Oxidative stress in liver disease patients

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Abstract

Aim: 1) To evaluate oxidative and antioxidant status in chronic liver disease.
2) To evaluate its inflammatory response in oxidative stress.

Methods: Samples were collected from Wenlock District Government Hospital, Mangalore (25 patients with chronic liver disease, 25 normal patients) from Aug. 2007 to Aug. 2008. The Parameters estimated are in plasma (total thiols, total proteins, antioxidant activity, AOPP). In RBC suspension % hemolysis, in whole blood (GSH), in serum, serum iron.

1) Statistical analysis was done by Mann Whitney U test to determine the significant of difference between two means. 2)Informed and written consent was taken from patients and ethical committee clearance was obtained.

Results: I. Non enzymatic antioxidants and TAA:

- Whole blood glutathione (GSH) decreased
- Plasma total thiols decreased
- Albumin decreased

II. Markers of oxidative damage:

- AOPP increased
- Percentage hemolysis no increase
- Iron increased

III. Plasma proteins as inflammatory markers:

- Total proteins decrease
- Globulin increased

Conclusion: In our study the non-enzymatic antioxidant metabolites were found to be decreased in chronic liver disease.

- There was very highly significant decrease blood GSH, plasma total thiols and albumin in chronic liver disease.
- No significant increase in AOPP, % hemolysis. Very high significant increase in serum iron in patients of chronic liver disease. There is oxidative stress in patients of chronic liver disease.

Key words: Oxidative stress, Chronic liver disease, Oxidative damage

Introduction

Chronic liver disease encompasses a large number of conditions having different etiologies and existing on a continuum between hepatitis infection and cirrhosis. Cirrhosis is a worldwide health problem affecting 15-20 percent of the total population. It is the end stage of liver fibrosis characterized by nodule formation[1].

Chronic alcoholism and chronic hepatitis C are the leading cause of cirrhosis and cirrhosis is the most common cause of portal hypertension [2]. Most types of cirrhosis may be classified by a mixture of etiologically and morphologically defined entities: alcoholic, cryptogenic and post hepatic, biliary, cardiac, metabolic, inherited and drug related [3]. The clinical signs of chronic liver disease can be visualized by observing spider angiomas, scleral icterus, jaundice, palmar erythema, gynecomastia, ascites, encephalopathy and aesterixis [2].

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Reactive oxygen species could be important causative agents of a number of human diseases[4]. Thus, antioxidants which control the oxidative stress state represent a major line of defense, regulating overall health [4]. Human plasma contains many different non-enzymatic antioxidants. The antioxidant status in human plasma is dynamic and may be affected by many factors. Thus, the relationship between non-enzymatic antioxidant capacity of plasma and their levels which are well know markers of oxidative stress (activity of plasma oxidized protein, lipid hydroperoxides, total thiol groups, glutathione level and plasma iron level) reflect health status [5,6,7].

Disturbances in the antioxidant system could play a role in pathogenesis

Inadequate removal of (ROS) Reactive Oxygen Species may cause cell damage by attacking membrane lipids, proteins and inactivating enzymes thus mediating several forms of tissue damage. The aim of our study was to evaluate the levels/activities of non-enzymatic antioxidants in blood of patients.

Aim

1) To evaluate oxidative and antioxidant status in chronic liver disease.
2) To evaluate its inflammatory response in oxidative stress.

Materials and Methods

A. Criteria for selection of subjects for study:

1) Test samples were collected from 25 patients aged 20 to 60 years, both male and female having chronic liver disease on clinical basis.
2) Control group samples were collected from age and sex matched 25 healthy individuals who are devoid of any diseased conditions like hypertension, diabetes mellitus, epilepsy, psychiatric disorders or history of any drug intake, alcoholism. Samples were collected from Wenlock District Government Hospital, Mangalore from Aug. 2007 to Aug. 2008.

B. Sample Collection and Processing:

6 ml blood was collected under aseptic conditions from both patients and normal persons after obtaining the informed consent. 3 ml of blood was collected without anticoagulant to separate serum. The remaining 3 ml of blood was collected with anticoagulant (EDTA). 0.2 ml of whole blood was taken for GSH estimation and the remaining volume was used to separate plasma. The separated RBC was washed thrice with cold normal saline (NS), after which they were suspended in an equal volume of normal saline. This 50% RBC suspension was used for estimation of % haemolysis.

C. The following parameters were estimated:

1) In Plasma

a) Total thiols by Ellman method.
b) Total proteins, albumin, globulin and A : G ratio by Lowry's method
c) Total Antioxidant activity by Koracevic method.
d) AOPP by modified Witko method.

2) In RBC suspension

% hemolysis by Kartha and Krishnamurthy method.

3) In whole blood

GSH by Ernest Beutler method.

4) In serum

Serum iron estimation by Kitzes method.

Statistical analysis:

Statistical analysis was done by Mann Whitney U test to determine the significance of difference between the two means.

Results

Non enzymatic antioxidants and (TAA) Total Antioxident Activity :

Whole blood glutathione (Table 1)

In comparison to controls, decrease in GSH activity in chronic liver disease is very highly significant

Table 1

Study group	N	Mean ± SD (mmol/L)	P	Remarks
Controls	25	45.0532 ± 10.4656		
Chronic liver disease	25	28.6154 ± 12.8992	<.001	VHS

N = Number of samples, SD = Standard Deviation, P = Probability of chance of significance of difference between two means, S = Significant, HS = Highly significant, VHS = Very highly significant, NS = Not significant

Plasma total thiols

In comparison to controls, decrease in total thiols in plasma of chronic liver disease patients, is very highly significant (Table 2)

Table 2

Study group	N	Mean ± SD (mmol/L)	P	Remarks
Controls	25	.5171 ± .1180		
Chronic liver disease	25	.2853 ± .1073	<.001	VHS

Albumin

There is a very highly significant decrease in albumin level in chronic liver disease when compared to the controls.(Table 3)

Table 3

Study group	N	Mean ± SD (mmol/L)	P	Remarks
Controls	25	4.6440 ± .7906		
Chronic liver disease	25	2.6252 ± .6534	<.001	VHS

Markers of oxidative damage:

(AOPP) Advanced Oxidation Protein Product

The increase in AOPP in patients of chronic liver disease is not significant when compared to controls. (**Table 4)**

Table 4

Study group	N	Mean ± SD (mmol/L)	P	Remarks
Controls	25	.1257 ± .0539		
Chronic liver disease	25	.1531 ± .0957	.483	NS

Percentage hemolysis

Percentage hemolysis, in chronic liver failure, both 0 hour and 2 hour sample show no significant increase in percentage hemolysis when compared to controls (Table 5 & 6).

Hemolysis at 0 hour in (%)

Table 5

Study group	N	Mean ± SD (mmol/L)	P	Remarks
Controls	25	5.2772 ± 2.8220		
Chronic liver disease	25	7.1765 ± 5.8475	.364	NS

Hemolysis at 2 hours (%)

Table 6

Study group	N	Mean ± SD (mmol/L)	P	Remarks
Controls	25	10.3671 ± 3.0950		
Chronic liver disease	25	14.1075 ± 9.4232	.400	NS

Iron

In chronic liver disease, increase in serum iron is very highly significant when compared to controls (Table 7).

Table 7

Study group	N	Mean ± SD (mmol/L)	P	Remarks
Controls	25	126.9824 ± 34.6687		
Chronic liver disease	25	227.7268 ± 76.0291	<.001	VHS

Plasma proteins as inflammatory marker

Total proteins

The decrease in serum total protein is very highly significant in chronic liver disease, when compared to controls. Decrease in albumin levels and increase in globulin levels is highly significant compared to controls (Table-8).

Table-8

Study group	N	Mean ± SD (mmol/L)	P	Remarks
Controls	25	7.8280 ± 1.0321		
Chronic liver disease	25	6.2800 ± 1.0598	<.001	VHS

Albumin

Albumin has already been discussed.

Globulin

Increase in serum globulin in highly significant in chronic liver disease when compared to controls (Table-9).

Table-9

Study group	N	Mean ± SD (mmol/L)	P	Remarks
Controls	25	3.0666 ± .6856		
Chronic liver disease	25	3.6608 ± .8102	.01	HS

(A : G Ratio) Albumin : Globulin

Change in A : G ratio is very highly significant in chronic liver disease, when compared to control (Table - 10).

Table-10

Study group	N	Mean ± SD (mmol/L)	P	Remarks
Controls	25	1.5176 ± .4783		
Chronic liver disease	25	.7212 ± .2976	<.001	VHS

Discussion

In a recent study it was calculated that (SH) Sulfhydryl groups contribute 52.9% to the measured serum total antioxidant capacity in healthy subjects [8].

The decrease in whole blood concentration of reduced glutathione might be attributed low its hepatic production in liver disease. The decreased reduced/total ratios and the increased total homocysteine concentration in plasma may indicates an increased oxidative activity in liver disease[9].

In patients of chronic liver disease the % hemolysis was not significant in both 0 hr and 2 hr. The % haemolysis estimated, indirectly measures the degree of erythrocyte membrane lipid peroxidation occurring in vivo.

Catalase activity was lower than normal. Lipid peroxidation was also observed in the RBC membranes and in the plasma [10]. The levels of cholesterol (C) and total phospholipids (PL) in (RBC) Red blood Cell membrane were found to be altered in liver cirrhosis patients. It has been shown by Grattaglianoa et al., that erythrocyte membrane modification is supposed to reflect hepatocytosis. The fluidity of (RBC) Red Blood Cell membrane which is also maintained by Cholesterol /Phospholipids ratio was found to be altered in patients with liver cirrhosis [11].

Increase values are reported in infective hepatitis, following release of strong iron from necrosed cell. Serum iron also falls considerably in infections (Cartwright and Wintrobe) and also in stressful condition. In chronic liver disease there is decreased synthesis of albumin. Hepatic fibrosis, characterized by excessive accumulation of extracellular matrix components in the liver, is a dynamic process, from chronic liver damage to end-stage liver cirrhosis. Iron-induced oxidant stress is involved in this process (1) as the primary cause of parenchymal cell mecrosis or (2) as activator of cells that are effectors (e.g., hepatic stellate cells, (myo) fibroblasts or key mediators (e.g., Kupffer cells) of hepatic fibrogenesis (or through both mechanisms). Beyond their effect as direct cytotoxic agents, iron and free radicals may trigger increased synthesis of collagen in myofibroblast – like cells as well as activate granulocytes and Kupffer cells, resulting in an increased formation of cytokines and eicosanoids and further reactive oxygen species. This may constitute a cascade of amplifying loops, which perpetuate the fibrogenic process. The fibrogenic potential of iron is even more dramatic when iron acts in concert with other hepatotoxins such as alcohol which is one of the most important cause for cirhossis. In this instance, even if tissue iron levels are only slightly elevated, the toxic effect of alcohol or its metabolities may be amplified and propagated with rapid acceleration of the liver disease [12].

Heavy alcohol intake can result in increased accumulation of iron in the liver, in both hepatocytes and Kupffer cells. Iron induced oxidative stress may promote the severity of alcoholic liver disease by (1) inducing NF-kappa B activation and subsequently increasing transcription of proinflammatory cytokines in Kupffer cells; (2) exacerbating CYP2E1-induced oxidative stress, especially in hepatocytes, through production of more toxic hydroxyl radicals; (3) stimulating hepatic stellate cells to produce excess amount of collagen and other matrix proteins that can lead to fibrosis; and (4) causing DNA damage and mutations that promote the development of liver cancer. Dietary iron supplements may further exacerbate the severity of alcoholic liver disease by increasing the magnitude of oxidative stress [13].

Summary and Conclusion

In our study the non-enzymatic antioxidant metabolites were found to be decrease in chronic liver disease.

There was a very highly significant decrease blood (GSH) Gultathione Sulfhydryl , plasma total thiols and albumin in chronic liver disease. There was no significant increase in (AOPP) Advanced oxidation protein product, marker of protein oxidation in patients of chronic liver disease.

There was no significant increase in % hemolysis in both 0 hour and 2 hour samples in patients with chronic liver disease. There was a very highly significant increase in serum iron in patients of chronic liver disease. It can be clearly concluded that there is oxidative stress in patients of chronic liver disease. In chronic liver disease increased serum iron

can lead to oxidative damage. We found a very highly significant hypoalbuminemia in patients with chronic liver disease and we also found a very significant hyperglobulinemia in patients with chronic liver disease. This shows elevated inflammatory response in patients with chronic liver disease.

References

1. Benyon, RC, Arthur MJ, "Extracelluar Matrix Degradation and the Role of Hepatic Stellate Cells". Semin Liver Dis., 2001; 21: 373-84.
2. Karnath. "Stigmata of Chronic Liver Disease", Review of Clinical Sign: pp. 14-16.
3. Harrison's Principles of Internal Medicine. "Cirrhosis and its Complications", 16th Ed.: Vol. 2, p. 1858.
4. Halliwell B, Gutteridge JMC. Eds. "Free Radicals in Biology and Medicine", Oxford: Clarendon Press (1989): pp. 416-508.
5. Vertuani S, Angusti A, Manfredini S. "The Antioxidants and Pro-Antioxidants Network: An Overview", Curr. Pharm. *Des.,* (2004); 10(14): 1677-94.
6. Chaudiere J, Ferrari-Iliou R. "Intracellular Antioxidants, from Chemical to Biochemical Mechanisms". Food Chem. Toxicol*.,* 37(9-10): 949-62.
7. Sies H. "Strategies of Antixoidant Defence". Eur. J. Biochem. (1993), 215(2): 213-219.
8. Carolina M, Costa Rita CC. Dos S, Emerson S L. "A Simple Automated Procedure for Thiol Measurement in Human Serum Sample", J. Bras Patol. Med. Lab. (2006), 42(5): 345-350.
9. Anders A, Arne L, Margret A, Hanne P, Bjorn H. "This as a Measure of Plasma Redox Status in Healthy Subjects and in Patients with Renal of Liver Failure". Clinical Chemistry, 45(1999): 1084-1087.
10. Krzysztof G. "Red Blood Cells Damage in Chronic Renal Failure". Cellular and Molecular Biology Letters, 191.
11. Geetha A, Lakshmi P, Annie JS, Surendran R. "Level of Oxidative Stress in the Red Blood Cells of Patients with Liver Cirrhosis". Indian J. Med. Res., 126, September 2007, pp. 204-210.
12. Pietrangelo A. "Iron Induced Oxidant Stress in Alcoholic Liver Fibrogenesis". Alcohol. 2003, Jun., 30(2): 121-9.
13. Purohit V, Russo D, Salin M. "Role of Iron in Alcoholic Liver Disease: Introduction and Summary of the Symposium". *Alcohol*, 2003 June, 30 (2): 93-7.

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