ANTIOXIDANT STATUS, OXIDATIVE STRESS, AND 5'-NUCLEOTIDASE ACTIVITY IN CHRONIC HEPATITIS B AND ALCOHOLIC PATIENTS

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ABSTRACT: Background: The present study was undertaken to estimate the 5'-nucleotidase enzyme in chronic hepatitis B and alcoholics. The oxidative stress, antioxidants and their correlation with 5'-nucleotidase was also included in this study. Methods: The blood samples of 25 subjects (age and sex matched) each from group I (control), group II (alcoholic) and group III (chronic hepatitis B) was taken and centrifuged for separation of plasma for analysis of 5'-nucleotidase. The separated cells were washed thrice with 0.9% w/v cold normal saline and used for the analysis of glutathione, malondialdehyde and superoxide dismutase. Results: The activity of serum 5'-nucleotidase was significantly increased in both chronic hepatitis B and alcoholics. The levels of malondialdehyde were also significantly increased in both chronic hepatitis B and alcoholic patients. The levels of glutathion and superoxide dismutase were significantly decreased in both chronic hepatitis B and alcoholics. Conclusions: From these findings it was concluded that the activity of serum 5'-nucleotidase rises consistently in chronic hepatitis B and alcoholics according to the extent of liver damage, hepatobiliary damage, and biliary stasis and can be a useful marker for diagnosis of hepatobiliary disorders.

Keywords: Alcoholics; Antioxidant Effects; Chronic Hepatitis B; 5'-Nucleotidase; Oxidative Stress.

INTRODUCTION

5'-nucleotidase (5 NT) is an intrinsic membrane glycoprotein, present as an ectoenzyme in a wide variety of mammalian cells, hydrolyzes 5'-nucleotides to their corresponding nucleosides (1). Elevations in the 5 NT serum level appear to be restricted to intrahepatic or extrahepatic obstructive disease with no change in other organs disease (2). It is measured as an indicator of liver damage resulting primarily from interference with the secretion of bile (2). Enzyme activity elevates in all diseases including liver cirrhosis, chronic hepatitis B, chronic alcoholism, neoplasms of liver and bile ducts, benign biliary disease but reaches its highest level in the presence of biliary stasis (3-4). 5 NT is also a sensitive marker for hepatic metastasis either by itself or in combination with other enzymes. The diagnostic value of 5 NT has been shown to be superior to other liver enzymes, especially in liver metastasis. Raised levels of 5 NT activities are found in 92 % of patient with obstructive jaundice, 70 % of patients with parenchymal liver disease and 81 % of patients with hepatic metastasis (5-6). It is also reported that serum 5 NT is clinically useful for differential diagnosis of hepatobiliary and osseous diseases, the enzyme activity being increased only in hepatobiliary disease (7). Because it is a plentiful primary liver enzyme, the 5 NT may be more readily influenced, by minute areas of obstruction than is the alkaline phosphatase (2, 8). Although determination of either oxidants or antioxidant components alone may give information about the oxidative stress, determination of oxidants along with antioxidants is more useful in this context (9). Oxidative stress has been implicated in liver cirrhosis due to chronic hepatitis B. Studies
suggested that evidence of oxygen free radical is found early in the development of fibrosis and cirrhosis of liver due to chronic hepatitis B (10). Patients suffering from liver disease either due to non-alcohol or excessive alcohol intake shows depletion of antioxidants such as glutathione (GSH) and superoxide dismutase (SOD) and increased concentration of product of lipid peroxidation such as MDA (11-19). Depletion of antioxidants such as GSH occurs as a result of decreased production from the diseased liver and consumption of antioxidants due to increased oxidative stress. Depletion of antioxidants renders the cell more susceptible to oxidative stress. Antioxidant and stress related enzymes might be able to determine the degree of liver damage (16-19). Hepatic fibrogenesis in alcoholic liver cirrhosis is an intricate process, which appears to involve a metabolic products of ethanol oxidation; cytochrome P450 induction, enhanced oxidative stress, depletion of antioxidant defenses, lipid peroxidation, generation of aldehydic products, the effects of mitogenic and fibrogenic cytokines and complex interactions between liver parenchymal and non-parenchymal cells, with hepatic stellate cells (Ito cells, hepatic lipocyte or fat storing cell) are now recognised as the primary source of extracellular matrix (20-21). There is evidence that the diseased liver of patients with cholestatic liver disease is exposed to oxidative stress associated with increased lipid peroxidation involving intraorgan generation of reactive oxygen species (ROS), possibly mediated by endotoxins, bile acids and accumulation of degradative products of lipid peroxidation, such as lipid peroxides and MDA (20, 22). Alcohol promotes the generation of ROS and / or interferes with the body’s normal defense mechanism against these compounds (23). Studies suggest that evidence of oxygen free radicals is also found early in the development of fibrosis and cirrhosis of liver due to chronic hepatitis B (10).

The present study is therefore undertaken to estimate the 5’-NT enzyme in liver cirrhosis due to chronic hepatitis B and alcoholics. The oxidative stress, antioxidants and their correlation with 5’-NT was also included in this study.

MATERIALS AND METHODS

Subjects
This study was conducted on 3 groups of 25 subjects each in the age group of 35-70 years. 13 males and 12 females were used in each group.

Group I: Consisted of 25 healthy individuals (13 males and 12 females) between the age group of 35-70 years having no history of any liver disease and no history of alcohol intake in the past or present, hereafter referred to as ‘control group’.

Group II: Consisted of 25 individuals (13 males and 12 females) between the age group of 35-70 years with a history of 10 years or more of alcohol intake without any history of liver disease or other hepatobiliary disorders hereafter referred to as ‘alcoholic group’.

Group III: Consisted of 25 patients (13 males and 12 females) diagnosed to have chronic hepatitis B with history of jaundice, hereafter referred to as ‘chronic hepatitis B’.

Selection of Cases
The test group III for this study consisting of 25 patients of chronic hepatitis B were taken from the following hospitals in Patna, India.
1) Nalanda Medical College Hospital
2) Patna Medical College Hospital
3) Kurji Holy Family Hospital
4) Tripolia Hospital
5) Arvind Hospital

The test group II for the study consisting of 25 individual with history of alcoholism were taken by personal contact from known alcoholics. While choosing the subject for the test and control groups, care was taken to eliminate subjects with habits like smoking, tobacco chewing, chronic inflammatory disease like tuberculosis, rheumatoid arthritis, diabetes mellitus and malignancy, all of which play a vital role in contributing to oxidative stress injury. Care was also taken to eliminate those with any bone disease or any other clinical conditions that might be involved in raised 5’-nucleotidase activity.

Approval to carry out these studies on human subjects was obtained from Institutional Clinical Ethics Committee of Nalanda Medical College, Patna, India and their guidelines were followed for these studies.

Sample collection
2 ml of venous blood was collected in a plain vial for serum separation for the analysis of 5’-nucleotidase. 5 ml of venous blood was collected in separate EDTA containers from the median cubital vein or basilic vein of the study subjects under strict aseptic precautions. From this 0.2 ml of whole blood was used for glutathione estimation; the rest of the sample was centrifuged at 3000 rpm for 10 minutes within 3 h of collection. Plasma was discarded. The sediment was used to prepare the erythrocyte suspension and used for the assay of malondialdehyde (MDA) and superoxide dismutase (SOD).

**Preparation of Erythrocyte Suspension**

The sediment separated was washed thrice with 0.9 % cold normal saline, after which they were suspended in an equal volume of the same saline solution. This was then stored at 50 % cell suspension at 4-5°C and was used for the assay of MDA and SOD.

**5’-Nucleotidase determination**

Serum 5’-nucleotidase enzyme was estimated using reported method (5).

Two test tubes were set up as follows:

a. Total activity: 0.2 ml of serum was added, 0.1 ml of 0.02M Manganous sulphate and 1.5 ml of 40 mM, pH 7.5 Barbitone buffer.

b. Non—specific alkaline phosphatase activity: 0.2 ml of serum was added, 0.1 ml of 0.02 M manganous sulphate, 1.3 ml of 40 mM, pH 7.5 barbitone buffer and 0.2 ml of 0.1M nickel chloride.

c. Both test tubes warmed to 37°C then was added 0.2 ml, 10mM adenosine 5’-Phosphate to each test tube and incubated at 37°C for 30 minutes. Then 2 ml of 10% Trichloroacetic acid (TCA) was added, mixed well and stand briefly then centrifuged. 2 ml of supernatant were taken (=0.1 ml serum) for estimation of inorganic phosphorous. For the blank and standard 1 ml of water and 1 ml of the phosphate standard (stock solution containing 6 mM/lit) were taken for use, each with 1 ml of TCA added. To all four tubes 3 ml 2M, pH 4.0 acetate buffer, 0.5 ml of 5% ammonium molybdate and 0.5 ml metol (2 gm metol and 10gms sodium sulphite in water made upto 100ml) mixed, allowed to stand for 10 minutes and then reading was taken using a red filter or at 680 nm.

**Malondialdehyde (MDA)**

Red cell lipid peroxidation was studied as thiobarbituric acid (TBA) reaction products. The method of Stocks and Dormandy was followed with certain modifications (24).

1 ml of erythrocyte suspension was added to 8.5 ml of 0.9 % normal saline and mixed well. Next 0.5 ml of 0.44 M H2O2 was added. From the above mixture, 2.5 ml of aliquot was immediately taken, to which 1 ml of 28 % TCA (trichloroacetic acid), in 0.1 M sodium metaarsenite was added. This was mixed well and allowed to stand for 10 minutes, after which it was centrifuged. 3 ml of the supernatant was then taken to which 1 ml of 1% TBA in 50 mM NaOH was added. This was then kept in a boiling water bath for 15 min and immediately cooled under tap water. The pink chromogen was read at 535 nm in a spectrophotometer. Values were expressed as nanomoles of MDA formed per / dl of RBC, taking the molar extinction co-efficient as 1.56 x105.

**Glutathione (GSH)**

Whole blood glutathione level was measured by the method of Beutler et al. (25).

To, 0.2 ml of whole blood, 1.8 ml of distilled water was added and mixed; 3 ml of precipitating solution was added, mixed and allowed to stand for 10 min at room temperature. This mixture was then centrifuged. To 2 ml of supernatent, 8 ml of phosphate solution (0.4M) and 1 ml of DTNB reagent was added. The absorbance was read at 412 nm.

**Superoxide Dismutase (SOD)**

The method of Fridovich was followed for estimation of SOD (26). Inhibition of the reduction of nitroblue tetrazolium (NBT) by superoxide radicals, generated by the illumination of riboflavin in the presence of oxygen an electron donor. Methionine was used as a basis for the assay of superoxide dismutase.

**Preparation of Hemolysate**

This was done by the method of McCord & Fridovich (27).

To 1 ml of erythrocytes (washed with 0.9 % normal saline) was added 1 ml of deionised water to lyse the cells. To this was added 0.5 ml of distilled ethanol followed by 0.3 ml of chloroform and mixed well. Allowed to stand for 15 min. Now added 0.2 ml of H2O and centrifuged at 40C. The supernatant contains SOD activity and was used for the assay of SOD, after dilution with potassium phosphate buffer (pH 7.8, 0.05 M) 0.1 ml of
hemolysate was diluted with 1.9 ml of potassium phosphate buffer. It was final diluted hemolysate that was used in the procedure given below.

Four test tubes were taken and labeled as ‘Test’, ‘Control’, ‘Test blank’ and control blank’ respectively. To the test 2.9 ml of reaction mixture with NBT containing 149 mg of methionine, 4.93 ml of NBT (1mg./ml), 0.63 ml of riboflavin (1mg/ml), and made up to 100 ml with potassium phosphate buffer (pH 7.8/0.05M) and 0.1 ml of diluted hemolysate was added. To the ‘Test Blank’ 2.9 ml of same reaction mixture without NBT and 0.1 ml of diluted hemolysate was added.

To the ‘Control Test’ 2.9 ml of the same reaction mixture with NBT and 0.1ml of potassium phosphate buffer (pH 7.8/0.05M) was added.

To the ‘Control Blank’ 2.9ml of the same reaction mixture without NBT and 0.1ml of potassium phosphate buffer(pH 7.8/0.05M) was added. Each of the these mixtures were now taken in a 10 ml beaker. The beakers were kept in an aluminium foil lined box fitted with a 15 W fluorescent lamp for 10 minutes. The absorbance was read at 560 nm in a spectrophotometer for all the four beakers.

**Estimation of Hemoglobin**

The hemoglobin content of erythrocytes was determined by the cyanmethaemoglobin method (28). Hemoglobin was treated with a reagent containing potassium ferricyanide, potassium cyanide and potassium dihydrogen phosphate (Drabkins Reagent). The ferricyanide oxidizes hemoglobin to methaemoglobin which is converted to cyanmethaemoglobin by the cyanide. Absorbance was measured at 540 nm in a spectrophotometer.

**Statistical Analysis**

All the biochemical parameters were compared using Fishers F test for analysis of variance (ANOVA). Fischer’s ‘F’ test was done for analysis of variance (ANOVA). The statistical software “SPSS Version 11” (statistical package for social sciences) was used for this purpose.

**RESULTS AND DISCUSSION**

In this study, the serum 5’- nucleotidase level, the product of lipid peroxidation and antioxidant level were compared between chronic hepatitis B patients, alcoholics and normal individuals taken as controls of the same age group.

5’-Nucleotidase (5’-NT)

The activity of this enzyme is distinguished from that of a non specific alkaline phosphatase by nickel inhibition and the inorganic phosphate released from adenosine monophosphate used as a substrate (29). This method was chosen due to its reliable analytical qualities and is appropriate for routine diagnostics (4). Serum 5’-NT activity in this study was highest in chronic hepatitis B group. Relatively high but less than chronic hepatitis B and more than control in alcoholic group, and it was found to be lowest in controls (Table 1). The values were statistically highly significant (P<0.001) on comparison between the three groups.

Its highest level (20.56 ± 2.16 IU/lit) in chronic hepatitis B group appeared to be indicative of greater sensitivity for intrahepatic obstruction or liver cell damage (2). Because it is a plentiful primary liver enzyme the 5’-NT may be more readily influenced by minute areas of obstruction. Its high level in chronic hepatitis B group is suggestive of intrahepatic obstruction of biliary canaliculi due to fibrosis as a result of liver cell injury. Measurement of 5’-NT has been used in the early detection of pericholangitis in patients with ulcerative colitis (Smith et al., 1996; Pagani & Pantighini, 2001). 5’-NT activity is also elevated early in congestive heart failure (CHF) with secondary passive congestion in the liver. 5’-NT, when abnormal in the non icteric patient with other normal liver function tests, would be highly suggestive of hepatic tumor (1-2). Its relatively higher activity (17.62 ± 1.73 IU/lit) in the alcoholic group found support in a previous report that increased nucleic acid catabolism in chronic alcohol toxicity lead to its elevation as the enzyme is related to the break down of nucleic acid, especially m-RNA. Chronic alcoholism produces a wide spectrum of liver and other organ diseases depending on the amount and duration of alcohol intake. The effect on liver range from fatty change to hepatitis and cirrhosis and the activity of 5’-NT will also be ranging as per the damage done to the liver cells and bile canicular membranes resulting in mild to moderate biliary stasis.

Of importance is the fact that increased 5’-NT activity in the serum of patients identified with high probability of the presence of liver disease confirms that any increase in 5’-NT activity is highly specific for hepatobiliary disease (1-2).
It has been observed and confirmed by several studies that serum 5'-NT is clinically useful for differential diagnosis of hepatobiliary and osseous diseases, the enzyme activity being increased only in hepatobiliary diseases (2).

**RBC Malondialdehyde (MDA)**
As a measure of oxidative stress, MDA, the end product of lipid peroxidation was estimated by the TBA method because of the ease with which this method can be used as an indicator of lipid peroxidation and free radical activity in biological samples.

RBC MDA levels in this study were highest in chronic hepatitis B group ($749.36 \pm 57.77$ nanomoles/100 ml) as shown in Table 2. MDA level was relatively lower in alcoholic group ($608.16 \pm 71.14$ nanomoles/100 ml), and it was found to be lowest in controls ($504.24 \pm 95.19$ nanomoles/100 ml). The values were statistically highly significant (P<0.001) on comparison between 3 groups.

Its high levels in both chronic hepatitis B and alcoholics were suggestive of increased oxidative stress in patients of chronic hepatitis B as well as in alcoholics (23). These findings agreed with the result of several studies which have confirmed involvement of free radicals in the pathogenesis of liver injury in case of chronic hepatitis B and chronic alcoholism (30-31).

**Glutathione (GSH) and Superoxide dismutase (SOD)**

Blood glutathione levels in this study were highest in control group ($57.52 \pm 5.96$ mg/dl), relatively less in alcoholic group ($43.75 \pm 5.79$ mg/dl) and lowest in chronic hepatitis B group ($23.23 \pm 6.97$ mg/dl) as shown in Table 3. The values were statistically highly significant (P<0.001) when a comparative analysis was done between the 3 groups.

The enzyme superoxide dismutase (SOD) activity was measured by the method of Fridovich.

The RBC superoxide dismutase (SOD) values were also highest in control group ($8075.60 \pm 399.85$ units/g), relatively less in alcoholic group ($6228.72 \pm 631.16$ units/g) and lowest in chronic hepatitis C group ($4945.64 \pm 442.44$ units/g) as shown in Table 4. The results were statistically very highly significant (P< 0.001) on comparative analysis.

Glutathione was chosen in this study because it represents the most abundant natural antioxidant in our body. Glutathione is of major importance in the reduction of hydrogen peroxide and organic peroxides (e.g. lipid peroxides) in a reaction that is catalyzed by selenium containing GSH peroxidase and by other proteins that also exhibit GSH-S-transferase activity (32). It is the major endogenous antioxidant produced by the cell. It participates directly in the neutralization of free radicals, reactive oxygen compounds and maintains exogenous antioxidants such as Vitamins C and E and also plays a role in the detoxification of many xenobiotics (23). It is an essential component of human immune response. It is also involved in the synthesis and repair of DNA.

Superoxide dismutase was chosen in this study as it plays a role in the removal of hydrogen peroxide (H2O2) formed in red cells and because hemoglobin and SOD have been shown to be in close association in red cells. In addition to this, some studies have documented that SOD is one of the most important enzymes in the front line of defense against oxidative stress and is more effective in protecting the RBCs against damage by exogenous hydrogen peroxide, especially at higher concentration.

The low levels of GSH and SOD in cellular and extracellular fluids reduce their oxygen derived free radical scavenging capacity making the tissues more vulnerable to oxygen derived free radical (ODFR) damage (33). The low level of GSH and SOD in chronic hepatitis B and alcoholics is indicative of increased oxidative stress in patients of chronic hepatitis B as well as alcoholics (30).

Increased lipid peroxides (MDA) and a decrease in antioxidants (GSH and SOD) in red blood cells of chronic hepatitis B patients shows an altered oxidant and antioxidant status (30).

Several factors contribute to the fall in GSH and SOD level in alcoholics and chronic hepatitis B patients, most important being oxidative stress, which occurred in this study. Depletion of GSH renders the cell more susceptible to oxidative stress.

Decrease of GSH in chronic hepatitis B patients is probably related to a reduced synthesis of the tripeptide by the diseased liver. A greatly increased production of reactive oxygen metabolites might overpower the capacity of the tissue to synthesize or regenerate sufficient amounts of GSH resulting in a decrease in GSH concentration (16, 19).

Low GSH in the presence of good nutrition in chronic hepatitis B and alcoholics suggest a decreased efflux of GSH from liver (16).
Our results show that antioxidant barrier in chronic hepatitis B is impaired, associated with decrease of glutathione level and also activity of the antioxidant enzyme SOD (18-19). Alcohol has been shown to deplete GSH levels, particularly in the mitochondria, which normally are characterized by high levels of GSH needed to eliminate the ROS generated during activity of the respiratory chain. Mitochondria can not synthesize GSH but import it from the cytosol (23).

Reduced glutathione in chronic hepatitis B and alcoholics was consistent with other reports (19). This observation may be explained on the basis of (i) its utilization in scavenging the free radicals, (ii) its involvement in maintaining non-GSH critical protein sulfhydrolys in reduced state, (iii) acting as co-factor for glutathione – S –transferase (GST) during detoxification of xenobiotics including alcohol, (iv) oxidation of glutathione to its oxidized form by glutathione peroxidase in detoxification of hydrogen peroxide and/or lipid peroxides, (v) suppression of glutathione synthesis by ethanol. Greater degree of reduction in GSH in alcoholic cirrhotsics may be because of synergistic action of alcohol and cirrhosis of liver.

Lowest level of glutathione found in our study in chronic hepatitis B indicate that glutathione antioxidant system in chronic hepatitis B is imbalanced and support the hypothesis that oxidative stress plays an important role in the development of liver cirrhosis (19). Low levels of SOD activity observed in chronic hepatitis B and alcoholics are indicative of oxidative stress that may be responsible for maximal destruction in liver architecture. A markedly lower SOD activity observed in chronic hepatitis B indicates that the oxidative stress is much profound in chronic hepatitis B than alcoholics, as liver cirrhosis is a common outcome of a variety of chronic liver diseases (22).

Significant increase in MDA levels in chronic hepatitis B and alcoholics suggest that chronic hepatitis B and alcoholic patients are subjected to more oxidative stress. Alcoholic cirrhotsics seem to have still greater degree of oxidative stress which may be due to compounding effect of alcohol. A decreased level of both GSH and SOD in the present study suggests that with increase in oxidative stress, there is corresponding proportionate decrease in antioxidant defense system in chronic hepatitis B and alcoholics. Antioxidants and stress related enzymes might be able to determine the degree of liver damage. The differences between the groups might be based on the type of liver pathology rather than its etiology (i.e. alcohol and non–alcohol related causes).

The implication of oxidative stress in alcoholic liver damage gives a rationale to the clinical application of therapies aimed to prevent or reduce ethanol induced oxidative damage by antioxidant compounds. The parameters of lipid peroxidation and antioxidant defenses may be useful surrogate markers for monitoring patients with liver disease during hepatoprotective treatment (15). Mapping the broader time structure with age and multifrequency rhythm characteristics of antioxidants and pro-oxidants is needed for exploring their putative role as markers in the treatment and management of liver cirrhosis due to chronic hepatitis B and alcohol.

In summary, our results show that the antioxidant potential in chronic hepatitis B and alcohol is unbalanced in antioxidant defense system in chronic hepatitis B and alcoholics the MDA level is increased, both GSH and SOD levels are decreased. These results are consistent with the finding of several studies already discussed.

The positive significant correlation between MDA and 5’-nucleotidase in chronic hepatitis B group suggest that in case of chronic hepatitis B in addition to increased level of MDA due to increased lipid peroxidation as a result of oxidative stress there is simultaneous consistent increase in the activity of 5’-nucleotidase as a result of biliary obstruction due to fibrosis of the canalicular membranes and sinusoids in the liver tissue. There is also positive significant correlation between SOD and 5’-nucleotidase in alcoholic group. This finding may be explained on the basis that in case of alcoholics the SOD activity was relatively lower than controls but more than chronic hepatitis B and the 5’-nucleotidase activity was also relatively lower than chronic hepatitis B but more than controls.

From these findings we can conclude that the activity of 5’-nucleotidase rises consistently in chronic hepatitis B and alcoholics according to the extent of damage to the liver and there is an increase in oxidative stress and decrease of antioxidant status in both chronic hepatitis B patients and alcoholics but the extent of oxidative stress is more profound in chronic hepatitis B than alcoholics.

CONCLUSION

This study attempts to establish the extent of rise of serum 5’-Nucleotidase activity in chronic hepatitis B patients and alcoholic in respect of normal individuals (controls). This study also attempts to establish the extent of oxidative stress and antioxidant status in chronic hepatitis B patients and alcoholics.
The results of the above study can be summarized as follows:
1. The activity of serum 5'- NT was significantly increased in both chronic hepatitis B and alcoholics but increase was very high in chronic hepatitis B patients.
2. The levels of MDA were significantly increased in both chronic hepatitis B and alcoholics but increase was very high in chronic hepatitis B patients.
3. The levels of GSH were significantly decreased in both chronic hepatitis B and alcoholics but decrease was more in chronic hepatitis B patients.
4. The levels of SOD were significantly decreased in both chronic hepatitis B and alcoholics but decrease was more in chronic hepatitis B patients.

From these findings we can conclude that the activity of serum 5'- NT rises consistently in chronic hepatitis B and alcoholics according to the extent of liver damage, hepatobiliary damage, and biliary stasis and can be a useful marker for diagnosis of hepatobiliary disorders. We can also conclude that there is an increase in oxidative stress and decrease of antioxidant status in both chronic hepatitis B of liver patients and alcoholics but the extent of oxidative stress is more profound in chronic hepatitis B than in alcoholics with or without any history of hepatobiliary diseases. The parameters of lipid peroxidation and antioxidant defense may be useful markers for monitoring patients with hepatobiliary disorders.

ACKNOWLEDGEMENT
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APPENDIX

Table 1: Mean 5'-nucleotidase of control, alcoholic and chronic hepatitis B group

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Mean (IU/l)</th>
<th>SD</th>
<th>P value</th>
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<tr>
<td>Control</td>
<td>25</td>
<td>12.98</td>
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<td>Alcoholics</td>
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<td>22.56</td>
<td>2.16</td>
<td>&lt;0.001</td>
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</table>

Name of the test used—Fishers ‘F’ test for analyses of variance (ANOVA), N= No. of subjects, SD=standard deviation, ‘P’ value=probability, HS=highly significant.

Table 2: Mean MDA levels of control, alcoholic and chronic hepatitis B group

<table>
<thead>
<tr>
<th>Groups</th>
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<th>Mean (Nanomoles/100 ml)</th>
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<td>71.14</td>
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Table 3 Mean glutathione level of control, alcoholic and chronic hepatitis B group

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<td>5.79</td>
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<td>Chronic hepatitis B</td>
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<td>6.97</td>
<td>&lt;0.001</td>
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Table 4 Mean SOD level of control, alcoholic and chronic hepatitis B group

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<th>Groups</th>
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<th>Mean (units/g)</th>
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