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BIOCHEMICAL DIAGNOSIS OF ALCOHOLISM

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ABSTRACT

Medically diagnosed alcoholics can be differentiated reliably from non-alcoholics using clinically laboratory tests. In the present study, patients with liver diseases either due to alcohol or without alcohol compared with a group of normal healthy persons. Heavy drinkers showed significantly lower body weight and percent body fat, and low BMI compared with other groups. The percentage of hemoglobin and total number of RBC were found to be significantly decreased, whereas mean corpuscular volume (MCV) significantly increased in alcoholic liver disease (ALD). Hyperbilirubinemia, hyperuricemia and hypoalbuminemia correlate with alcohol intake. Albumin / globulin ratio significantly decreased in ALD. In acute liver injury AST/ ALT ratio is ≤ 1.0, whereas in alcoholic hepatitis it is always > 1.0. Moderately elevated level of ALP and high GGT values are good discriminator of alcoholic patients. Alcohol-induced liver injury is linked to oxidative stress as observed by decreased level of reduced glutathione and ascorbic acid, and increased level of thiobarbituric acid reactive substances.

KEY WORDS

Alcohol, Biochemical marker, g-Glutamyltransferase, Aminotransferase, Glutathione

INTRODUCTION

Alcohol abuse is a major public health problem in Sikkim. In comparison to national average of alcohol consumption (17%), the average value for Sikkim is higher (51%). In addition to this, the relapse to alcohol abuse, after de-addiction, is also as high as 35% (1). Severity of liver damage is often correlated with the amount of alcohol consumption in patients with a history of heavy alcohol abuse (2). However, alcoholic liver disease (ALD) not only depends on the total amount of alcohol consumed; drinking patterns and type of alcoholic beverage ingested are also playing important role in the development of ALD (3). Most patients develop fatty liver, which reverses on withdrawal of alcohol and is unlikely to progress to liver cirrhosis. In the liver, the acetaldehyde produced by oxidation of ethanol interacts with lipids and proteins, generating free radicals and impairing protein function (4).

Physicians have long sought an accurate and inexpensive means of identifying persons who consume excessive amounts of ethyl alcohol. It has been reported that medically diagnosed alcoholics can be differentiated reliably from non-alcoholics using clinical laboratory tests. Moreover, distinguishing alcoholic from non-alcoholic liver disease has important implications for treatment and management (5). The most widely used tests for this purpose are standard liver function tests, g glutamyl transferase (gGT), and mean cell volume (MCV) using an electronic cell counter. Although gGT is a sensitive indicator of excessive alcohol intake, it is also raised in a variety of non-alcoholic liver diseases (6).

In the present study we have investigated changes in biochemical parameters in patients with alcoholic liver injury (both moderate and heavy drinkers), and non-alcoholic liver diseases. These parameters were compared with normal values obtained from normal healthy people. Biochemical parameters included common laboratory tests.

MATERIALS AND METHODS

A total of 181 subjects were studied: 81subjects (43 males and 38 females) with alcoholic liver disease (ALD); 45 patients (24 males and 21 females) with non-alcoholic liver disease (NALD); and 55 normal healthy presenters (33 males and 22 females) without any reported disease. Alcoholic patients were further classified as described by Paton (7) into: a) High alcohol intake group (ALD-H; those had been drinking more than 80g alcohol per day for at least one year), and b) Moderate alcohol intake group (ALD-M; those had been drinking less than 80g alcohol per day). 24 male and 22 female were in High alcohol intake group;

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while 19 male and 16 female were in Moderate alcohol intake group. These patients were selected from those who had visited Central Referral Hospital, Gangtok; and categorized on the basis of oral questionnaires following Michigan Alcoholism Screening Test (MAST) and laboratory findings. All participants were within same age group, similar economic status, non-smokers, and similar dietary habits. The individuals who were diabetic, or had essential hypertension, thyroid disease, nephritic disease, and pregnant women were excluded from this study. Consent was obtained from every subject.

Their mean age, height, and weight were noted. Body mass index (BMI) was measured by the formula: BMI= weight in kg/ (Height in meters)^2.

Percent fat in body was calculated from BMI using the following formula of Foster, (8).

In males, % fat = 1.218 (BMI) - 10.13; and in females, % fat = 1.48 (BMI) – 7.

Blood was collected after an overnight fast in heparinised and unheparinised tubes. Plasma, serum and hemolysate were prepared and stored at -4°C for further analysis as given below.

Hemoglobin concentration was determined using cyanmeth reagent (9) and other hematological parameters were measured. Urea in serum was estimated by the kinetic method (10) and creatinine level was estimated by rate of change in absorbance using alkaline picrate (11). Uric acid was estimated as described by Gochman and Schmitz (12). Bilirubin (total, conjugated and nonconjugated,) levels of serum were measured by the method of Jendrassik and Groff (13). Serum albumin and protein levels were measured by using bromocresol green (14) and biuret reagent (15) respectively. Serum phosphatase levels (alkaline phosphatase, EC 3.1.3.1; acid phosphatase, EC 3.1.3.2) activity was determined by the method as employed by Linhardt and Walter (16). Activities of alanine aminotransferase (EC 2.6.1.2) and aspartate aminotransfease (EC 2.6.1.1) in serum were determined by the method as described elsewhere (17). The g-glutamyltransferase activity of serum was assayed by the method of Gowelock (18). Ascorbic acid content was determined using 2,4-dinitrophenyl hydrazine (19). Extent of lipid peroxidation (20) and reduced glutathione content (21) were also determined. All chemicals used were of analytical grade.

Results have been expressed as mean±SEM (standard error). Statistical significance was determined by Student's 't' test for unpaired data. The values of significance were evaluated with 'p'values. The difference were considered significant at $p<0.05$.

RESULTS AND DISCUSSION

In the present study, patients with liver diseases either due to alcohol or without alcohol were compared with a group of normal healthy persons. Their mean age, height, weight, body mass index (BMI), and percent body fat were determined (Table 1). Heavy drinker ALD patients had significantly low body weight, low BMI, and lower percent body fat compared to other groups. Addolorato *et al* (22) observed that alcoholics, as compared with social drinkers showed a lower body weight due essentially to fat mass reduction. The same may be true for the present study also. World *et al* (23) concluded that a fall in body weight was the best clinical indicator of apparently continuing alcohol abuse. Reduced adipose tissue is one cause of lower body weights in such patients. Loss of adipose tissue in chronic alcoholics who continue to drink is probably due to simultaneous inadequate nutritional intake. As ethanol can supply >50% of the dietary energy in alcoholics, body composition alterations may easily occur (24). BMI was highly correlated with PBF in the patient population studied.

Alcohol has a variety of pathologic effects on hematopoiesis. It directly damages erythroid precursors, thereby contributing to macrocytosis and the anemic state of chronic alcoholics. Ethanol induces sideroblastic anemia, perhaps by direct interference with heme synthesis. Further, chronic ingestion of alcohol can lead to various types of hemolytic anemia caused by alterations in the erythrocyte membrane lipids that occur in association with alcoholic liver disease (25). In the present study (Table 2), the percentage of hemoglobin and total number of RBC were found to be significantly decreased, whereas mean corpuscular volume (MCV) significantly increased in alcoholic liver disease with heavy alcohol intake in comparison to other groups. Because the red blood cell survive for 120 days after it has been released into the circulation, an MCV result may remain elevated for up to 3 months after a person has stopped drinking. But increase in MCV has been reported in other conditions such as thyroid disease, folate deficiency, recent blood loss and a number of hematological conditions, and liver disease from other causes (5, 26). Thus it cannot be taken as a sole parameter for alcoholic liver disease. However, no significant variation in either of the groups tested was observed in case of polymorphonuclear cells, lymphocytes, eosinophil and ESR values.

Patients with various forms of liver disorders showed hyperbilirubinemia. In the present study, there is an increase of two and seven times increase in conjugated bilirubin level of serum of moderate and heavy alcoholic patients, respectively. However, the increase in unconjugated bilirubin levels of serum was far less (Moderate: 2% and Heavy: 71%) in both the

groups of alcoholics (Table 3 and Fig.1). Ahlgren *et al* (27) also found elevated serum bilirubin level in non-alcoholic liver disease. They further observed that the maximum patients were showing either elevated monoconjugated or albumin-bound bilirubin in serum. Uptake and excretory functions of liver is constrained depending upon the increment of bilirubin level of serum.

The urea level was found to be within normal range in all the tested groups (Table 3). Though the increases in creatinine level of non-alcoholic liver disease group was found to be statistically significant in comparison to other groups, but no significant change was observed in alcoholic liver disease groups when compared with normal healthy persons (Table 3). Significant increase in uric acid level was observed in alcoholic liver disease groups with respect to other two groups, and high alcohol intake group showed significantly higher uric acid value when compared with moderate alcohol intake [ALD-M] group (Table 3). Therefore, the bilirubin level in association with urea, creatinine, and uric acid may be used as markers in combination for ALD.

Though only ALD-H group showed significant increase in globulin level in comparison to other groups (Fig 2), the albumin level was found to be significantly decreased in all the tested groups when compared with normal group. In ALD-H group hypoalbuminemia was observed. Like the albumin values, same pattern of alterations was observed in albumin: globulin ratios (Table 3 and Figure 2). Common features of chronic alcoholic liver disease are progressive hypoalbuminemia (5, 28). Acute exposure to alcohol depressed albumin. The decrease in serum albumin

Table 1. Demographic profile: Age, Height, Weight, Body mass index (BMI) and body percent fat of normal healthy persons, and patients with non-alcoholic liver disease and alcoholic liver disease.

Group	Age (yrs)	Height (cm)	Weight (kg)	BMI (Kg/m2)	Body fat (%)
Normal Healthy Persons (n=55)	38.37 ± 1.14	161.49 ± 0.61	62.29 ± 0.65	23.85 ± 0.25	23.12 ± 0.46
Non Alcoholic Liver Disease (n=45)	39.86 ± 1.84	161.68 ± 0.65	60.48 ± 0.65	23.34 ± 0.25	22.02 ± 0.66
Alcoholic Liver Disease (Moderate Alcohol Intake) (n=35)	40.53 ±1.74	161.81± 0.88	61.56 ± 0.56	23.61 ± 0.24	22.68 ± 0.85
Alcoholic Liver Disease (High Alcohol Intake) (n=46)	44.73 ± 1.83	161.68 ± 0.65	58.25 ± 0.54*#@	22.24 ± 0.21*#@	20.96 ± 0.61*

Values are mean ± SEM of number of observations (n). * indicates $p < 0.05$ when compared with normal healthy control, # indicates $p<0.05$ when compared with non alcoholic liver disease, and @ indicates $p<0.05$ when compared with alcoholic liver disease with moderate alcohol intake.

Table 2. Hematological profile of normal persons, and patients with alcoholic liver disease and non-alcoholic liver disease

	Normal Healthy Persons (n=55)	Non Alcoholic Liver Disease (n=45)	Alcoholic Liver Disease (Moderate Alcohol Intake) (n=35)	Alcoholic Liver Disease (High Alcohol Intake) (n=46)
Hb (gm%)	14.91± 0.11	14.75± 0.12	14.23 ± 0.15*#	12.86 ± 0.26*#@
RBC count (x 10^6) (cells / cu.mm.)	5.02 ± 0.04	5.04 ± 0.04	4.84 ± 0.03*#	4.49 ± 0.04*#@
Polymorpho-nuclear cells (%)	59.18 ± 0.41	58.7 ± 0.38	57.76 ± 0.72	57.68 ± 0.72
Lymphocytes (%)	35.89 ± 0.51	36.04 ± 0.59	36.34 ± 0.75	34.85 ± 0.54
Eosinophils (%)	2.89 ± 0.23	3.29 ± 0.22	3.25 ± 0.26	3.26 ± 0.19
ESR (mm/ h)	2.53 ± 0.19	2.5 ± 0.22	2.75 ± 0.28	2.74 ± 0.24
MCV (fl)	84.36 ± 0.70	91.09 ± 0.53*	94.63 ± 0.64*#	101 ± 0.6*#@

Values are mean ± SEM of number of observations (n). * indicates $p < 0.05$ when compared with normal healthy control, # indicates $p<0.05$ when compared with non alcoholic liver disease, and @ indicates $p<0.05$ when compared with alcoholic liver disease with moderate alcohol intake.

level is attributed to nutritional status of the subjects (5, 29). On the other hand, the albumin is a potential subject of formation of adduct by acetaldehyde, an alcohol metabolite. This albumin or other protein adducts can stimulate the formation of immunoglobulins, thus causing a rise in serum globulin level (5, 30). In the present study significant decrease in total protein was observed in all the tested groups when compared with normal healthy group, and heavy drinkers were found significantly low value compared to any other groups. Ethanol consumption slows down the rate of hepatic protein catabolism.

Significant increase in alkaline phosphatase, g-glutamyltransferase, aspartate aminotransferase and alanine aminotransferase activities were observed in alcoholics in comparison to healthy controls as well as in heavy drinkers in comparison to moderate drinkers (Table 4). A similar change was also noted in case of ratio of aspartate aminotransferase and alanine aminotransferase (Figure 3). Alanine amino transferase (ALT) and aspartate amino transferase (AST) are present in high concentration in hepatocytes. These enzymes leak into the circulation when hepatocytes or their cell membranes are damaged. Although these aminotransferases are sensitive indicators of liver cell damage, neither alone is an ideal marker. In the non-alcoholic liver disease, the activities of AST, and ALT were increased significantly in comparison to normal healthy individuals. Though significant increase in activities of these enzymes were also observed in the moderate and heavy alcohol intake groups in comparison to normal healthy individuals, the rise was significantly less than the non alcoholic liver disease. Serum aminotransferase concentrations are moderately raised in chronic and milder cases of acute viral or drug-induced hepatitis, autoimmune hepatitis, and alcoholic liver disease (31). Slightly raised serum aminotransferase levels

Table 3. Urea, creatinine, uric acid, total bilirubin, total protein, albumin: globulin ratio of normal healthy persons, alcoholic liver disease and non-alcoholic liver disease patients.

	Normal Healthy Persons (n=55)	Non Alcoholic Liver Disease (n=45)	Alcoholic Liver Disease (Moderate Alcohol Intake) (n=35)	Alcoholic Liver Disease (High Alcohol Intake) (n=46)
Urea (mg/dl)	21.96± 0.75	22.19 ± 0.85	20.86 ± 1.13	18.65 ± 1.01*#
Creatinine (mg/dl)	0.72 ± 0.05	1.05 ± 0.03*	0.82 ± 0.05#	0.81 ± 0.03#
Uric acid (mg/dl)	4.17 ± 0.06	4.15 ± 0.06	4.76 ± 0.11*#	5.25 ± 0.11*#@
Total bilirubin (mg/dl)	0.66± 0.019	4.26 ± 0.16*	0.96 ± 0.07*#	2.08 ± 0.15*#@
Total protein (g/dl)	7.28 ± 0.05	6.96 ± 0.04*	7.04 ± 0.07*	6.55 ± 0.08*#@
Albumin/ Globulin	1.31 ± 0.01	1.26 ± 0.01*	1.21 ± 0.02*	0.97 ± 0.02*#@

Values are mean ± SEM of number of observations (n). * indicates $p < 0.05$ when compared with normal healthy control # indicates $p<0.05$ when compared with non alcoholic liver disease and @ indicates $p<0.05$ when compared with alcoholic liver disease with moderate alcohol intake.

Table 4. Serum enzyme activities for healthy, non-alcoholic liver disease and alcoholic liver disease patients.

Group	AST (IU/L)	ALT (IU/L)	ACP (IU/L)	ALP (IU/L)	γGT (IU/L)
Normal Healthy Persons (n=55)	19.07 ± 0.58	18.49 ± 0.49	5.93 ± 0.27	209 ± 3.38	14.35 ± 0.43
Non Alcoholic Liver Disease (n=45)	196.09 ± 16.24*	248.14 ± 19.55*	5.67 ± 0.36	603.57 ± 18.85*	28.68 ± 1.52*
Alcoholic Liver Disease (Moderate Alcohol Intake) (n=35)	54.44 ± 2.36*#	47.56 ± 2.24*#	5.69 ± 0.29	296.5 ± 12.19*#	52.16 ± 3.14*#
Alcoholic Liver Disease (High Alcohol Intake) (n=46)	134.52 ± 12.49*#@	91.7 ± 8.88*#@	5.81 ± 0.34	400.2 ± 30.56*#@	242.48 ± 29.22*#@

Values are mean ± SEM of number of observations (n). * indicates $p < 0.05$ when compared with normal healthy control # indicates $p<0.05$ when compared with non alcoholic liver disease and @ indicates $p<0.05$ when compared with alcoholic liver disease with moderate alcohol intake.

characterize cirrhosis, non-alcoholic steato hepatitis, cholestatic liver disease, fatty liver, and hepatic neoplasms (31).

Figure 3 represents the variation in AST / ALT ratio among different groups of study. The ratio of aspartate amino transferase (AST) to alanine amino transferase (ALT) in serum may help in the diagnosis of some liver diseases. The entire study groups showed a significant alteration in comparison to normal healthy individual (1.04 ± 0.03). A significant reduction was observed in non alcoholic liver disease group (0.8 ± 0.02), whereas significant increases were observed in alcoholic liver disease groups with both moderate (1.16 ± 0.02) and heavy (1.5 ± 0.04) alcohol intake. Alcoholic liver disease group with heavy alcohol (ALD-H) intake showed significant elevation in the AST/ALT ratio in comparison to alcoholic liver disease with moderate alcohol intake (ALD-M) group. Deficiency of pyridoxal-5'-phosphate, a necessary coenzyme for both aminotransferases, is common in alcoholic liver disease. This deficiency decreases hepatic ALT to a greater extent than AST, with corresponding changes in serum concentration (5, 32). Hence, the AST/ALT ratio is a good marker of ALD.

In the present study, the serum ALP level was significantly higher in both moderate group (42%) and heavy group (91%) of alcoholics in comparison to healthy volunteers, and significantly lower than the non-alcoholic liver disease group. This study shows that chronic intake of ethanol increases serum activities of enzymes originating from liver plasma membranes but has different effects on the enzyme activity in liver

Table 5. Plasma ascorbic acid, thiobarbituric acid reactive substance (TBARS), and reduced glutathione (GSH) content of normal healthy persons, and patients with alcoholic liver disease and non alcoholic liver disease.

Group	Ascorbic acid (mg/dl)	TBARS (nmol/ml)	GSH content (µg/ mg protein)
Normal Healthy Persons (n=55)	1.28 ± 0.17	3.43 ± 0.23	3.58 ± 0.25
Non Alcoholic Liver Disease (n=45)	1.12 ± 0.16	8.7 ± 0.47*	2.93 ± 0.13*
Alcoholic Liver Disease (Moderate Alcohol Intake) (n=35)	0.94 ± 0.11	6.36 ± 0.29*#	3.16 ± 0.16
Alcoholic Liver Disease (High Alcohol Intake) (n=46)	0.75 ± 0.09*#	11.7 ± 0.67*#@	2.72 ± 0.15*@

Values are mean ± SEM of number of observations (n). * indicates $p < 0.05$ when compared with normal healthy control # indicates $p<0.05$ when compared with non alcoholic liver disease and @ indicates $p<0.05$ when compared with alcoholic liver disease with moderate alcohol intake.

Figure. I

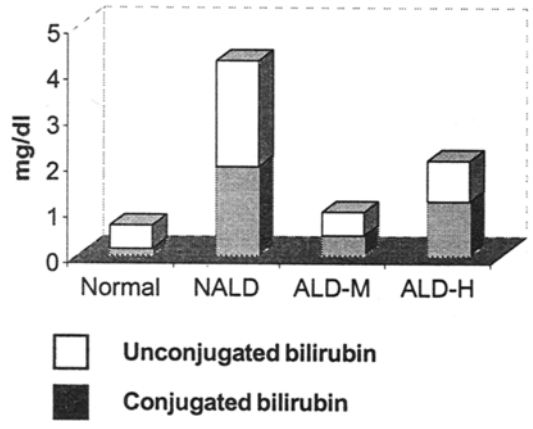

Figure I. Unconjugated and Conjugated bilirubin levels of normal healthy persons, alcoholic liver disease and non-alcoholic liver disease patients.

Values are mean of number of observations (n).

Figure. 2

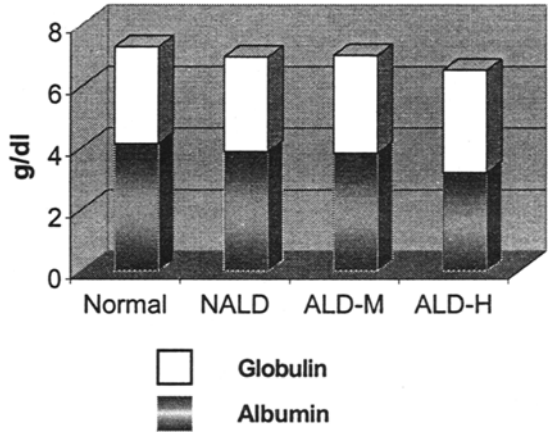

Figure 2. Albumin and globulin levels of normal healthy persons, alcoholic liver disease and non-alcoholic liver disease patients.

Values are mean of number of observations (n).

Figure 3.

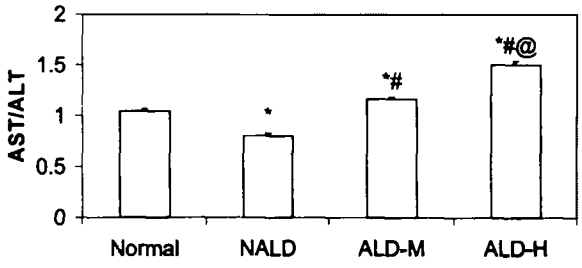

Figure 3. Ratios of AST and ALT in different groups of study. Each bar represents mean + SEM of number of observations (n).

* indicates $p < 0.05$ when compared with normal healthy control,

# indicates $p<0.05$ when compared with non alcoholic liver disease, and

@ indicates $p<0.05$ when compared with alcoholic liver disease with moderate alcohol intake.

plasma membranes itself, suggesting that the alcohol-mediated increase of serum activities of various enzymes originating from liver plasma membranes might be due to different mechanisms. Monitoring alkaline phosphatase level is also helpful in identifying the cause and severity of liver damage. No significant change was observed in case of ACP in any of the group studied.

In case of γGT, both moderate and heavy alcohol intake groups of alcoholic liver disease groups showed significantly nigher activities than the non-alcoholic liver disease group and normal healthy volunteers (Table 4). γ-glutamyltransferase (γGT) is a biliary canalicular enzyme, which is induced by alcohol, and serum levels also rise in response to acute hepatocellular damage. γGT characterizes chronic, long-term misuse of alcohol. Even significant difference was also observed between the moderate and heavy alcoholics. Experimental evidence present that the determination of γGT activity in serum is useful in the assessment of alcohol-induced liver disease. However, Krastev et. al. (33) did not find any significant correlation between the severity of liver damage and the extent of γGT increase at the beginning and at the end of the follow-up period. With several studies, serum γGT is the most sensitive, moderate specificity, most widely employed marker of alcohol consumption (34). Recently it has been suggested that subjects with very high γGT seem to demonstrate a more intense vulnerability to alcohol, a characteristic that appears to be stable over time (35). Thus in the present study, moderately high level of γGT in Moderate group of alcoholics and very high levels of γGT in Heavy group of alcoholics indicating that these patients are consuming high amount of alcohol and they are really at the risk or suffering from liver disorders.

Plasma levels of ascorbic acid, thiobarbituric acid reactive substances and reduced glutathione of the entire groups were presented in Table 5. Patients with various forms of liver disorders showed increased levels of plasma lipid peroxides. Significant increases in TBARS content was observed in all the study groups when compared with the normal healthy group. Though the moderate alcohol intake group showed significantly lower level of TBARS in comparison to non alcoholic liver disease, the high alcohol intake group of alcoholic liver disease showed a significant rise in TBARS level with respect to both non alcoholic liver disease group and moderate alcohol intake of alcoholic liver disease group. Chronic ethanol consumption has been associated with increased lipid peroxidation (36). Experimental studies strongly favor the possibility that the pathogenesis of ALD is due to peroxidation of polyunsaturated fatty acids.

Only high alcohol intake group of alcoholic liver disease showed significant decrease in ascorbic acid content of serum in comparison to normal healthy persons and non-alcoholic liver disease groups. These findings indicate that, with the heavy dose of alcohol, subjects are facing oxidative stress.

Glutathione (GSH) plays a major role in cellular protection against oxidative damage, and is critical in preserving the proper cellular redox balance. In comparison to normal healthy persons, the glutathione levels of non-alcoholic liver disease and alcoholic liver disease (High alcohol intake) groups showed significant decrease. However, no significant alteration in glutathione level was observed in case of Moderate alcohol intake group. Depletion of hepatic GSH by chronic ethanol ingestion induced oxidative stress is well reputed (31, 36). Hepatic GSH has an especially important relationship with lipid peroxidation because of its ability to bind with free radicals that may initiate peroxidation (37). Patients with alcoholic liver disease have lower hepatic GSH levels, which appear to be independent of nutritional status and probably reflect increased oxidative stress (38). Several factors contribute to the fall in hepatic GSH level in alcoholic liver disease.

CONCLUSION

Alcohol consumption is associated with a number of changes in cell functions and the oxidant- antioxidant system. Body weight, percent body fat, body mass index and hematological parameters are affected. Hyperbilirubinemia, hyperuricemia, hypoalbuminemia, high erythrocyte mean corpuscular volume, and normal urea and creatinine levels are common features of

alcoholics. Monitoring γGT, ALP, AST and ALT in combination is a sensitive means of detecting severity of alcohol induced liver damage. Increased lipid peroxidation and depletion of reduced glutathione could occur as a consequence of free radical generation due to alcohol consumption. All these parameters in combinations may be useful indicator for identification and determination of severity of alcoholic liver diseases.

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