

RESEARCH ARTICLE

A COMPARATIVE STUDY OF OXIDATIVE STRESS PARAMETERS IN HEPATITIS B VIRUS INFECTION & ALCOHOLIC HEPATITIS***Ghosh Chinmoy¹, Pal Santasmita¹, Roy (Adak) Shiuli², Bose Mohua³, Paul Suhrita⁴**¹M.D; Demonstrator, Department of Biochemistry, Medical College Kolkata²M.D; Associate Professor, Department of Biochemistry, North Bengal Medical College³M.D; Associate Professor, Department of Microbiology, Murshidabad Medical College⁴M.D; Professor, Department of Pharmacology, Medical College Kolkata**E-Mail of the Corresponding Author: bappa_amichin@yahoo.co.in***ABSTRACT**

The liver has a critical role in metabolism, digestion, detoxification and elimination of substances from the body. Hence in diseased conditions, the products of free radicals from the liver increase which results in further damage to the vital organs. This study is done to compare the oxidative stress parameters in hepatitis B virus infected patients and alcoholic hepatitis patients. The study included 150 individuals (39 females) aged 25-75years of which the Group A included normal healthy volunteers; Group B consisted of alcoholic hepatitis patients and Group C included hepatitis B patients. When compared to control, Erythrocyte MDA was significantly high and erythrocyte GSH was significantly low in both Group B and Group C whereas Plasma Ascorbic Acid level & Serum α -Tocopherol were low in both the groups. When group B and Group C were compared, MDA and GSH showed no significant difference ($p>0.05$) while α -Tocopherol and ascorbic acid levels varied significantly ($p<0.005$). The liver enzymes were also significantly raised in both forms of Hepatitis, when compared to the control. The AST/ALT ratio was reversed (>1) in Group B (Alcoholic Hepatitis) as compared to Group C (Hepatitis B) and normal individuals (<1). It is suggested that GSH, MDA in erythrocyte, Plasma Ascorbic Acid & Serum α -Tocopherol can be made an effective tool in assessing the progression of the liver disease for timely intervention.

Key words: Alcoholic Hepatitis, Ascorbic Acid, α -Tocopherol, Glutathione (GSH), Hepatitis B virus infection, Malondialdehyde (MDA).

INTRODUCTION

Free radicals are small, highly reactive, molecules that are naturally generated in small amounts during the body's metabolic reactions and can react with and damage complex cellular molecules such as fat, protein or DNA. The potential toxicity of free radicals is counteracted by a large number of cytoprotective enzymes and antioxidants that limit the damage. This protective mechanism functions co-operatively in form of a cascade in which antioxidants α -tocopherol, ascorbic acid and reduced glutathione act in combination.¹ A homeostasis between rate of formation of free radicals and the rate of their neutralization if not maintained, oxidative damage accumulates, known as Oxidative Stress.²

The liver plays a central and critical biochemical role in the metabolism, digestion, detoxification and elimination of substance from the body. Products derived from digestion of food are processed, transformed and stored in the liver. It is also the main site of metabolism of exogenous compounds, such as drugs and toxins.³ While there are many causes of liver disease, they generally present clinically in a few distinct patterns, usually classified as hepatocellular, Cholestatic (Obstructive) or mixed.⁴ However all aetiologies lead to liver damage that ranges from acute hepatitis to hepatocellular carcinoma, through many inflammatory processes, apoptosis, necrosis, fibrosis that involve hepatocyte, Kupffer, stellate and endothelial cells. Any insult to the liver cells produce reactive oxygen and nitrogen species (ROS, RNS), which

in turn, is responsible for the induction and progression of liver disease, independent of its aetiology. These free radicals are involved in the transcription and activation of a large series of cytokines and growth factors that, in turn, can contribute to further production of ROS and RNS.⁵ Thus the vicious cycle continues. The extent of cellular damage is manifested by either an increase in the oxidation products or a decrease in the antioxidant levels or both in our body. Hence by measuring the oxidative stress parameters, the extent of liver damage can be assessed.

Globally, many people are addicted to alcoholism resulting in significant number of people suffering from alcoholic liver diseases. The number of people infected with Hepatitis B virus infection, all over the world, is also on the rise. Both the forms of hepatitis lead to hepatic damage of varying intensity, ranging from mild hepatitis to gross hepatic failure. Although a lot of information is available regarding the biochemical changes and Oxidative Stress Parameters in Alcohol Induced Hepatitis (chemical injury) and Hepatitis B virus infection (biological injury) separately, but a comparative evaluation of the above mentioned parameters is lacking. The present study was undertaken to note if any difference in the extent of oxidative stress caused by chemical and biological agents responsible for hepatitis exists and these tools may further be used as markers in determining extent of the disease and guide to the appropriate intervention to be taken in time.

MATERIALS AND METHODS

This prospective study was undertaken by department of Biochemistry, Medical College, Kolkata between January 2012 to December 2012. Permission of the Institutional Ethics Committee was obtained. A preliminary screening of Study subjects was done from the Medicine out patients department of the Institution. Subjects diagnosed to be suffering from Alcohol induced hepatitis and Hepatitis B were selected for the study. A total of 150 subjects (age 25-75 years of either sex) were enrolled into the study. Group A consisted of 50 healthy volunteers and acted as control. Group B consisted of 50 clinically proven alcoholic hepatitis patients while Group C consisted of 50 clinically and serologically proven cases of Hepatitis B patients.

Inclusion Criteria

Group A (Healthy volunteers)

1. Age 25-75years
2. Free of any disease as per routine clinical, biochemical and microbiological Examination
3. Non smoker
4. Non alcoholic

Group B (Alcoholic Hepatitis)

1. Age 25-75years
2. History of regular alcohol intake for at least 2 years
3. Clinical features of Alcoholic hepatitis
4. Laboratory findings of Alcoholic hepatitis
5. No other disease apart from disease under study

Group C (Hepatitis B)

1. Age 25-75years
2. Clinical features of Viral hepatitis
3. Laboratory findings correlating to Viral hepatitis
4. Serological tests positive for Hepatitis B
5. No other disease apart from disease under study

Exclusion criteria

Group A:

1. Alcoholics,
2. Patients on chronic drug therapy
3. Patients suffering from any other disease

Group B:

1. Patients on chronic drug therapy,
2. Patients suffering from any other disease apart from alcoholic Hepatitis,
3. Hepatitis due to any other cause apart from alcoholic Hepatitis.

Group C:

1. Patients on chronic drug therapy,

2. Hepatitis due to any cause other than Hepatitis B,
3. Patients suffering from any other disease apart from Hepatitis B.

Methods

About 5ml of venous blood sample was taken from all the subjects under aseptic precautions. Of the 5ml of blood sample, 2.5 ml was collected in EDTA containing tubes for analysis of erythrocytic Glutathione (GSH), erythrocytic (MDA) and plasma ascorbic acid and the remaining 2.5 ml in plain vacutainers for analyzing Liver Enzymes and α -tocopherol.

Preparation of blood for analysis

Blood sample from the anticoagulant containing vacutainer was centrifuged at 3000rpm for 10 minutes; supernatant plasma was used for ascorbic acid estimation. The buffy coat was discarded. The packed cells were suspended in equal volume of cold phosphate buffer saline and re-centrifuged. The supernatant was discarded. The washing of packed cells was repeated twice, the packed cells were used for analysis of GSH and MDA.⁶ The blood sample from plain vacutainers was centrifuged at 3000 rpm for 10 minutes; the serum was aspirated and was further used for analyzing liver enzymes and α -tocopherol.

The Oxidative Stress Parameters that were assayed included:

- Erythrocyte Glutathione (GSH) was estimated by DTNB method.
 - Malondialdehyde (MDA) in Erythrocyte was evaluated by measuring the Thiobarbituric Acid reacting substances (TBARs).
 - Plasma Ascorbic Acid was estimated by DNPH method.
 - Serum α -tocopherol was estimated by Baker and Flank method.
- ### The Liver Function Tests taken up for study:
- Aspartate Amino Transferase (AST)
 - Alanine Amino Transferase (ALT)
 - Gamma Glutamyl Transferase (GGT)
 - Alkaline Phosphatase (ALP)

The above were measured by using standard methods adapted in the clinical laboratory.

Statistical analysis

The values of all parameters were statistically analyzed using SPSS 15.0 software. A comparison of the mean values of oxidative stress parameters and liver enzymes were done separately between group A and B, Group A and C, group B and C using independent t test.

RESULTS

Table 1 shows the mean \pm S.D of Erythrocyte Glutathione (GSH), Erythrocyte Malondialdehyde (MDA), Serum α -tocopherol and Plasma Ascorbic Acid in Group A as 11.94 \pm 1.86, 8.67 \pm 1.29, 8.72 \pm 2.75, 1.14 \pm 0.21

respectively; Group B were 6.99 ± 2.02 , 12.43 ± 2.01 , 5.41 ± 1.93 , 0.92 ± 0.23 respectively and Group C were 7.69 ± 2.13 , 12.42 ± 2.18 , 8.23 ± 2.45 and 1.07 ± 0.19 respectively. When compared to control, Erythrocyte GSH was significantly low and MDA was significantly high in

both groups B & C ($p < 0.005$). Plasma Ascorbic acid and Serum α -tocopherol levels were decreased in both the groups B & C when compared to Group A but was significantly low ($p < 0.005$) only in group B (alcoholic hepatitis).

Table 1: Comparison of Oxidative stress parameters in group A (Control), group B (Alcoholic Hepatitis) and group C (Hepatitis B); Results expressed as Mean \pm SD

Groups (n=50)	Glutathione (mg/gm Hb)	MDA (nmoles /gm Hb)	α - Tocopherol (mg/l)	Ascorbic Acid (mg/dl)
Group A	11.94 ± 1.86	8.67 ± 1.29	8.72 ± 2.75	1.14 ± 0.21
Group B	6.99 ± 2.02	12.43 ± 2.01	5.41 ± 1.93	0.92 ± 0.23
Group C	7.69 ± 2.13	12.42 ± 2.18	8.23 ± 2.45	1.07 ± 0.19
Gr A Vs Gr B	$p < 0.005$	$p < 0.005$	$p < 0.005$	$p < 0.005$
Gr A Vs Gr C	$p < 0.005$	$p < 0.005$	$p > 0.05$	$p > 0.05$
Gr B Vs Gr C	$p > 0.05$	$p > 0.05$	$p < 0.005$	$p < 0.005$

Table 2 depicts the measure of liver enzymes. Mean \pm S.D of serum Aspartate Transaminase (AST) in groups A, B and C was 28.12 ± 10.21 , 182.36 ± 96.14 , 240.1 ± 192.40 respectively; serum Alanine Transaminase (ALT) were 32.40 ± 11.90 , 147.70 ± 95.98 , 297.50 ± 221.3 respectively; serum Alkaline Phosphatase (ALP) was 150.84 ± 41.49 , 279.24 ± 120.97 , 248.84 ± 91.78 respectively and Gamma Glutamyl Transferase (GGT) 25.38 ± 13.63 ,

138.84 ± 67.12 , 72.42 ± 42.61 respectively. The ratio of AST/ALT was 0.96 ± 0.5 , 1.38 ± 0.37 and 0.81 ± 0.18 respectively. Liver enzymes showed high significance ($p < 0.005$) in group B and C patients when compared individually with group A. When we compared between Groups B and C serum AST and GGT showed significant change ($p < 0.005$) where as changes of serum ALT and ALP were insignificant ($p > 0.05$).

Table 2: Comparison of liver enzymes in group A (Control), group B (Alcoholic Hepatitis) and group C (Hepatitis B); Results expressed as Mean \pm SD

Groups (n=50)	AST (IU/L)	ALT (IU/L)	AST/ALT	ALP (IU/L)	GGT (U/L)
Group A	28.12 ± 10.21	32.4 ± 11.9	0.96 ± 0.5	150.84 ± 41.49	25.38 ± 13.63
Group B	182.36 ± 96.14	147.7 ± 95.98	1.38 ± 0.37	279.24 ± 120.97	138.84 ± 67.12
Group C	240.1 ± 192.4	297.5 ± 221.3	0.81 ± 0.18	248.84 ± 91.78	72.42 ± 42.61
Gr A vs Gr B	$p < 0.005$	$p < 0.005$	$p < 0.005$	$p < 0.005$	$p < 0.005$
Gr A vs Gr C	$p < 0.005$	$p < 0.005$	$p < 0.005$	$p < 0.005$	$p < 0.005$
Gr B vs Gr C	$p > 0.05$	$p < 0.005$	$p < 0.005$	$p > 0.05$	$p < 0.005$

DISCUSSION

Free radical mediated damage to macromolecule plays a crucial role in the pathophysiology of atherosclerosis, inflammation, carcinogenesis, ageing, drug reactions and toxicity.⁷ Liver injury due to acute and chronic abuse has been proved to be dependent on its oxidative metabolism at the cytosolic, peroxisomal and/or microsomal levels.

The present study showed that in the control group which included normal, healthy individuals; Oxidative stress parameters which included Glutathione (GSH), Malondialdehyde (MDA), α - Tocopherol and Ascorbic Acid were found to be in the considered normal range, which was concurring with the data from other studies.^{8,9}

The present study revealed that the patients suffering from liver disease either due Hepatitis B virus infection or excessive alcohol intake showed significant

depletion ($p < 0.005$) of GSH level when compared with controls, which is similar to the findings from other studies.¹⁰ Several factors contribute to the fall in GSH level. Most important is oxidative stress, which consumes GSH. Depletion of GSH renders the cell more susceptible to oxidative stress.¹¹ Decreased Glutathione Reductase (GR) activity may be predominant cause of GSH depletion within RBC leading to serious consequences like increased lipid peroxidation and haemolysis.¹⁰ The other reasons for GSH depletion being, it acts as a co-factor for Glutathione Transferase (GST) during detoxification of xenobiotics including alcohol and also suppression of glutathione synthesis by ethanol.^{12, 13} However, when compared between group B and C, the mean values of GSH showed no significance ($p > 0.05$).

Results also revealed a significant rise ($p < 0.005$) in the MDA levels in both group B and group C patients compared to group A, which is well in term with other

studies.⁵ The raised MDA level reflects the oxidative injury due to alcohol and hepatitis B virus infection, which is attributed to free radical formation that subtracts hydrogen atoms from lipoproteins causing lipid peroxidation, of which MDA is the main product. But the levels of MDA did not vary significantly ($p>0.05$) when compared in group B and group C cases.

The study revealed that although α -Tocopherol levels decreased significantly ($p<0.005$) in alcoholic hepatitis, they showed a slight decrease ($p>0.05$) in patients with Hepatitis B infection.¹⁴

Another relevant finding in the study was that the Ascorbic Acid levels were significantly lowered ($p<0.005$) in Alcoholic patients compared to controls, but the levels were insignificantly reduced ($p>0.05$) in case of Hepatitis B virus infection. These results corroborated well with previous studies.^{10,13} However, a comparison of the Ascorbic Acid levels in group B and group C, showed high significance ($p<0.005$). The possible explanation for this is that apart from the free radicals mediated depletion of ascorbic acid in hepatitis, alcohol directly reduces ascorbic acid levels in blood by impairing its absorption by damaging cell lining the stomach and intestines thus disabling transport into the blood and also by altering Vitamin C metabolism and utilization.^{15,16,17}

The measure of liver enzymes revealed a significant rise in the levels of AST ($p<0.005$), ALT ($p<0.005$), ALP ($p<0.005$) and GGT ($p<0.005$) in both Hepatitis B virus infection and Alcoholics, compared to controls which also confirms the literature evidence.^{18,19,20} The AST/ALT ratio was found to be <1 in controls as well in Hepatitis B, but >1 in Alcoholic Hepatitis patients. This reversal of AST/ALT ratio in Alcoholic Hepatitis patients is correlating with previous studies.^{20,21} This is due to

release of mitochondrial AST by alcohol itself or through its toxicity by its metabolites and/or oxidative stress²¹ While in Hepatitis B, ALT is typically higher than AST because of slower clearance.⁵

This study substantiates a certain degree of oxidative stress and free radicals mediated damage in both Alcoholic Hepatitis and Hepatitis B virus infection. Earlier authors like Albano et al have postulated in their study that the generation of ethanol-derived free radicals, in alcoholism, is mainly due to the increased activity of cytochrome P-450 dependent monooxygenase system (cytochrome P-450 2E1).²² The first breakdown product, acetaldehyde initiates much of the inflammation and fibrosis in alcoholic hepatitis. Acetaldehyde and lipid peroxidation products recruit leukocytes, resulting in production of more inflammatory cytokines. This elicits a vicious cycle of inflammation that culminates in fibrosis and loss of hepatocytes.²³ Thus hepatic ethanol overload is followed both by an increase in reactive oxygen species and by a decline of antioxidants. Obviously the latter can further deteriorate once lipid peroxidation has been stimulated. Researchers Rahamani et al and Waris et al in their works have shown that in Hepatitis B virus infection, HBx, the X gene product of Hepatitis B Virus genome, interacts with an outer mitochondrial voltage-dependent anion channel (VDAC3) and that this association leads to a decrease in the mitochondrial membrane potential and causes elevation of Reactive Oxygen Species.^{24,25}

It was seen that the extent of damage caused due to oxidative stress was similar in both chemically and biologically induced hepatitis patients. Thus it can be recommended that further supplementation of antioxidants, along with other treatment modalities can yield better results and improve the prognosis of the patients.

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