

SIGNIFICANCE OF DE RITIS RATIO AND SERUM GGT/ALP RATIO IN DIFFERENTIAL DIAGNOSIS OF ALCOHOLIC LIVER DISEASE

Biochemistry

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ABSTRACT

Alcoholic liver disease (ALD) is one of the most prominent hepatic disorders found in chronic alcoholics. According to the Centres for Disease Control and Prevention (CDC), the number of deaths due to chronic alcoholism and ALD is increasing worldwide. Traditionally clinicians employ a combination of hepatic ultrasound, liver function tests (LFT), and histology for differential diagnosis of ALD and non-alcoholic fatty liver disease (NAFLD). In the present study, we report the significance and usefulness of the De Ritis ratio and serum GGT/ALP ratio in the differential diagnosis of ALD. The study group included 70 cases (38 ALD and 32 NAFLD), and 50 healthy controls. We investigated their hepatic enzymes and correlated it with serum CRP, sonography, and clinical history findings. Our findings show that both the De Ritis ratio and GGT/ALP ratio are elevated (> 2.5) in ALD when compared to that of control and NAFLD. A significant reduction in the GGT/ALP ratio (0.15) is seen in NAFLD, which helps us to differentiate NAFLD pathology from that of ALD. The serum CRP level is found to be elevated both in ALD and NAFLD; however, it could not differentiate from one another. Our findings could be used as a potential index in the differential diagnosis of chronic ALD and NAFLD.

KEYWORDS

De Ritis ratio, GGT, ALP, ALD, NAFLD.

INTRODUCTION

Alcoholic liver disease (ALD) or alcohol-related liver disease (ARLD), is a liver disorder with multiple manifestations including fatty liver, hepatitis, fibrosis, and hepatocellular carcinoma (HCC) due to alcohol overconsumption¹. Studies show that 90% of chronic and heavy drinkers develop fatty liver and about 25% develop more severe alcoholic hepatitis, and 15% cirrhosis². There are several risk factors known to contribute to ALD and related complications that include, the amount of alcohol consumed, drinking pattern, gender, viral infections (hepatitis C virus), diet, iron overload, and genetic factors³.

According to the World Health Organization (WHO), the total per-capita consumption of alcohol by individuals above 15 years of age is 6.2 L of pure alcohol per year, which translates to about 13.5 g of pure alcohol per day⁴. Recent studies show that alcohol consumption is increasing in India, and its pattern and practices vary across different parts of the country because of various socio-cultural diversity and the difference in laws governing individual states within India⁵. The nature of alcohol-related liver injury varies from alcoholic fatty liver disease (or steatosis) to alcoholic liver cirrhosis. There is a greater chance of getting ALD for those who consume at least 80 g of alcohol daily for a few years. Fatty liver is found to set up in about 90% of individuals who drink more than 60 g/day of alcohol. However, this condition is reversible with complete alcohol abstinence after about 4 to 6 weeks¹. Studies show that at least 80% of heavy drinkers develop steatosis, 10% to 35% develop alcoholic hepatitis and approximately 10% will develop liver cirrhosis⁶. Chronic alcoholism is a potential risk of developing liver cirrhosis (the most severe form of alcoholic liver injury) in both men and women where dose plays an important role as well⁷. A rise in the serum gamma-glutamyl transferase (GGT), alkaline phosphatase (ALP), transaminases, and the De-Ritis ratio (serum aspartate transaminase (AST) to alanine transaminase (ALT) ratio) explains the potential injuries in the hepatocytes at any stage of alcoholic liver disease⁸. These intracellular enzymes are released into the circulation upon any tissue injury.

The present study is mainly aimed at revealing the significance of the De-Ritis ratio and serum GGT/ALP ratio in the differential diagnosis of ALD and NAFLD.

MATERIALS AND METHODS

The study was conducted in the Department of Biochemistry, Government Medical College, Kota, Rajasthan with all the necessary

formalities of the institutional ethics committee (IEC). The study group was constituted of newly diagnosed ALD (n=38) and NAFLD (n=32) cases and healthy controls (n=50). Patients were recruited according to the pre-designed protocol where all ALD patients were men, and hence only men (age-matched) were included (Fig. 1A) in the whole study group. The group was categorized according to the smoking habit (Fig. 1B) to check any possible potential relation between smoking and liver pathology. Subjects with severe diseases including cardiac disorders, malignancy, and terminal liver failure were excluded.

About 5 mL of blood was collected aseptically by venipuncture in the plain vial as per the standard protocol. The serum was used for the study of routine liver function tests (LFT) and the lipid profile. A fasting plasma sample was also drawn to study the variations in glucose levels. The routine chemistry parameters were studied on a fully automated biochemistry analyzer, XL-640 (Transasia Bio-Medicals Ltd, Mumbai, India) based on photometric principles. The serum CRP levels were measured on an autoanalyzer, Cobas c501 (Roche Diagnostics, Mannheim, Germany) based on the immunoturbidimetric method (latex agglutination principle).

Samples were run in triplicates and the mean results obtained are represented as mean $\pm$ SD. We have used a nonparametric statistical tool, the student t-test for comparing one variable between two independent samples or groups. Correlation analysis was done by the Spearman rank correlation method. A p -value < 0.05 was considered to be significant, and a p -value of < 0.01 was considered to be highly significant for a given hypothesis testing. All statistical analyses were performed using GraphPad Prism Ver.6.0 (GraphPad Software, Inc., CA, USA) and Microsoft Excel, MS office 2019 (Redmond, WA, USA).

RESULTS

In the current study, we had 38 ALD patients (32%), and 32 NAFLD patients (27%) compared with 50 healthy age-matched control (41%) subjects (Fig. 1A). No significant variation in the FPG level (Fig. 1C), was found in ALD (102.3 ± 19.2 mg/dL) or NAFLD (104.2 ± 23.2 mg/dL) patients as compared to control (95.7 ± 16.9 mg/dL) subjects. The serum CRP level (Fig. 1D), in both ALD patients (4.14 ± 1.11 mg/dL) and NAFLD patients (3.99 ± 1.8 mg/dL) showed a very significant variation when compared to that of the control group (0.21 ± 0.03 mg/dL).

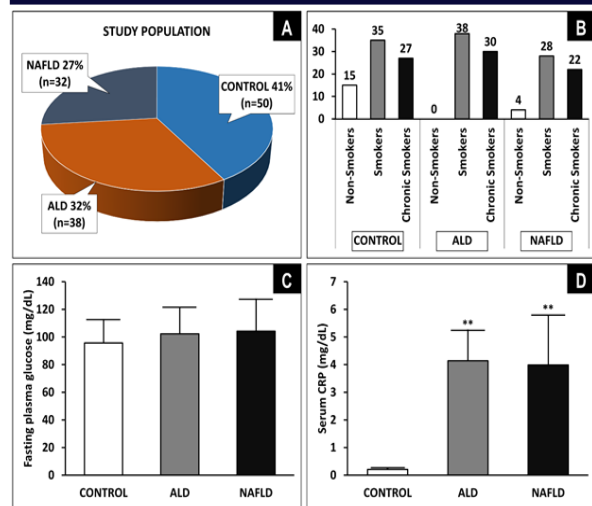


Fig. 1: (A) The study population shows the number of cases (ALD and NAFLD) and controls. **(B)** Smoking status of the study population. **(C)** Comparison of fasting plasma glucose levels. **(D)** Comparison of serum CRP levels. ***p*-value < 0.01.

The AST levels (Fig. 2A), showed a significant variation in ALD (58.7 ± 6.5 U/L) and NAFLD (36.8 ± 5.5 U/L) patients as compared to the control (16.2 ± 2.1 U/L) subjects. The ALT levels (Fig. 2B), also showed a significant variation in ALD (22.2 ± 4.1 U/L) and NAFLD (34.4 ± 4.6 U/L) patients as compared to the control (15.6 ± 3.7 U/L) group. The serum ALP levels (Fig. 2C), showed significant elevation in ALD (55.2 ± 11.6 U/L) and NAFLD patients (145.7 ± 20.1 U/L) when compared to the control group (44.6 ± 7.2 U/L). Similarly, the serum GGT (Fig. 2D) was found to be significantly elevated in ALD patients (168.7 ± 12.6 U/L), but not in NAFLD patients (22.3 ± 6.3 U/L) when compared to the control group (21.2 ± 3.2 U/L).

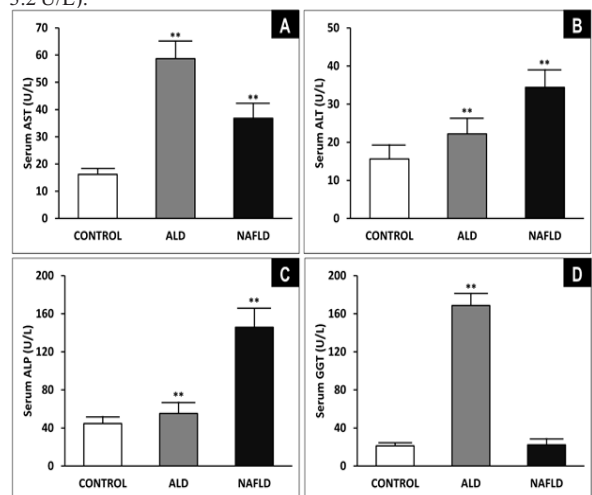


Fig. 2: (A) Comparison of serum AST levels. **(B)** Comparison of serum ALT levels. **(C)** Comparison of serum ALP levels. **(D)** Comparison of serum GGT levels. ***p*-value < 0.01.

The AST/ALT ratio (Fig. 3A) in ALD patients (2.64 ± 1.7) was found to be highly significant when compared to that of the control group (1.04 ± 0.9). There was no significant variation in this ratio among the NAFLD patients (1.07 ± 0.8) and the control subjects. The serum GGT/ALP ratio (Fig. 3A) was found to be significantly elevated in ALD patients (3.06 ± 0.97), while the same was found to be decreased in AFD patients (0.15 ± 0.1) when compared to that of the control group (0.48 ± 0.27). Serum total bilirubin (Fig. 3B) was significantly elevated in ALD (1.9 ± 0.6 mg/dL) and NAFLD patients (2.3 ± 0.4 mg/dL) when compared to controls (0.9 ± 0.3 mg/dL). The direct bilirubin was elevated only in NAFLD patients (0.31 ± 0.11 mg/dL), but not in ALD patients (0.22 ± 0.17 mg/dL) when compared to control subjects (0.18 ± 0.10 mg/dL). The indirect bilirubin was significantly elevated in both in ALD (1.68 ± 0.58 mg/dL) and NAFLD cases (1.99 ± 0.53 mg/dL) when compared to the control group (0.72 ± 0.19 mg/dL).

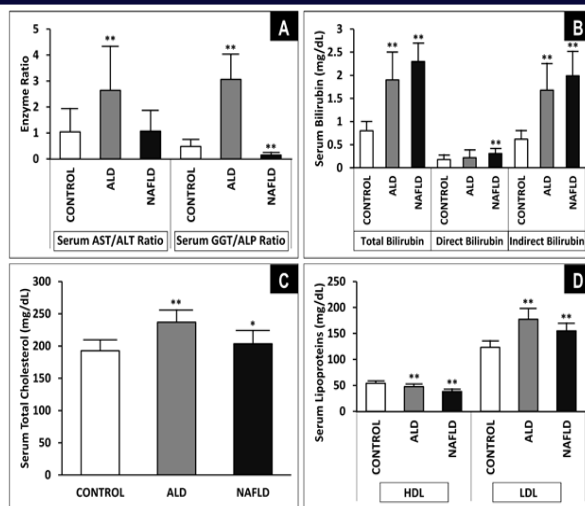


Fig. 3: (A) Comparison of enzyme ratios. **(B)** Comparison of serum bilirubin levels. **(C)** Comparison of serum total cholesterol levels. **(D)** Comparison of serum lipoprotein levels. ***p*-value < 0.01, **p*-value < 0.05.

The serum total cholesterol (Fig. 3C) was found to be elevated in ALD cases (236.8 ± 19.2 mg/dL) and NAFLD cases (203.5 ± 20.6 mg/dL) when compared to the controls (192.6 ± 16.9 mg/dL). The serum HDL levels (Fig. 3D) were significantly reduced in both ALD (47.6 ± 5.2 mg/dL) and NAFLD patients (38.2 ± 4.8 mg/dL) when compared to the control group (54.2 ± 4.5 mg/dL). On the other hand, the serum LDL levels were elevated significantly in ALD (177.4 ± 20.7 mg/dL) and NAFLD patients (155.1 ± 14.8 mg/dL) when compared to the control subjects (123.3 ± 12.7 mg/dL).

DISCUSSION

The mean age of ALD, NAFLD cases, and the controls in the present case-control study was 49.3 ± 4.9 years, 46.7 ± 5.8 years, and 47.5 ± 6.6 years respectively. Among 38 ALD patients, 30 (78.9%) were chronic drinkers, who used to consume at least 5 times a week. The rest of the 8 patients (21.1%) used to drink occasionally, once or twice a month. The liver function was remarkably affected both in ALD and NAFLD patients as shown by marked elevation of serum bilirubin, serum transaminases, serum ALP, and serum GGT. We noticed that the extent of liver damage was higher in chronic ALD alcoholics, as revealed in their sonography reports.

The damage that happened to hepatocytes during alcohol-induced liver injury causes the release of liver-specific non-functional enzymes into the bloodstream, that in turn leads to elevation of their serum levels beyond normal. A normal De Ritis ratio should be <1. Studies show that, in patients with alcoholic liver disease, this ratio rises above 1.0 in 92% of patients, and above 2.0 in 70%¹⁰. A recent study by Parmar *et al* shows that the De Ritis ratio is markedly increased in ALD, and significantly increased in NAFLD¹¹. They also found that this ratio is reduced in viral hepatitis and unaltered in other liver disorders like cholestasis. Present study results are also in line with these findings. Certain patients showed a low or normal level of serum ALT. This may be due to an alcohol-induced deficiency of pyridoxal phosphate found in chronic alcoholism¹². Extensive hepatocellular damage including mitochondrial disruption could increase the cellular AST/ALT ratio above normal^{13,14}. A hospital-based study in Nepal showed that the De Ritis ratio could also be used for predicting the development of ALD in alcoholics¹⁵.

Alcohol, being a potential inducer of GGT, causes its overproduction at the genetic level. The excessive production of this ectoenzyme causes its cellular leakage and subsequent serum elevation. Studies show that, in alcoholic hepatitis, the ALP levels may be normal or disproportionately lower than GGT^{16,17}. In the present study, the GGT/ALP ratio is elevated in ALD but significantly reduced to 0.15 in NAFLD. The latter might have been caused by a sudden elevation of serum ALP. Our study results are comparable with these recent studies. Both ALP and GGT are useful enzymes to assess liver function. However, a marked elevation of serum GGT with a fair elevation in serum ALP suggests ALD. Elevation of serum ALP with normal serum GGT is found in other liver disorders like biliary obstruction, viral hepatitis, and cholestasis^{18,19}.

Our results show that the serum CRP was also found to be elevated significantly in ALD and NAFLD patients. There was a positive correlation between smokers and their elevated serum CRP levels ($r = 0.7$). The rise in serum CRP was proportionate to the degree of hepatic inflammation as correlated with their sonography results. Recent studies revealed the association between elevated liver-specific enzymes and CRP in liver disorders. They identified the possible hepatic contribution to systemic inflammation in metabolic syndrome²⁰. Animal model studies show that increased levels of serum CRP are an early sign of alcoholic fatty liver in rats. The abnormally elevated CRP induced by ethanol can be used as a biomarker to distinguish alcoholic fatty liver from normal or other liver disorders²¹.

CONCLUSION

The present study reveals the usefulness and the significance of the De Ritis ratio and the GGT/ALP ratio in the differential diagnosis of ALD and NAFLD. The transaminase ratio (AST/ALT) holds its relevance, especially in the differential diagnosis when used in the correct combinations. An elevated De Ritis ratio > 2.0 indicates inflammation of the liver, and > 2.5 suggests severeness of the condition. This ratio is elevated in both ALD and NAFLD. However, a reduced GGT/ALP ratio < 0.25 or fewer indicates the NAFLD.

Limitations

The current study is mainly focused on men of middle age in the South-East Rajasthan area. More statistically valid details would have been obtained if the sample size was higher and geographically wider in recruitment.

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Conflict Of Interests

The authors hereby declare that they have no conflict of interests related to this original work.

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