

A Study of Biochemical and Hematological Markers in Alcoholic Liver Cirrhosis

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Received July 04, 2014; Revised July 14, 2014; Accepted July 21, 2014

Abstract Progressive fibrosis and cirrhosis, clinically presenting as end-stage liver disease are common outcomes in alcoholic Liver disease (ALD) patients. A variety of laboratory tests are available to assist in the progression and diagnosis of cirrhosis to end stage liver disease. The aim of this study is to identify potential novel biomarkers for progression of cirrhosis to end-stage liver cirrhosis. The biomarkers evaluated in this study included liver function indicators including serum ferritin, prothrombin time, albumin, total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT), renal parameters (urea and creatinine) and red blood cell counts, hemoglobin and blood glucose. The study included two groups based on severity of cirrhosis of liver; categorized as compensated and decompensated liver cirrhotic patients based on child Pugh criteria. All decompensated cirrhotic patients in the study group had significantly elevated biomarkers levels ($P < 0.001$) than those with compensated cirrhotic patients and control group who were not suffering from liver cirrhosis. Thus these results suggest that elevated and altered liver and hematological biomarkers are associated with pathogenesis and progression of liver cirrhosis.

Keywords: alcohol, Biochemical marker, haematological markers, γ -Glutamyltransferase, Aminotransferases

Cite This Article: Neelesh Deshpande, Sabitha Kandi, Manohar Muddeshwar, Rajkumar Das, and K V Ramana, "A Study of Biochemical and Hematological Markers in Alcoholic Liver Cirrhosis." *World Journal of Nutrition and Health*, vol. 2, no. 2 (2014): 24-27. doi: 10.12691/jnh-2-2-3.

1. Introduction

Alcoholism is condition resulting from excess drinking of beverages that contain alcohol. The major health risk of alcoholism includes liver disease, heart disease, pancreatitis, central nervous system disorders and certain forms of cancer [1]. Alcohol can be manifested in liver damage from fibrosis to end stage of cirrhosis and may eventually lead to carcinoma of liver. The liver is particularly vulnerable to disease related to heavy drinking, most commonly termed as alcoholic hepatitis or cirrhosis. The progression of alcoholic liver disease is characterized by steatosis, inflammation, necrosis and cirrhosis. When severe Cirrhosis occurs, death is the outcome [2]. Chronic consumption of alcoholic beverages is a primary cause of liver injury. Chronic and excessive consumption of alcoholic beverages provokes membrane lipid-peroxidation due to triglyceride accumulation in hepatocytes [3]. The study underway can serve as potential diagnostic tools for more specific biomarkers of ethanol-induced diseases. Hence, an attempt has been made to evaluate the effect of chronic alcohol consumption on blood, renal and hepatic biomarkers against worsening child pugh criteria.

2. Materials and Methods

2.1. Design and Subjects

A comparative study was carried out in a sample of 85 cirrhotic patients with chronic alcoholism of 31-54 years of age, and a mean consumption of ethanol 153.4 ± 36.9 g/d during the past 10 years (without alcohol ingestion in the past 30 days) and without additional diseases,

GROUP 1: (n=35)

Patients who were having with clinical, biochemical and ultrasonographic evidence of cirrhosis of liver without evidence of ascites, hepatic encephalopathy, GI bleed were classified as compensated cirrhotic patients with a mean age of 42.1 ± 10.19 years.

GROUP 2: (n=50)

Patients with clinical, biochemical, ultrasonographic evidence of cirrhosis of liver with evidence of GI bleed, hepatic encephalopathy & ascites were classified as decompensated cirrhotic patients with mean age of 42.5 ± 8.1 [4].

An equal number of aged matched normal healthy adults of with a mean age of 41.0 ± 9.4 years, with chronic alcoholism. All subjects had lived in central India for the past 10 years at the time of the study. The subjects agreed

to participate in the study after giving their informed consent.

2.2. Blood and Biochemical Analyses

Patients were obtained from gastroenterology ward of super specialty hospital with proven history of liver cirrhosis on the basis of clinical, biochemical and imaging methods and endoscopic signs. Cirrhosis was related to chronic alcohol intake. The severity of the disease was evaluated according to the Child-Pugh classification [4]. Heparin was the anticoagulant agent used. Blood samples containing heparin were analyzed using complete hemoglobin test protocol and ferritin [5,6]. The serum obtained from samples containing no anticoagulant agent was subjected to the following tests: urea, creatinine, albumin, total bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT) [6-13]. All reagents employed in biochemical tests were obtained from Randox Laboratories, Ltd. These tests were used as screening measurements for diagnosis of liver injury prevalent in different stages of cirrhotic patients.

2.3. Statistical Analysis

Data were processed by use of standard statistical software Open epi. The results are presented as mean \pm SD. The exact measures of associations in results between patients and control were compared using chi square test and fisher statistics. The significance was taken at $P < 0.001$.

3. Results

3.1. Effect of Alcohol on Serum Levels of Liver Chemistries.

Table 1 indicates the levels of serum bilirubin, albumin, GGT and ALT/AST ratio of compensated and decompensated cirrhotic groups as compared to that of control. The serum concentration of bilirubin was found to be significantly altered in patients with decompensation (group 2) as compared to compensated (group 1) and controls. The serum bilirubin levels were significantly elevated in patients consuming alcohol for the past 10

years Group 2 as compared to Group 1 and the control subjects ($P < 0.001$). The albumin concentration was significantly altered in compensated and decompensated patients (group 1 & 2) as compared to control group. The serum concentration of albumin was significantly decreased ($P < 0.001$) and the serum GGT Levels was significantly elevated ($P < 0.001$) and gradually declined with progression of cirrhosis in patients consuming alcohol for the past 28- 45 years. Also the serum levels of ALT, AST and AST/ALT ratio was significantly altered in patients consuming alcohol with more pronounced in decompensated subjects (Group 2) as compared to control subjects.

3.2. Effect of Alcohol Intake on Renal Markers

Table 2 shows the activities of serum urea and creatinine compared to control subjects respectively. There was significant change in serum urea and creatinine levels of patients consuming alcohol for the past 10 years in decompensated (Group 2) as compared to the compensated and control subjects. The serum levels of urea and creatinine showed pronounced elevation in decompensated cirrhotic patient as compared to compensated and control subjects.

3.3. Effect of Alcohol Intake on Haemoglobin RBC Count and Serum Ferritin

Table 3 shows the level of total RBC count, Hb concentration, PT values and Serum Ferritin of control compensated and decompensated respectively as compared to that of control. The levels of total RBC count, Hb content showed depleted values against serum ferritin levels which showed steep elevation in decompensated, as against compensated and control groups. The two groups of patients (i.e. compensated and decompensated) were consuming alcohol for the past 10 years as compared to the control subjects. The prothrombin time was significantly altered in decompensated patients than in compensated and decompensated patients. The serum concentrations of cirrhotic patients were reflected according to progression and gradation of cirrhosis and significantly elevated as compared to control subjects.

Table 1. Serum ALT, AST, T.Bilirubin, Albumin, GTT levels of patients

Patients	ALT (mean \pm SD)	AST (mean \pm SD)	T.Bilirubin (mean \pm SD)	Albumin (mean \pm SD)	GGT (mean \pm SD)
Control	29.18 \pm 8.23	34.04 \pm 6.88	0.62 \pm 0.16	3.65 \pm 0.23	22.47 \pm 3.30
Deompensated	79.52 \pm 14.69	132.3 \pm 42.88	6.1 \pm 3.22	2.70 \pm 0.29	34.48 \pm 19.52
Compensated	56.32 \pm 10.09	120.49 \pm 29.54	1.32 \pm 0.36	3.20 \pm 0.20	44.90 \pm 10.59

ALT=Alanine aminotransferase; AST=Aspartate aminotransferase; GGT=gamma glutamyl transeptidase

Table 2. Values of serum urea, creatinine in cirrhosis groups

KIDNEY FUNCTION TESTS	CONTROLS n =90	COMPENSATED CIRRHOSIS n =35	DECOMPENSATED CIRRHOSIS n =50
UREA	23.43 \pm 7.27	40.17 \pm 8.41	59.51 \pm 32.09
CREATININE	0.90 \pm 0.22	1.20 \pm 0.34	1.80 \pm 1.18
GLUCOSE	89.09 \pm 9.19	82.52 \pm 9.12	70.11 \pm 5.63

Table 3. Values of RBC counts, Ferritin and Hemoglobin in cirrhotic patients

Patients	RBC Count 10 ⁶ (mean \pm SD)	Ferritin (ng/ml) (mean \pm SD)	Hb (g/dl) (mean \pm SD)	PT (mean \pm SD)
Control	5.27 \pm 1.54	68.85 \pm 19.39	15.12 \pm 0.51	12.66 \pm 0.75
Compensated	4.12 \pm 0.42	154.82 \pm 19.67	14.11 \pm 0.36	14.00 \pm 0.51
Decompensated	3.10 \pm 0.70	432.61 \pm 71.57	8.82 \pm 1.39	19.94 \pm 4.59

RBC counts=red blood cell counts; Hb=Hemoglobin; PT=Prothrombin time

4. Discussion

Excessive chronic consumption of alcohol results in profound alterations in the blood chemistries which may be associated with alterations in metabolic activities of cell resulting in several clinical and/or biochemical changes.

4.1. Effect of Chronic Alcohol Consumption on Liver Chemistries

The serum gamma glutamyl transferase (γ GT), aspartate aminotransferase, bilirubin and albumin are considered to be well known markers of cirrhosis [14]. We have measured liver function tests, albumin and gamma glutamyl transferase, with worsening child Pugh classification in the present study, considering γ GT as the most sensitive markers for acute hepatocellular damage. Our results revealed that levels of γ GT were high in patients with severe alcoholic liver disease and low in the later stages of cirrhosis (Table 1). This pattern of rise in γ -GT levels were in congruence with the earlier study showing rise in compensated cirrhosis and fall in decompensated cirrhosis [15]. However some researches depicted controversial results [16]. The rise in the levels of γ GT as also concluded by certain other studies [15] in alcoholic liver diseases may probably be due to inductive action of alcohol. Hyperbilirubinemia and hypoalbuminemia were also observed to be common features with alcoholics in our study. The decrease in serum albumin level can be attributed to nutritional status of the subjects as also reported by Das et. al. earlier [14]. Albumin has been known as a potential subject for the formation of adduct by acetaldehyde, an alcohol metabolite. Ethanol consumption also slows down the rate of hepatic protein catabolism and may be related to degree of ethanol-induced Liver injury [14]. The serum transaminases viz, aspartate aminotransferase (AST) and alanine amino-transferase (ALT) are significantly elevated in decompensated cirrhosis as compared to controls and compensated cirrhosis. The ratio of AST to ALT may help in the differential diagnosis of alcoholic liver disease. The ratio is generally 1 or less with acute liver injury [4]. Following heavy alcohol consumption there has been evidence of mitochondrial damage. In Mitochondrial damage, the elevation in ratio of mitochondrial AST to total AST (mAST: tAST) has also been reported in heavy alcohol consuming individuals. This rise is proportionately higher in alcoholic individuals than healthy individuals which contribute only 10% of mitochondrial AST activity in serum [14]. The pattern of aberration in AST and ALT observed in our study (Table 1) has been correlated well with the reports in the past [16]. Ozenirler et. al. [16] reported rise in values of ALT in decompensated cirrhosis than that of compensated cirrhosis. The values of total bilirubin as reported by Agnieszka-Szuster-Ciesieka et. al. in compensated cirrhosis showed upward trend with progression of cirrhosis [15]. The Bilirubin levels observed by us were low in compensated cirrhosis than that of decompensated cirrhosis. Ozenirler et. al. [16] showed similar pattern of bilirubin values in compensated cirrhosis. Serum albumin as reported by Agnieszka-Szuster-Ciesieka et. al. [15] and

Ozenirler et. al. [16] was found to be lowered with progression from compensated to decompensated cirrhosis which matched with our study as evident from Table 1.

4.2. Effect of Chronic Alcohol Consumption on Renal Chemistries and Glucose Levels

Our present findings of kidney profile comprising slightly elevated blood urea and serum creatinine in later stages of decompensation as compared to controls and compensated cirrhosis are quite similar to earlier reports of Das et al [14]. The values of bilirubin are associated with urea and creatinine as observed by us may be used as markers in combination for diagnosis for ALD. It has been reported that liver disease has been associated with renal disorders [17]. We also have noticed hypoglycemia in patients associated with liver cirrhosis with increasing Child –Pugh score (Table 2). The finding is well supported by SQ Siler et al [18] who suggested that inhibition of gluconeogenesis and low intake of carbohydrates and other nutrients, associated with alcohol intake results in hypoglycemia.

4.3. Effect of Chronic Alcohol Consumption on Haematological Values

Alcohol has a variety of pathologic effects [17] in general including hematological abnormalities. On hematopoiesis, it has been found that alcohol has direct action on erythroid precursors, thereby contributing to macrocytosis and the anemic state of chronic alcoholics [19]. Ethanol induces sideroblastic anemia due to interference with heme synthesis. The hematological study in the present work showed Prothrombin time (PT) elevated with increasing Child Pugh score thereby showing clear sign of liver injury (Table 3). The percentage of hemoglobin and the total number of RBC were found to be significantly decreased with heavy alcohol intake as a result of hemodilution [18]. Iqbal et. al [20] Reported decrease in hemoglobin values in decompensation which increased on liver transplantation. RBC count showed marked decrease with growing decompensation as compared to control and compensated cirrhosis (Table 3). The degree of liver impairment showed direct relation with decrease in RBC count. Das et. al [18] suggested that chronic ingestion of alcohol causes alterations in erythrocyte membrane lipids which occur with the progression of alcoholic liver disease. Thus this leads to hemolytic anemia.

In our study we also made it a point to note that with the increase in consumption of alcohol and the level of liver injury in cirrhotic patients was profoundly more severe which was evident with elevated serum transaminases levels and other biomarkers (table1,2,3).

5. Conclusion

In conclusion, it is evident from the results of this study and the existing literature that there was a compromise of Liver function system with variation in other related biomarkers of injury with respect to different organs and body systems. The two groups based on child-Pugh classification also suggested compromise of liver function with increase in alcohol consumption. The variation in

liver functions with elevated Ferritin levels and corresponding reduction in RBC'S and hemoglobin values also revealed risk to liver injury and renal functions related to excessive alcohol intake when compared with control group. Glucose levels were decreasing from control to compensated through decompensated phases reiterating the role of alcohol in glucose metabolism. Our results reemphasize the fact that with rising levels of transaminases and variation in other biomarkers of injury reflecting iron as central cofactor in producing injury, alcohol consumption and iron levels play a key role in the progression of liver disease and pathogenecity. Regular monitoring of these markers and iron overload indicators in alcoholic patients is necessary for better patient management and to minimize the morbidity and mortality related to liver injury.

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