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Enzymatic Exploration of Alcoholic Liver Disease

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Original Research Article

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Abstract: Alcoholic liver disease (ALD) is the most common liver disease in world. For many reasons, it is underestimated and underdiagnosed. Liver disease is the most likely diagnosis if the AST level is more than twice that of ALT, a ratio some studies have found in more than 80 percent of alcoholic liver disease patients. An elevated level of the liver enzyme GGT is another gauge of heavy alcohol use and liver injury. A group of blood tests called liver function tests can be used to diagnose liver disease. Other blood tests can be done to look for specific liver problems or genetic conditions. Imaging tests like ultrasound, CT scan and MRI can show liver damage. An early diagnosis is absolutely essential as alcohol is a hepatotoxin that is commonly consumed worldwide and is associated with a spectrum of liver injury including simple steatosis or fatty liver, alcoholic hepatitis, fibrosis, and cirrhosis. Alcoholic liver disease (ALD) is a general term used to refer to this spectrum of alcohol-related liver injuries. The two major end points of ALD are alcoholic liver cirrhosis and the rare and clinically-defined alcoholic hepatitis (AH). The prediction and early diagnosis of both entities is still insufficiently solved and usually relies on a combination of laboratory, clinical and imaging findings. It is not widely conceived that conventional screening tools for ALD such as ultrasound imaging or routine laboratory testing can easily overlook ca. 40% of manifest alcoholic liver cirrhosis. Non-invasive methods such as transient elastography (Fibroscan), acoustic radiation force impulse imaging or shear wave elastography have significantly improved the early diagnosis of alcoholic cirrhosis. Present algorithms allow either the exclusion or the exact definition of advanced fibrosis stages in ca. 95% of patients. The correct interpretation of liver stiffness requires a timely abdominal ultrasound and actual transaminases levels. Other non-invasive methods such as controlled attenuation parameter, serum levels of M30 or M65, susceptometry or breath tests are under current evaluation to assess the degree of steatosis, apoptosis and iron overload in these patients. Liver biopsy still remains an important option to rule out comorbidities and to confirm the prognosis namely for patients with AH.

Keywords: Alcoholic hepatitis, Alcoholic steatohepatitis, Alcoholic liver disease, Non-invasive, Liver stiffness, Serum marker, Steatosis.

INTRODUCTION

Although alcohol ingestion is required to develop alcoholic liver disease, not everyone who consumes the "threshold dose" of alcohol will develop the disease. Amazingly, nearly 50% of individuals who ingest large amounts of ethanol are spared serious injury. In addition to the amount and duration of alcohol use, several other factors have been linked to an increased risk for the development of liver disease. These include genetics, gender, viral liver disease, nutrition, and exposure to other hepatotoxin. Genetics Polymorphisms exist in the enzymes ADH, CYP2E1, and ALDH. Differences in ADH and ALDH certainly contribute to the negative association with ethanol dependence in some Asian populations. HLA phenotypes, a genetic predisposition toward alcoholism and female gender may also contribute to overall risk. Viral Liver Disease Concurrent viral hepatitis increases the incidence of liver injury in alcoholics. Studies have shown that alcoholics co-infected with hepatitis C virus (HCV), (but not necessarily hepatitis B virus), develop liver injury at a younger age and with a lower cumulative dose of alcohol than those not infected with HCV. These patients also have a much higher chance of developing cirrhosis and hepatocellular cancer compared to alcoholics without hepatitis C. As the liver becomes more severely damaged, more obvious and serious symptoms can develop, such as: yellowing of the skin and whites of the eyes (jaundice) swelling in the legs, ankles and feet caused by a build-up of fluid (edema) swelling in abdomen caused by a build-up of fluid known as ascites. As the name implies, alcoholic liver disease is liver injury attributed to alcohol abuse [2]. The majority of Americans manage to drink alcohol

without serious consequences. Research suggests, however, that liver disease may begin to develop after a "threshold" dose of alcohol has been consumed—generally assumed to be four drinks a day (four 12 ounces beers, four glasses of wine, or four ounces of hard liquor) for men, and one half that quantity for women. Nearly everyone who consumes this amount or more will have some evidence of liver injury, although less than 50% will develop serious liver disease.

Symptoms

The range of clinical features of alcoholic liver disease varies, from asymptomatic to end-stage liver disease with portal hypertension, jaundice and encephalopathy. Liver pain is felt in the upper right area of the abdomen, just below the ribs. Usually, it is a dull, vague pain though it can sometimes be quite severe and may cause a backache. Sometimes people perceive it as pain in the right shoulder. Patients may present with nonspecific digestive tract symptoms such as nausea, dry retching, diarrhea, anorexia, and abdominal pain—but often they wait until severe liver decompensation forms before consulting a physician. Patients may also seek medical attention as a result of the consequences of alcoholism, which may include accidents, violent behavior, depression, tremors, poor work performance, or social disruptions. Fatty liver is usually asymptomatic. On evaluation hepatomegaly is present in 70% of patients and there may be mild abnormalities in transaminases [3]. Patients with alcoholic hepatitis may also be asymptomatic. Hospitalized patients usually have jaundice and hepatomegaly and may exhibit ascites, encephalopathy, and fever depending on the severity of their disease. Most patients with alcohol-induced cirrhosis have hepatomegaly and/or splenomegaly. Clinical presentation is similar to other forms of end-stage liver disease but may be accompanied by concurrent alcoholic hepatitis. Spider angiomata are frequently found in this patient population, along with palmar erythema, enlargement of parotid and lachrymal glands, testicular atrophy, ascites, venous collaterals, jaundice and encephalopathy. Various enzymatic studies reveal stat diagnosis [4]. GGT levels become elevated after 24 hours to 2 weeks of heavy alcohol consumption and return to normal within 2 to 6 weeks of abstinence, which allows them to detect binge drinking. Liver blood tests are designed to show evidence that abnormalities, for example, inflammation, liver cell damage, has or is occurring within the liver. The blood tests most frequently used for liver disease are the aminotransferases (alanine aminotransferase or ALT and aspartate aminotransferase or AST) [5].

Table-1: Mean values and rise (as times normal) of serum levels of GGT, 5'NT and ALP in different groups

Different Groups	GGT		5'NT			ALP
	Mean Value (Szasz)	Rise as times normal	Mean Value (IU/L)	Rise as times normal	Mean value (KA Units)	Rises as times normal
Control group	22.54	-	9.18	-	7-10	-
Study group (56)	64.94	2.88	18.20	1.98	11.60	1.63
Anicteric Subgroup (33)	36.03	1.60	12.75	1.39	8.81	1.24
Icteric subgroup (13)	116.33	5.16	27.89	3.04	16.56	2.33

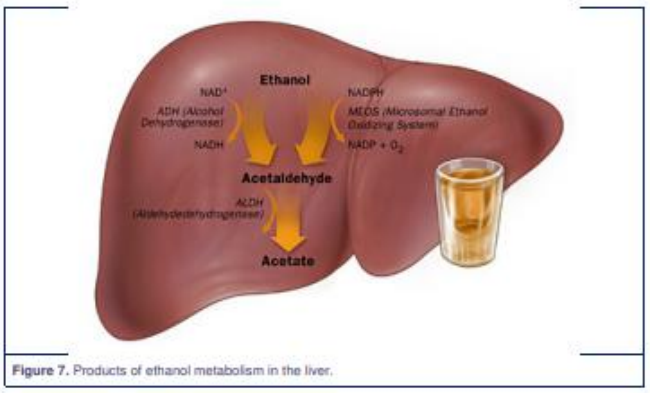


Figure 7. Products of ethanol metabolism in the liver.

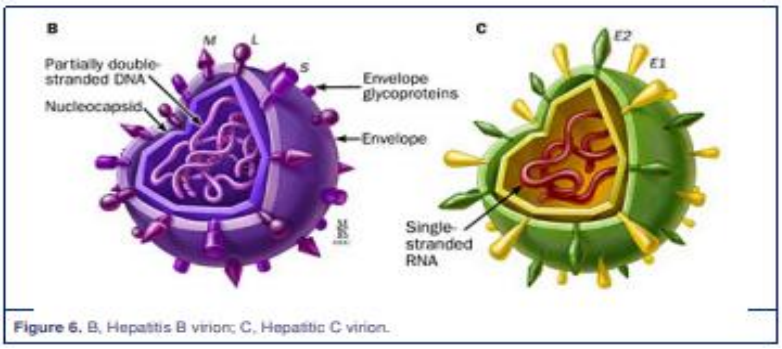
Vicious cycle between alcohol and liver

Table-4: Mean Values and rise of serum, 5'NT and ALP in different groups

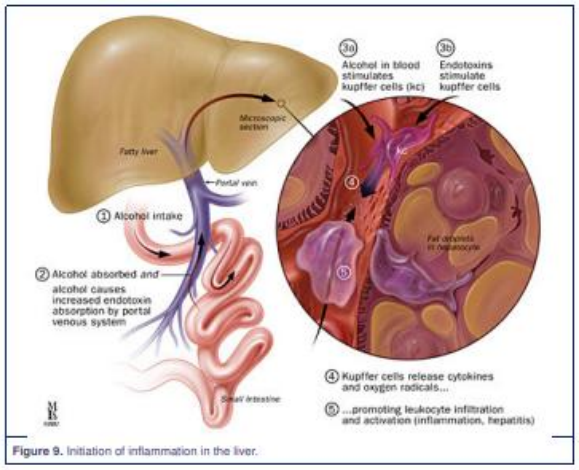
	GGT			5'NT	ALP	
	Mean value (Szasz)	Rise as normal times	Mean Value (IU/L)	Rise as normal times	Mean value (KA)	ALP
Control Group (56)	22.54	-	9.18	-	7.00	-
Study Group	64.94	2.88	18.98	1.87	10.98	1.43
Anicterics (34)	36.03	1.60	13.98	1.32	9.12	1.76
Icterics (22)	116.33	5.16	29.98	17.87	17.09	1.12

MATERIALS AND METHODS

Most patients with alcohol-induced cirrhosis have hepatomegaly and/or splenomegaly. Clinical presentation is similar to other forms of end-stage liver disease but may be accompanied by concurrent alcoholic hepatitis [6]. Spider angiomata are frequently found in this patient population, along with palmar erythema, enlargement of parotid and lachrymal glands, testicular atrophy, ascites, venous collaterals, jaundice and encephalopathy. 56 patients suffering from various biliary diseases attending OPD or indoor patients in Govt. Medical College, Amritsar were enrolled for the study. A detailed history was taken, complete physical examination conducted and the required investigations, and including an ultrasound abdomen were done to confirm the diagnosis. 10 ml of venous blood was taken for analyzing GGT, 5'NT, ALP and bilirubin levels. The values were measured in serum, after allowing the sample to clot and then centrifuging at 3000 rpm for 10 minutes to separate the serum. GGT was measured by the method of Szasz and Klin.6 5'NT was measured by the method of Campbell and ALP by the method of Kind and King [7]**.** 50 healthy controls matched for age and sex were enrolled in the control group, and it was ensured that none had any hepatobiliary disorder (previously or present), coronary artery disease, diabetes or prolonged drug therapy. The mean values of the three enzymes in this group were taken as normal. Bilirubin levels of below 1 mg/dL were taken as normal and such patients were considered an-icteric, whereas patients with levels more than this were considered icteric [7]. The study was cleared by the research and ethics committee of the institute where the work was carried out and the patients/subjects signed an informed consent regarding participation in the study.



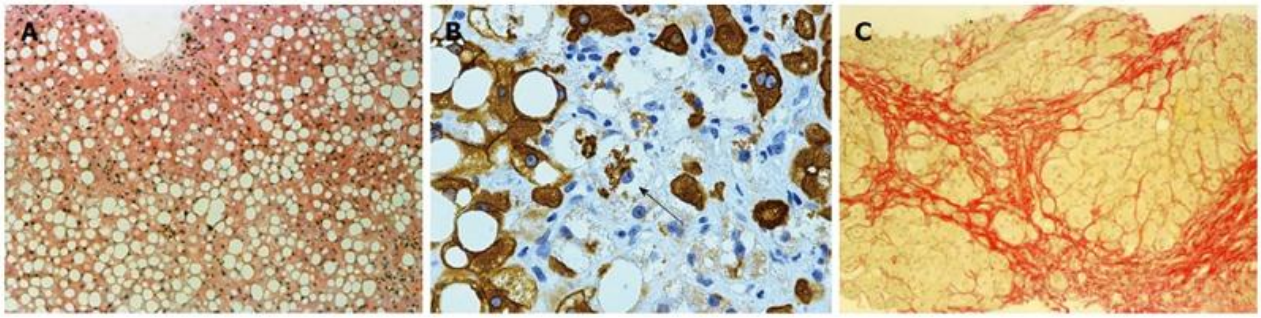
Microscopic Picture of Liver in Hepatitis C



Hepatoportal Shunt

RESULTS

The 56 patients comprising the study group had an average age of 48 years, with a range of 16 to 70 years. The group had 29 (58%) females and 21 (42%) males. Alanine aminotransferase levels were significantly raised in alcoholic disease patients. The levels being 258.2±91.73, 79.66±28.63, and 50.73± 8.4 respectively as compared to normal control (11±3.42) Aspartate aminotransferase levels were significantly raised in alcoholic liver disease [8]. The levels being 132.80±62.4, 174±58.32, and 56±7 respectively as compared to normal control (13±3.54) Alkaline phosphatase levels were significantly raised in viral hepatitis, alcoholic liver disease and cirrhosis patients. The levels being 208±54.4, 132.33±33.29, and 116±11.98 respectively as compared to normal control (19.20±9.32) Gamma glutamyl transpeptidase levels were significantly raised in viral hepatitis, alcoholic liver disease and cirrhosis patients. The levels being 115.33±28.31, 181.33±60.66, and 248.66± 43.52 respectively as compared to normal control (26.73.±4.03). The control group had 50 healthy subjects with a comparable age and sex distribution. In the control group the mean value of the three enzymes GGT, 5'NT and ALP were 22.54 Szasz units, 9.18 IU/L and 7.1 KA units respectively, which is within the reported normal range for each enzyme (Table I). Further, in the control group, the enzymes did not show any particular trend with age and also there was no variation among males and females. Out of the total 56 patients, there were 32 patients with normal bilirubin levels forming the an-icteric group and this group showed a mean bilirubin value of 0.75 mg/ dL. 18 patients with elevated bilirubin levels formed the icteric group with an average bilirubin level of 4.95 mg/dL. Mean values of all the three enzymes i.e. GGT, 5'NT and ALP showed a rise in the study group as a whole, as well as the two subgroups. Table-1 shows the mean values of the three enzymes in various groups as well as the rise as times normal in the study group and an-icteric and icteric subgroups. Among the three enzymes, the rise was highest for GGT and least for ALP in the study group as well as both the an-icteric and icteric subgroups. The elevation in the mean values of enzymes was further evaluated statistically for significance in all the different groups. Table-2 shows the significance/ insignificance of the rise in the levels of different enzymes in various groups at different "p" values. In the icteric subgroup, the rise in all the three enzymes was highly significant (p value < 0.001). However, in the an-icteric subgroup the rise was significant only for GGT and 5'NT ($p < 0.001$ for GGT and $p < 0.01$ for 5'NT) and not for ALP ($p > 0.01$).



Histology of Alcoholic Fatty Liver. A: Macrovesicular steatosis in alcoholic fatty liver (HE stain, × 218); B: Ballooned hepatocyte (arrow) containing a Mallory Denk body in alcoholic hepatitis (CAM5.2 stain for cytokeratins 8 and 18, × 218); C: Collagen surrounds nodules of hepatocytes in alcoholic cirrhosis (Serius red stain, × 872).

Table-3: Significance (+) insignificant (-) level of GGT, 5'NT, ALP in various groups

	P < 0.01			P<0.001		
	GGT	5'NT	ALP	GGT	5'NT	ALP
Study Group (56)	+	+	+	+	+	+
Anicterics (34)	+	+	-	+	-	-
Icterics (22)	+	+	+	+	+	+

DISCUSSION

The liver associated enzymes, Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), and gamma glutamyl transferase (GGT) are measures of liver homeostasis [9]. Serum amino transferases such as alanine aminotransferase (ALT) and aspartate aminotransferase (AST) indicate the concentration of hepatic intracellular enzymes that have leaked into the circulation. These are the markers for hepatocellular injury [10]. The aminotransferases (transaminases) are sensitive indicators of liver cell injury and are most helpful in recognizing acute hepatocellular diseases such as hepatitis [11]. The pattern of the aminotransferase elevation can be helpful diagnostically. In most acute hepatocellular disorders, the ALT is higher than or equal to the AST. An AST:

ALT ratio>2:1 is suggestive while a ratio >3:1 is highly suggestive of alcoholic liver disease. The AST in alcoholic liver disease is rarely >300 U/L and the ALT is often normal.

Table-2: Enzyme values among patients of viral hepatitis, Alcoholic liver disease and Liver cirrhosis

Investigation	Control (15)	Viral Hepatitis (15)	Alcoholic liver Disease (15)	Liver cirrhosis (15)
ALT	11.20 ±3.43	258.20±91.73 P=000	79.66 ±28.63 P=000	50.73±7.98 P=001 (all)
AST	13.00±3.54	157±67.81 P=000	164.00±54.36 P=000	62.12±11.8 P=000 (all)
ALP	36.20±9.54	208±54.40 P=000	180.33±33.9 P=000	116.00±12 P=000 (all)
GGT	26.73±4.02	115±28.31 P=000	181.34±59.9 P=001	248.66±34 P=002 (all)

p value comparing patients, Alcoholic liver disease, Liver cirrhosis to healthy controls
p<05 significant

A low level of ALT in the serum is due to an alcohol induced deficiency of pyridoxal phosphate [12]. In this study, Table-3 shows the AST: ALT ratios 1 for normal, 0.65(<1) for viral hepatitis, consistent with F. De Ritis *et al*., [11], >2 for ALD group, which similar to reported by several other studies conducted earlier [13], and 1.24 in cirrhosis , > 1 but < 2 also documented by Nyblom *et al*., [14] and others . This helps to differentiate ALD from other liver diseases. In this study AST, ALT ALP, GGT levels were significantly raised in alcoholic liver disease and cirrhosis patients as compared to control. AST, ALT and ALP Levels were significantly high as compared to alcoholic liver disease and cirrhosis. Moreover alcoholic liver disease patients have more AST, ALT and ALP as compared to cirrhosis. The peak levels of Transaminases have been reported to vary from 400- 4000 IU/l or more [14]. In alcoholic liver disease AST activity has been reported to be greater than ALT and usually does not exceed 300 IU/L. AST/ALT ratio is greater than 2 because of existing mitochondrial damage [15]. This study also confirms that in cirrhosis AST and ALT levels are normal or slightly elevated. If the etiological factors are present or with active alcohol abuse increases AST and ALT levels [15]. The ALP activity has been reported by various workers minimally increased usually upto 200 - 300 U/L in viral hepatitis and in alcoholic liver disease ALP usually up to 300 U/L. In cirrhosis ALP is either normal or slightly elevated [15], increased in serum ALP is associated with liver disease is caused by intra or extra hepatic cholestatis and some destruction of hepatic cell membrane [16]. Elevation of ALP is observed in patients who have some form of extra hepatic and intra hepatic bile duct obstruction. Any mechanism that impaired excretion of ALP in bile will result in regurgitation of enzyme into circulation via the hepatic sinusoid. The increased ALP present in the patients with disease closely resembles the ALP that can be extracted from liver [7]. The increased cholestatis stimulates the synthesis of ALP by the bile ductules cell [17] providing more ALP which ultimately enters the bloods, the amphillic nature of bile salts facilitates the release of ALP from its membranes bound site and entry into blood [18]. 5' Nucleotidase level was found to be elevated were found to be elevated but GGT was more sensitive specific in patients of Alcoholic liver Disease.

CONCLUSION

There are 12 fold elevations in mean value of AST in Viral hepatitis, 12.6 folds elevation in alcoholic liver disease and 4.7 fold in cirrhosis ALT showed the elevation in mean value of 23 fold in viral hepatitis, 7.11 fold elevations in alcoholic liver disease and 4.5 fold in cirrhosis. There is 5 fold rises in alcoholic liver disease and 3.2 fold in cirrhosis [18]. The serum GGT showed elevation in mean value of 4 fold in viral hepatitis, 6 fold in alcoholic liver disease and 9 fold in cirrhosis Liver associated enzymes tests are used to detect, specifically diagnose, and estimate the severity of hepatic disease. 5' Nucleotidase level was found to be elevated were found to be elevated but GGT was more sensitive specific in patients of Alcoholic liver Disease.

Recognizing the different patterns of liver injury can be used as a guide to direct further evaluation of diseases that affect the liver. In combinations with the physical examination and history, the evaluation of other serum enzymes should aid in differentiating the source of increased Liver associated enzymes level. GGT was useful for evaluation of not only obstructive biliary disease patients, but also for the patients with biliary disease who are an-icteric [9]. Further they score over ALP, both for patients with or without biliary obstruction. Among GGT and 5'NT, former has got a better diagnostic relevance in evaluation of biliary disease patients.

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