending different hospitals in Mosul city, was conducated. A control group, consisting of ten healthy males in the same above age range were used .

Clinical laboratory examinations on serum including, peroxy nitrite as an oxidant indicator and the antioxidants like (SOD, Vitamin C, Vitamin E), Total protein, albumin, globulin, total bilirubin, Iron, Sulfate, triglycerides and cholesterol levels were evaluated .

Peroxy nitrite activity was measured by modified method described by [18]. Serum Vitamin C [19] and Vitamin E [20] were measured spectro-photometrically .

The activity of SOD in blood serum was determined using photochemical method described by [21], this methods depends on an indirect approach to determine the

SOD activity through the change in formazene absorbance formed from the reduction of $O_2^{\cdot-}$, which is produced by radiating the sample of serum with light, for nitroblue tetrazolum (NBT) dye. Decreased difference in formazene absorbance means increased SOD activity. Serum total protein was determined by using manual Biuret method [22], while serum Albumin was determined by bromocresol green (BCG) dye binding method [23] .

The level of serum total bilirubin and direct bilirubin were based on the reaction of bilirubin (conjugated and unconjugated) with diazotized sulfanilic acid (Diazo method) [24]. Serum Iron was determined colorimetrically [25]. Serum sulfate was assayed using a turbidometric method [26] . Cholesterol and Triglycerides levels were determined colorimetrically by [25] and [27] respectively .

The statistical methods used to analyse the data include mean, standard deviation, minimum and maximum, while t-test was used to compare between control and patients at $P < 0.05$ and $P< 0.01$ and $P < 0.001$.

Correlation coefficient was obtained to find the relationship between extent of liver injury and assayed biochemical parameters .

Results and Disscusion

Production of reactive species, including free radicals, is an integral part of human metabolism. Because of the high potential to damage vital biological systems, reactive species have now been incriminated in aging and in more than 100 disease states [28,29] .

A complex system of neutralizing antioxidants exists in plasma and intra- and extra cellular fluids, but an imbalance (oxidant stress) between free radical production and use can cause damage to DNA, lipids, proteins, and other biomolecules [30] .

Never the less, most markers of oxidative damage have not been fully validated [31]. Endogenous antioxidant defenses (SOD, H_2O_2 removing enzymes, metal binding proteins) are inadequate to prevent damage completely, so diet-derived antioxidants are important in maintaing health [29,32]. The sum of endogenous plus food-derived anti-oxidants represents the total antioxidant capacity may give more relevant biological information compared to that obtained by the measurement of individual parameters, as it considers the cumulative effect of all antioxidants present in plasma and body fluids [33] .

In the present study, we have assayed the concentrations of antioxidants in patients with liver cirrhosis .

Table (1) indicates that certain parameters of liver function test like total bilirubin, Triglycerides and total cholesterol were increased in cirrhotic patients as compared to normal control.

Table (1) also shows a significant change in the values of Vitamin E and Vitamin C, Total protein, Globulin, Albumin, Peroxy nitrite and Iron in patients when compared with normal control.

Moreover, no significant change was observed when cirrhotic patients was compared with normal control for SOD activity and sulfate concentrations .

Albumin, vitamin C and bilirubin contribute to the total anti-oxidant capacity of human plasma [34] .

Increased serum bilirubin level is a result of liver dysfunction and hyperbilirubinemia is a weak

antioxidant defense mechanism by itself. An increasing body of experimental evidence incriminates oxidative stress as a pivotal signal for liver fibrogenesis [35] .

Our study showed increased triglycerides (TG) and total cholesterol (TC) levels ($P < 0.05$) in liver cirrhosis patients. This suggests that cirrhosis is commonly associated with dyslipidemia, characterized by the elevation of TG and TC [36] this finding was consistent with that reported by [37,38] indicating that the content of cholesterol and TG in liver was positively correlated with plasma TG and TC levels, however lipids levels were in normal range in early liver cirrhosis and where higher in advanced liver cirrhosis.

Because albumin is made by the liver, decreased serum albumin (hypoalbuminemia was observed or may be a sign of liver disease [1] .

Table (1) : The measured Biochemical parameters in normal control & Cirrhotic patients

Parameters	Control (n=10)	Cirrhotic patients (n=21)
Bilirubin (µmol/L)	11.8 ± 1.923	38.28 ± 15.232***
Albumin (g/L)	52.8 ± 13.1	21.26 ± 4.01*
Globulin (g/L)	30.8 ± 4.38	14.119 ± 3.05*
Total protein (g/L)	83.60 ± 8.561	35.38 ± 6.64*
Vitamin E (µmol/L)	19.20 ± 0.91	11.311 ± 2.56*
Vitamin C (µmol/L)	34.10 ± 4.9	17.85 ± 2.16*
TG (mmol/L)	2.14 ± 0.36	5.72 ± 1.37*
Cholesterol (mmol/L)	5.98 ± 0.82	13.26 ± 2.46*
Iron (µmol/L)	18.3 ± 1.68	6.90 ± 1.15*
SOD	0.176 ± 0.08	0.1577 ± 0.005(NS)
peroxy nitrite (µmol/L)	82.25 ± 3.83	78.36 ± 3.86**
Sulfate (mmol/L)	1.32 ± 0.24	1.417 ± 0.27(NS)

- Values are mean ± SD, P values when cirrhotic were compared with control *P < 0.05 ; **P < 0.01 ; *** P < 0.001 .
- SOD activity expressed by the difference in formazene absorbance before and after irradiation. (Increased absorbance means decreased SOD activity) .
- (NS) = not significant .

Symptoms of cirrhosis are usually caused by the loss of functioning liver cells or organ swelling due to scarring, patients may experience anemia [39]. Iron deficiency is the commonest cause of anemia worldwide. Cirrhosis has many causes, including some inherited diseases such as hemochromatosis, a disease that occurs when the body absorbs to much iron and stores the excess iron in the liver .

In human, iron is an essential component of proteins involved in oxygen transport. It is also essential for the regulation of cell growth and differentiation [40]. A deficiency of iron limits oxygen delivery to cells, resulting in fatigue, poor work performance, and decreased immunity [25] .

*Sulfate is generated in the liver from methionine, cystine and cysteine [41]. Sulfate can be increased in liver disease [42] .

Patients with liver cirrhosis had plasma cholesterol sulfate concentrations significantly higher than those of control subject [43] .

Serum levels of peroxy nitrite was significantly decreased in patients with cirrhosis vs controls. However,

Peroxy nitrite is a powerful oxidant shown to damage membranes. Previous studies [44,45] observed a reduction of peroxy nitrite in plasma of patients with acute liver necrosis, peroxy nitrite reactions involved oxidation and nitration .

The chemical properties depend on the presence of CO_2 and metallic compounds as well as the concentrations of reagents and kinetic laws. This complex chemistry can be explained by the formation of several structural forms and active intermediates released from peroxy nitrite [46] .

Nitric oxide (NO) may play a role in mediating hepatic injury because the liver is the only organ that is normally exposed bacterial endotoxin, an indicator of nitric oxide synthase, and high concentration of circulatory endotoxin is frequently found in patients with hepatic cirrhosis [47] .

Several previous studies showed that serum (NO) levels are elevated in hepatic cirrhosis [47, 48, 49] .

Some several studies of the possible involvement of oxidative stress in cirrhosis reported that SOD activity was significantly lower in erythrocytes and hepatic tissue of cirrhotic patients, compared to control subjects [50,51] .

Significant decreased in the activity of SOD was observed in cirrhotic patients[52] .

NO and superoxide radical interact to form peroxy nitrite, which is an important mediator of free radical toxicity, the body protect itself from oxygen free radical toxicity by enzymatic antioxidant mechanisms (e.g., SOD) and by nonenzymatic antioxidants (e.g., Vitamins, Albumin, Bilirubin and many others) [35] .

the serum level of SOD was slightly decreased in patients when compared with control .

SOD and hydrogen peroxide have been reported to cause rapid breakdown of NO and formation of peroxy nitrite [53] .

SOD, the first line of defense against oxygen-derived free radicals, converts superoxide anion into H_2O_2, forming as neutral products O_2 and H_2O [54] .

The state of oxidative stress exists when there is imbalance between pro-oxidant and antioxidant chemical species. There is insufficient knowledge about antioxidant defense mechanisms, particularly the enzymatic components, in the pathogenesis of cirrhosis. In our study we observed decreased serum peroxy nitrite, and a slight but statistically insignificant decreased of serum SOD activity in patients, this finding may indicate that the hepatic antioxidant enzymatic defense system in patients is impaired .

Many patients with cirrhosis have protein-calorie malnutrition at base line, one study randomized hospitalized patients with hepatic encephalopathy to receive either a normal-protein diet or a low- protein diet .

The liver plays a vital role in synthesis of proteins, detoxification and storage (e.g., Vitamin A). In addition, it participates in metabolism of lipids and carbohydrates .

Vitamin C is also reducing tissues damage and reformation of vitamin E from tocopherol radical which form as a result of lipid peroxidation, so that vitamin C contributes with vitamin E in protect cell membranes from damage and

removing free radicals (e.g., $O_2^{\cdot -}$, OH^-) [56]

Reduced levels of vitamins in general and vitamin E in particular have been found in serum of liver cirrhosis patients, this may reduce antioxidant capacity and promote generation of free radicals and lipid peroxides resulting in tissue damage and disease [55]. There is some preliminary laboratory evidence that various antioxidant supplements including vitamin E may help against liver damage and cirrhosis [57,58] .

These results suggest that disturbances of antioxidative mechanisms may diminish hepatic resistance to oxidative stress, thereby contributing to development of fibrogenesis .

We perceive that failure antioxidant defense mechanisms against oxidative stress may be an important factor in the pathogenesis of liver diseases. Treatment approaches that address the antioxidant enzymes and the antioxidant vitamins may be helpful in the therapy of cirrhotic patients .

Table (2) : The relation between period of Infection and the biochemical parameters measured in the liver cirrhosis patients

Peroid of Infection	Vitamin C	TG	Chols.	Iron	Total protein	Vitamin E	Bilirubin	Albumin	Globulin	peroxy nitrite	SOD	Sulfate
≤ 5 years (n = 10)	-0.559	0.438	0.780**	-0.873**	-0.160	-0.776**	0.301	-0.492	-0.370	0.341	-0.644**	-0.465
> 5 years (n = 11)	-0.651**	0.852**	0.598	- 0.279	-0.443	-0.030	0.324	-0.68**	-0.715**	0.067	-0.505	-0.063

n = number of samples .

** correlation is significant at the 0.01 level (2-tailed) .

The results in table (2) showed that there is no correlation between (total protein, bilirubin, peroxy nitrite and sulfate) with period of liver injury in patients with cirrhosis, this mean that these parameters did not affect by disease progress, while there was a significant correlation between period of infection and (triglycerides and cholesterol) but negative correlation with (vitamin C, vitamin E, iron, albumin, globulin and SOD) .

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