

## **A Cross Sectional Study on Utility of Conventional Serum Liver Enzymes and De Ritis Ratio as Affordable Diagnostic and Prognostic Markers in Alcoholic Liver Disease (ALD) Patients of A Tertiary Care Teaching Hospital in Uttarakhand, India**

Javin Bishnu Gogoi<sup>1</sup>, Kailash Gairola<sup>1\*</sup>, Kingshuk Lahon<sup>2</sup>

<sup>1</sup>Department of Biochemistry, Veer Chandra Singh Garhwali Government Institute of Medical Sciences & Research, Srikot, Srinagar-Garhwal, Uttarakhand-246174, India

<sup>2</sup>Department of Pharmacology, Veer Chandra Singh Garhwali Government Institute of Medical Sciences & Research, Srikot, Srinagar-Garhwal, Uttarakhand-246174, India

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**\*Corresponding author**

*Kailash Gairola*

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**Abstract:** Several new biochemical and hematological parameters are available to diagnose and monitor alcoholic liver disease (ALD), but none are independently sufficient for the purpose. Serum enzymes - Alanine aminotransferase (ALT), Aspartate aminotransferase (AST), Alkaline phosphatase (ALP), Gamma glutamyl transferase (GGT) and AST:ALT ratio (De Ritis Ratio) are conventionally used as markers for diagnosis, treatment, prognosis and monitoring of ALD, based on their correlation with values before and after treatment or abstinence from alcohol intake. We wanted to establish the utility and affordability of estimation of these serum enzymes in ALD. To assess the levels and analyse the costs of estimation of conventional serum liver enzymes in ALD. After IEC clearance, this hospital based study was carried out on 30 male patients of diagnosed ALD and 30 normal control (non-alcoholic, healthy male) subjects between 18-65 years without history of liver disease, HIV, upper gastrointestinal bleeding, shock and/or ischemia to the liver, hepatotoxic medication use or exposure to high levels of environmental hepatotoxins, renal disorders, diabetes mellitus, hypertension. Serum AST, ALT, ALP, GGT levels were estimated using commercially available Roche diagnostic kits and fully automated biochemistry analyser (eCOBAS 6000 c501, Roche). Enzyme levels were expressed as Mean  $\pm$  SD in IU/ml. Statistical analysis of differences in Means was done with Unpaired Student's 't' test, using GraphPad Quickcalcs online software, considering significance level as  $P < 0.05$  at 95% confidence interval. Cost calculation of all the tests was done. Serum AST, ALT, ALP, GGT levels were highly significantly raised in ALD patients compared to healthy controls. De Ritis ratio was greater than 2 in 70% ALD patients. Cost calculation revealed that each patient had to pay a total of Rs 80/- for the tests. Conventional serum liver enzyme estimation and calculation of De Ritis ratio are useful and affordable diagnostic and prognostic markers in ALD.

**Keywords:** ALD, AST, ALT, ALP, GGT, De Ritis ratio

### **INTRODUCTION**

Alcohol use disorders affect millions of individuals worldwide. Chronic alcoholism is considered as a social disease or health related problem inflicting a substantial population in almost all the countries of the world [1]. In India, Uttarakhand is one of the leading states with regard to alcohol consumption, where almost one-third of the male population (32%) consume alcohol as per observations of the National Institute of Medical Statistics, New Delhi [2]. Kumar S, *et al.* have found high prevalence of alcohol use in Srinagar Garhwal [3].

The liver is an organ that is primarily affected by alcohol. Alcoholic liver disease (ALD) is a highly serious and potentially fatal consequence of alcohol abuse. ALD comprises of three different conditions - fatty liver, alcoholic hepatitis and cirrhosis. Excessive alcohol consumption leading to ALD comprises a significant burden of the leading causes of preventable morbidity and mortality worldwide [4,5]. The diagnosis and management of the complications of ALD are important for alleviating the symptoms of the disease, improving quality of life, and decreasing mortality [6]. Alcoholic hepatitis is defined as a clinical syndrome

with characteristic biochemical and histological changes with severe chronic alcoholism. As it is an emerging health problem globally, there is a need for rapid, reliable and inexpensive tests for the diagnosis and monitoring of these cases for better patient care.

The diagnosis of ALD is based on history of alcohol intake, clinical features and laboratory tests. In general, ALD should be suspected in patients with a significant history of alcohol use who present with abnormal serum transaminases, particularly if the level of aspartate aminotransferase (AST) is greater than that of alanine aminotransferase (ALT), hepatomegaly, clinical signs of chronic liver disease, radiographic evidence of hepatic steatosis or fibrosis/cirrhosis, or who have had a liver biopsy showing macrovesicular steatosis or cirrhosis [7]. Many other parameters like carbohydrate deficient transferrin (CDT), beta-hexosaminidase, acetaldehyde adducts, urinary ratio of serotonin metabolite, 5-hydroxytryptophol, 5-hydroxyindoleacetic acid are used to diagnose and monitor the prognosis of alcoholic hepatitis. Chronic alcohol consumption also may be associated with hypertriglyceridemia, hyperuricemia, hypokalemia and low magnesium level, as well as an elevated mean corpuscular volume (MCV). Hyperuricemia and hypertriglyceridemia often normalize with abstinence, and hypokalemia normalizes with adequate potassium replacement. Elevated MCV often is found in people who ingest >50 grams of alcohol per day, with sensitivity of 27-52% and specificity of 85-90%. The blood protein known as carbohydrate-deficient transferrin (CDT) frequently is used to detect current or recent alcohol abuse, especially consumption in excess of 60 grams per day [8,9], but there are no ideal tests to identify continuing alcohol intake. Leukocytosis and thrombocytopenia are common in alcoholic hepatitis. Thrombocytopenia may be transitory, but in patients with concomitant cirrhosis, it is persistent. Markers of severe alcoholic hepatitis or cirrhosis include hyperbilirubinemia, prolonged prothrombin time (PT), and hypoalbuminaemia. The most commonly used prognostic index in alcoholic hepatitis is Maddrey's Discriminant Function (DF), which is calculated by this equation:

$$[\text{PT}(\text{patient}) - \text{PT}(\text{control})] + \text{Total bilirubin (mg/dl)}$$

If this value exceeds 32, the mortality rate during a current hospitalization may exceed 50 % [10,11]. There is also evidence that blood concentrations of proteins (i.e., cytokines) that promote inflammation—such as tumor necrosis factor alpha (TNF- $\alpha$ ), interleukin-6, and interleukin-8 correlate with mortality in patients with alcoholic hepatitis, but levels of these cytokines are not determined in routine clinical practice [12].

Liver biopsy mainly is used to clarify atypical cases, to better define the contribution of alcohol in patients with possible non-alcohol-related coexisting conditions (e.g., hepatitis C, use of lipid-lowering medications), and to determine the severity of liver disease. Many laboratories are conducting research to evaluate biomarkers or identifier proteins for detecting ongoing alcohol abuse and ALD. The importance of genetic variations in alcoholism and ALD among individuals is also under active investigation. New tests may provide novel way of identifying alcohol abuse, susceptibility to liver injury, and mechanisms of liver injury, and of detecting and monitoring liver injury [6]. Recently many tests related to urine, breath and sweat analysis have been introduced for the diagnosis of alcohol consumption but still there are great controversies regarding the usefulness of these tests [13]. Moreover, the newer tests are expensive and are not available in all laboratory settings.

The conventional serum enzymes like ALT, AST, ALP (alkaline phosphatase) and GGT (gamma glutamyl transferase) are used in the diagnosis of ALD. But it should be noted that patients with ALD may or may not have elevated serum aminotransferase levels [7]. When present however, the pattern of elevation in transaminases is helpful in making a diagnosis of liver injury due to alcohol as AST is typically two to three times greater than ALT in alcoholic liver injury [14]. The levels of both AST and ALT will be below 300 international units per milliliter (IU/ml). The AST:ALT ratio (De Ritis Ratio) when properly interpreted is also an indicator of ALD, although originally it was applied in viral hepatitis patients [15]. ALD patients will also typically have an elevated serum GGT [16]. These simple biochemical parameters also help in the treatment and monitoring of the patients of alcoholic hepatitis. They have the ability to determine response to various therapies and evaluate not only disease progression but also possible regression [17]. Though these tests have limited sensitivity and specificity when used alone, they can play a great role if they are used together and correlated with the clinical findings correctly. Moreover, they are cheap and widely available in diagnostic laboratories.

Hence, we undertook this study with the aim of establishing the utility and affordability of conventional serum liver enzymes and De Ritis ratio as diagnostic and prognostic markers in ALD patients visiting our tertiary care teaching hospital.

## MATERIALS AND METHODS

This hospital based study was carried out in the Clinical Biochemistry laboratory of our institute and HNB Base Hospital, Srinagar, Garhwal after institutional ethical committee (IEC) approval. Written informed consent was taken from all the participants.

**Inclusion Criteria**

Male patients between 18-65 years, attending the outpatient/inpatient services of Department of Medicine with diagnosed ALD during the period 1<sup>st</sup> July to 31<sup>st</sup> December 2014.

Non-alcoholic apparently healthy males between 18-65 years without any liver disease who were ready to volunteer were included as a control.

**Exclusion Criteria**

Patients with infective hepatitis, HIV, hepatic or extrahepatic malignancy with hepatic metastasis, overt upper gastrointestinal bleeding, shock and/or ischemia to the liver, use of hepatotoxic medication or exposure to high levels of environmental hepatotoxins, renal disorders, diabetes mellitus and hypertension.

**Study procedure**

All patients satisfying the inclusion/exclusion criteria were enrolled if they gave written informed consent after explaining the purpose of the study to them. Similarly we recruited the normal control group of apparently healthy volunteers between 18-65 years and took written informed consent from them as well.

60 subjects, 30 each in the ALD group and in the healthy, non-alcoholic control group were included in the study.

**Sample collection and estimation**

5 ml of blood was drawn from the patients and healthy volunteers by venipuncture under aseptic conditions. The serum was separated and was transferred into a test tube. The estimation of serum AST, ALT, GGT and ALP was done by using commercially available Roche diagnostic kits and the readings were taken on fully automated biochemistry analyser (eCOBAS 6000 c501, Roche).

**Statistical analysis**

Enzyme levels were expressed as Mean  $\pm$  SD in IU/ml. The statistical analysis of differences in Means was done with Unpaired Student's 't' test, using GraphPad Quickcalcs online software, considering significance level as  $P < 0.05$  at 95% confidence interval. Cost calculation of all the tests was done.

**RESULTS**

The results of the study are summarized in Tables 1 and 2 below.

**Table-1: Levels of serum liver enzymes in ALD and Normal Control group**

SERUM LEVELS	ALCOHOLIC LIVER DISEASE (ALD) GROUP (N=30) MEAN $\pm$ SD (IU/ML)	NORMAL CONTROL GROUP (N=30) MEAN $\pm$ SD (IU/ML)	P VALUE	95% confidence interval
AST	133.50 $\pm$ 51	21.27 $\pm$ 5.75	<0.0001 (#)	93.47 - 131.00
ALT	71.87 $\pm$ 30.62	17.48 $\pm$ 4.05	<0.0001 (#)	42.90 - 65.87
ALP	186.00 $\pm$ 68.00	90.00 $\pm$ 19.00	<0.0001 (#)	70.20 - 121.80
GGT	125.00 $\pm$ 58.00	16.00 $\pm$ 4.00	<0.0001 (#)	87.75 - 130.25

Values are expressed as Mean  $\pm$  SD; Unpaired t test \* =  $P < 0.05$  (significant), # =  $P < 0.0001$  (highly significant) Serum AST, ALT, ALP, GGT were raised

highly significantly ( $p < 0.0001$ ) in patients with ALD when compared with the normal control group.

**Table-2: AST: ALT ratio (De Ritis ratio) in ALD and Normal Control group**

AST:ALT ratio or De Ritis ratio	ALCOHOLIC LIVER DISEASE (ALD) (N=30)	CONTROL GROUP (N=30)
<2	30%	100%
$\geq 2$	70%	0%

The increase in De Ritis ratio was  $>2$  in 70% patients with ALD when compared with control group ( $<2$ ) in all the samples.

Simple cost calculation of all the tests revealed that the total cost per patient was only Rs 80/-.

**DISCUSSION**

ALT, AST, ALP, GGT and De Ritis Ratio are conventionally used as markers for diagnosis, treatment, prognosis and monitoring of ALD. Although many new biochemical and haematological tests are used

nowadays for diagnosing ALD, no test can be used independently as a sole marker for this purpose. Moreover, these tests are generally expensive and are not available in all laboratory settings. In this background, we wanted to establish the utility and affordability of the conventional serum enzymes AST, ALT, ALP, GGT and the De Ritis ratio as diagnostic and prognostic markers in ALD.

We observed highly significant rise in AST, ALT, ALP, GGT among patients of ALD compared to the normal control group. Rise in AST, ALT and high

De Ritis ratio in ALD patients were similar to the observations of several researchers [18-26]. GGT and ALP levels in ALD group were comparable to the findings of other researchers [27-31]. When two or more liver enzymes are abnormal, the patient undergoing testing has a high likelihood of having hepatic or biliary tract disease. The finding of only one abnormal liver enzyme is more difficult to interpret. In our study, all four enzymes were elevated and accurately reflected the clinical diagnosis in ALD group.

The elevation of AST was more than that of ALT in ALD cases, reflecting their leakage into the serum from damaged hepatocytes [32]. ALT is a purely cytosolic enzyme whereas AST is both cytosolic as well as mitochondrial in origin. The excessive alcohol consumption causes damage to the hepatocytes leading to secretion of mitochondrial AST in the blood. The serum AST level depends mostly on the degree of liver damage by the alcohol abuse and also on how recently the alcohol has been consumed by the person [33].

In alcoholic patients, mitochondrial damage in hepatocytes seems to be more extensive, and the ALT level is lower because of pyridoxine deficiency which commonly accompanies alcohol abuse. The excess alcohol leads to increased oxidative stress, cell membrane permeability, cell necrosis and leakage of mitochondrial AST into the blood [24,34]. Mitochondrial AST appears to be especially useful in identifying patients who are alcoholic with or without alcoholic hepatitis [35].

The serum levels of AST and ALT are raised to some extent in all types of liver diseases. The maximum level of elevation ( $>1000$  U/L) occurs in viral hepatitis, drug- or toxin-induced hepatic necrosis, shock and/or ischemia to the liver. In most other liver diseases the serum levels of AST and ALT rise only to mild or moderate levels. AST or ALT level that crosses beyond  $1000$  U/L secondary to viral or drug induced hepatitis typically return to normal over a period of weeks or months. On the other hand, the marked elevation of these enzymes due to ischemia reverts back to normal within few days. The absolute level of enzyme elevation does not correlate with the extent of hepatocyte damage or prognosis [36].

Mild elevation of serum transaminases ( $<2$ -3 folds) may be false positive secondary to laboratory error, statistical quirk, or disease of another organ system, or they may often be explained by inconsequential liver disease such as fatty liver. On the other hand, elevation of aminotransferases  $>2$ -3 folds, or elevation that occurs in association with abnormalities of other liver function tests are most likely explained by significant underlying liver

diseases. A careful analysis of aminotransferases is to be carried out to rule out the underlying disease process [36]. In our study, we found that rise in AST was almost twice as much as in ALT, but the levels were below  $300$  IU/ml, which is similar to the observations of Tourellas C, *et al.* and Diehl, DM [7,14]. Mild to moderate increase of AST or ALT are seen in alcoholic liver disease, with values  $>500$  U/L being clearly unusual. In such conditions, aminotransferases are usually accompanied by abnormalities of other tests, such as GGT, HDL-cholesterol, erythrocyte mean corpuscular volume and iron [37].

The determination of the De Ritis ratio in ALD patients can be considered as a dependable marker, along with increase serum levels of GGT which is also considered as another reliable marker for detection of ALD if correlated correctly [38]. In ALD, De Ritis ratio may be helpful diagnostically [34] as a value of  $>2$  is suggestive of ALD. In one study, over  $80\%$  of patients attained this ratio [39]. In our study, it has been observed that the De Ritis ratio is  $>2$  in  $70\%$  cases of ALD, which is similar to the findings of another observer [40]. A low De Ritis ratio has been advocated as a good index of viral hepatitis. However, in general, an ALT of  $>300$  U/L is more discriminatory for hepatitis than the De Ritis ratio [6,27,41,42]. The high De Ritis ratio in ALD is basically due to a decrease in hepatic activity of ALT, deficiency of pyridoxal-5-phosphate in alcoholics and damaged hepatocytes leading to an increase in the leakage of mitochondrial AST in blood of patients with ALD [19,35,43,44].

Elevations of alkaline phosphatase (ALP) have been observed most commonly in patients with hepatobiliary diseases, as observed by Rekha M, *et al.* [31,45] as well as physiologic bone growth, and benign and malignant bone diseases [45]. Less common causes of an elevated ALP include infarction of several organs (myocardium, lung, spleen, kidney or bowel), ectopic production by carcinoma, ulcerative colitis, sepsis, hyperthyroidism, congestive heart failure and acute bone fracture [46]. An elevation of ALP with elevation of other LFTs, such as aminotransferases or bilirubin, is most often due to hepatobiliary disease. Mild elevations of ALP are common in physiologic conditions such as age greater than 50, pregnancy and physiologic bone growth. In addition, a number of mild transient elevations of ALP occur for reasons that are unexplained or secondary to other coexistent disease processes. Finally, elevation of ALP greater than 1.5-fold or 2-fold, particularly when associated with abnormalities of other liver tests, strongly point to the presence of hepatobiliary disease. Serum GGT is often used to confirm that an elevated ALP is secondary to hepatobiliary disease rather than to bone or other organ disease [36]. In our study, mean ALP was more than

twice that of control group and AST, ALT and GGT were also raised.

Serum GGT is elevated in association with hepatobiliary disease and generally parallels activity of alkaline phosphatase [47]. However, routine screening may lead to the difficult problem of interpreting the significance of an isolated elevation of GGT. GGT may also be elevated in injury to other organs from conditions such as pancreatic disease, myocardial infarction, renal failure, chronic obstructive pulmonary disease and diabetes [41,48]. Serum GGT is the most sensitive indicator of hepatobiliary disease; however, its poor specificity limits its usefulness. In addition, GGT is inducible by alcohol and a number of enzyme-inducing drugs [41,48,49]. Some investigators have advocated the use of GGT for the detection of surreptitious alcohol ingestion. In general, GGT levels were elevated 2-3 times the upper limits of normal in patients without clinically obvious liver disease but to 8-10 times normal in those with obvious liver disease [16,50]. The variable sensitivity and lack of specificity, however, suggests that GGT levels alone are not helpful in detecting alcohol ingestion [41]. Elevated blood levels of GGT indicate heavy alcohol use and liver injury. This test has greater ability to correctly test positive (i.e., sensitivity) but less ability to correctly test negative (i.e., specificity) than AST or ALT tests. Of the three enzymes, GGT is the best indicator of excessive alcohol consumption, but GGT is present in many other organs and because some drugs raise GGT levels, high GGT levels are not necessarily an indicator of alcohol abuse [6,51]. Thus, the extreme sensitivity and poor specificity make the interpretation of an isolated GGT elevation fraught with difficulty. The use of alcohol or enzyme-inducing drugs will elevate GGT in the absence of liver disease. The primary clinical usefulness of GGT is to confirm the hepatic origin of alkaline phosphatase. Moreover, marked elevation suggests active alcoholic liver disease, biliary tract obstruction or hepatic metastasis [36].

We found that the cost per patient for the serum liver enzymes was only Rs 80/- which is affordable for a vast majority of the population. Botros M, *et al.* have previously reported that serum transaminase estimations are one of the cheapest laboratory tests available and all laboratory information systems are capable of calculating and comparing simple ratios like AST:ALT [52].

## CONCLUSION

The traditional liver enzymes AST, ALT, ALP, GGT are significantly elevated in ALD and being affordable by all socioeconomic categories of patients, these are of immense utility in detecting and monitoring of ALD if correlated carefully with the clinical findings. Interpretation of De Ritis ratio is also an affordable

diagnostic and prognostic enabler in ALD. Though GGT is a good marker for detecting alcohol consumption, it is always advisable to interpret the findings by analyzing other liver parameters like AST, ALT and the De Ritis ratio in particular along with ALP. This simple, time saving, affordable and reliable parameters can also be used for screening of ALD in general population.

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