UTILITY OF GAMMA-GLUTAMYL TRANSPEPTIDASE AND MEAN CORPUSCULAR VOLUME IN ALCOHOLIC LIVER DISEASE

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Abstract. The present study was designed to establish the role of Gamma-glutamyl transpeptidase (γ-GT) and mean corpuscular volume (MCV) in alcoholic liver disease (ALD). Serum γ-GT, total and direct bilirubin, albumin, total protein, AST, ALT and ALP were assayed by standard methods in a clinical chemistry autoanalyser. MCV, Hb, PCV and RBC were measured by an automated cell counter. Activity of γ-GT and MCV levels were significantly higher in the patients with ALD compared to controls. A γ-GT level of ≥ 25 U/l was found to be significantly associated with ALD. MCV level ≥ 100 fl/l showed a significant association with ALD. An AST to ALT ratio > 1 was found in 92% of the patients. None of the patients showed an ALT level ≥ 300 IU/l. The degree of AST elevation in the patients with ALD was higher (3.7 times) then ALT (3.2 times). A γ-GT level ≥ 25 IU/L and an MCV level ≥ 100 fl/l stand as markers of heavy alcohol consumption in this study. An AST to ALT ratio > 1 was present in most of the patients with ALD. The degree of elevation of AST was higher than ALT in the patients with ALD.

INTRODUCTION

Alcohol use is rising rapidly in developing regions and is a major concern among indigenous people around the world, showing a higher prevalence of use and associated problems. The global burden of disease project estimated alcohol to be responsible for 1.5% of all deaths and 3.5% of those who live life with disability (WHO, 2001). It is also a major concern among the Nepalese who show a higher prevalence of use and abuse. Alcoholism is a chronic, progressive and potentially fatal disease characterized by tolerance, physical dependency and/or pathologic organ changes or either directly or indirectly due to alcohol ingested. It has a detrimental effect on the body in the gastrointestinal tract, and related organs, such as the liver and pancreas. The liver is one of the organs most significantly damaged and physiologically deranged as a result of alcohol ingestion (Ryback *et al*, 1982).

Three major histologic changes have been associated with chronic alcohol consumption: alcoholic fatty liver (alcoholic steatosis), alcoholic hepatitis (alcoholic steatonecrosis) and alcoholic cirrhosis. Alcoholic fatty liver is a condition of accumulation of fat in the liver, reversible upon discontinuation of alcohol use. Alcoholic hepatitis is a second major histopathologic lesion due to alcohol. It occurs much less often than alcoholic fatty liver and cirrhosis. Alcoholic cirrhosis is the third major histologic pattern of liver injury due to alcohol. It occurs in about 15% of heavy drinkers. Acetaldehyde formed of ethanol oxidation stimulates collagen synthesis. It is an irreversible stage of alcoholic liver damage, and is of the micronodular type (Matloff *et al*, 1979).

Alcoholic liver disease causes elevations of serum aspartate transaminase (AST) and alanine transaminase (ALT). More than 80% of patients with alcoholic liver disease have an AST:ALT ratio of 2 or more (Daeppen *et al,* 1999). Hyperbilirubinemia is frequent in alcoholic liver disease. Tests for alkaline phosphatase, γ-glutamyl transpeptidase (γ-GT), serum albumin, and prothrombin time, are indicator tests of altered hepatic activity (Eckardt *et al,* 1981). Hematologic tests, namely, RBC counts, WBC counts, hemoglobin levels and mean corpuscler volumes are strong indicators of alcoholic liver disease as

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reported by several researchers (Ryback *et al*, 1982).

Though numerous data are available regarding alcoholic liver disease with biochemical and hematologic indicators, very few are available in the Nepalese context. Hence, an effort has been made to see the role of γ-GT and MCV in alcoholic liver disease among the Nepalese subjects.

MATERIALS AND METHODS

This case-control study was conducted on 187 subjects; 87 patients and 100 control subjects, at the Department of Biochemistry, BP Koirala Institute of Health Sciences (BPKIHS), Dharan, in collaboration with the departments of Pathology and Forensic Medicine. Patients with ALD who presented to the medical outpatient department were enrolled in the study. Controls were chosen from healthy petients and relatives of BPKIHS staff.

Patients with firm livers if palpable, or reduced liver spans, splenomegaly, if present, ascites, if present, low serum albumins, elevated serum globulins, with or without elevated bilirubins or transaminases, and suggestive ultrasonography results (USG), were included in the study (Laing *et al*, 1989). Alcoholic cirrhosis was diagnosed according to the criteria of Laing *et al* (1989) with the help of clinical, biochemical and USG findings. Non-cirrhotic alcoholic liver disease (fatty liver and hepatitis) were diagnosed with the help of clinical, biochemical and USG findings, suggestive of diffuse liver disease, but not cirrhosis (Laing *et al*, 1989). Healthy subjects who did not have any features suggesting abnormalities related to biochemical, liver or hematological parameters, were enrolled in the study as controls. Individuals with autoimmune disease, hemolytic anemia, or infections of the liver, were excluded from the study.

Serum γ-GT, total and direct bilirubin, albumin, total protein, AST, ALT and ALP were assayed by respective Ecoline kits (MERCK, India) in a Vitalab Selectra 2 discrete random access clinical chemistry auto analyzer. MCV, Hb, PCV and RBC were measured by an automated cell counter (Coulter Counter, Human, Germany). The ratio of AST to ALT was calculated. The sensitivity and specificity of γ-GT and MCV were calculated in the ALD patients.

A one way ANOVA was used to test the hypothesis between all the parameters of controls and patients. A Pearson correlation analysis was done to see the association among parameters. Chi-square analysis was done to evaluate associations of γ-GT and MCV with alcoholic liver disease. The statistical calculation were performed using SPSS version 10.0.

RESULTS

The study consisted of 56.3% male and 43.7% female patients with 59% male and 41% female control subjects. There were no statistically significant differences in age and sex between patients and controls (Table 1). Out of 87 patients, cirrhotic and non-cirrhotic patients numbered 31 and 56, respectively.

The levels of alkaline phosphatase, AST, ALT and γ-GT were significantly higher ($p<0.01$) in the patients as compared to the non-ALD controls (Table 2). MCV and PCV levels were also found to be significantly higher ($p<0.01$) in the patients. The patients had significantly lower ($p<0.01$) RBC levels (Table 3). The Hb levels in the patient and control groups not significantly differed ($p>0.05$). Total protein and albumin were significantly ($p<0.01$) lower in the patients. The

Table 1
Sex distribution of patients with ALD (n = 87) and non-ALD controls (n = 100).

Sex	Patients (n = 87)			Controls (n = 100)		
	No.	%	p-value	No.	%	p-value
Male	49	56.3	> 0.05	59	59	> 0.05
Female	38	43.7	> 0.05	41	41	> 0.05

p>0.05 = statistically non-significant

Table 2
Biochemical parameters among ALD and control groups.

Test	Non-ALD control (n=100)	Patients with ALD (n=87)	p-value
Alkaline phosphatase (IU/l)	187 ± 55.69	450.07 ± 129.82	< 0.01
ALT (IU/l)	31.3 ± 17.30	72.39 ± 22.27	< 0.01
AST (IU/l)	28.86 ± 11.05	131.6 ± 20.06	< 0.01
Gamma-GT (IU/l)	24.41 ± 11.79	351.4 ± 71	< 0.01
Total bilirubin (mg/dl)	0.615 ± 0.143	4.62 ± 2.38	< 0.01
Direct bilirubin (mg/dl)	0.165 ± 0.052	2.84 ± 1.82	< 0.01
Total protein (g/dl)	6.71 ± 0.535	6.18 ± 0.98	< 0.01
Albumin (g/dl)	4.14 ± 0.492	2.6 ± 0.58	< 0.01
AST : ALT	1.16 ± 0.84	1.59 ± 0.52	< 0.01

$p < 0.01$ = Statistically highly significant

Table 3
Hematological parameters.

Parameters	Non-ALD controls	Patients with ALD	p-value
RBC (million/mm^3)	3.24 ± 0.375	2.84 ± 0.43	< 0.01
Hb (g/dl)	12.36 ± 1.03	12.08 ± 1.49	> 0.05
PCV (%)	32.6 ± 1.41	37.41 ± 4.23	< 0.01
MCV (fl/l)	98.66 ± 7.65	126 ± 13.17	< 0.01

Table 4
Distribution of AST and ALT.

Criteria	ALD group,%	Non-ALD group,%
Ratio, AST to ALT >1	92	40
Ratio, AST to ALT >1 and ALT <300 IU/l	80	40
Ratio, AST to ALT ≤1 and AST + ALT ≥300 IU/l	0	0

AST to ALT ratio was significantly higher ($p < 0.01$) in the patient group (1.586) compared to the control (1.16). Eighty patients (92%) with alcoholic liver disease had an AST/ALT ratio > 1, but no one in the group had an AST or ALT level ≥ 300 IU/l (Table 4). The degree of elevation in the AST in the patients was higher (3.7 times) than the ALT (3.2 times).

The specificity of measuring γ-GT levels was higher (62.5%) than MCV (41.7%), but the sensitivity of the γ-GT was lower (81.0%) than the MCV (85.3%).

DISCUSSION

The present study reveals that the γ-GT in the patients with ALD was significantly higher than in the controls. Several researchers have reported a significant elevation in γ-GT in patients with ALD, and even in light and moderate drinkers (Daeppen *et al*, 1999). Studies by Cushman *et al* (1984) and Poikolainen *et al* (1985) showed that elevated serum γ-GT levels in drinkers were related more closely to the biological effects of alcohol consumption rather than to the amount of alcohol consumed.

A study by Sharpe *et al* (1996) reported γ-GT as the best marker in distinguishing consumption of alcohol. Cushman *et al* (1984) reported a low sensitivity of γ-GT, and only 49% of the alcoholic liver diseased patients showed abnormally high γ-GT. Our study showed the sensitivity of γ-GT to be 81.0%, and 96.5% of the patients showed abnormally high γ-GT. This study showed a γ-GT level ≥ 25 IU/l was significantly associated with ALD patients.

The present study showed significantly el-

evated MCV levels ($p < 0.01$) compared to controls. A study by Pasqualetti *et al* (1995) showed MCV as a marker of alcoholism in chronic liver disease. The same study showed a significant ($p < 0.001$) decrease in MCV of about 3% after alcohol was withdrawn.

Clerment and Chalmers (1967) reported that the relationships between AST and ALT can be used to determine the etiology of liver disease. The ALT level is a more specific indicator of hepatic disease than AST (Magarian *et al*, 1992). The ALT levels were usually elevated to a lesser degree than the AST levels and may be within normal limits. Typically the AST/ALT ratio is approximately 1.5-2 to 1 in alcoholic liver disease, even when neither level is elevated (Magarian *et al*, 1992). Our study showed the ratio of AST to ALT to be 1.59, along with elevations of both AST and ALT levels. The patients showed a greater degree of elevation in AST levels than ALT levels, though the ALT levels were also significantly increased. Alcoholic liver disease has been characterized by an AST to ALT ratio greater than 1.0 when accompanied by a sum of the AST and the ALT of less than 300 IU/l (Cohen *et al*, 1975). Levin and colleagues (1979) reported that an AST to ALT ratio of less than 1.0 when accompanied by an ALT of more than 300 IU/l confirms alcoholic liver disease. Our study showed only 8.0% of patients with ALD had an AST to ALT ratio < 1. None of the patients presented an ALT level > 300 IU/l. Hence our study is much closer to the findings of Cohen and Kaplan (1975) rather than to Levin and colleagues (1979).

This study shows the increased levels of γ-GT and MCV in ALD patients. γ-GT ≥ 25 IU/l and MCV ≥ 100 fl/l show a strong association with ALD. The study showed an AST to ALT ratio > 1 is strongly associated with ALD patients along with significant elevations of both parameters. The degree of elevation of AST was higher compared to ALT.

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