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ORIGINAL RESEARCH ARTICLE

ALBUMIN TO GLOBULIN RATIO AND ITS ASSOCIATION WITH LIVER ENZYMES IN ALCOHOLIC LIVER DISEASE

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ABSTRACT

Background: High alcohol consumption may lead to alcoholic liver disease, the diagnosis of which is made by using clinical laboratory tests among which liver enzymes, De Ritis ratio, and Albumin to Globulin ratio are more reliable. The main aim of this study was to evaluate the albumin to globulin ratio and its correlation with the liver enzyme panel in patients with alcoholic liver disease.

Methods: A prospective cross-sectional study was conducted among the patients visiting the hospital from November 2022 to September 2023 after getting ethical approval. Non-probability sampling method and convenience sampling technique was used for enrolling the participants in this study. After performing specific biochemical tests, results are expressed as mean ± standard deviation for continuous data. In addition, Pearson correlation coefficient was used to measure the correlation of albumin to globulin ratio with liver enzymes.

Results: Liver enzymes, such as AST and ALT were significantly raised in patients with alcoholic liver disease. A rise in AST activity was greater than that of ALT activity resulting in a ratio of AST to ALT (De Ritis ratio) greater than two but a ratio of serum albumin to globulin is lesser than one in more than two-thirds (67.60%) of the patients and the ratio of serum albumin to globulin is negatively correlated with liver enzymes.

Conclusions: The albumin to globulin ratio is decreased in the majority of alcoholic liver disease patients. Therefore, along with De Ritis ratio, the albumin to globulin ratio can be used as a marker for alcoholic liver disease.

INTRODUCTION

Alcoholic liver disease (ALD) refers to the spectrum of alcohol-related liver injuries namely hepatic steatosis, alcoholic hepatitis, fibrosis, and cirrhosis.[1, 2] A damage to liver cells results in a derangement in liver function. One way of assessing it is by estimating serum aminotransferases- Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT), Gamma glutamyltransferase (GGT), Alkaline phosphatase (ALP), and serum proteins such as Albumin, etc.[3]

Besides, the calculation of AST to ALT, known as De Ritis ratio, is more diagnostic to distinguish different types of liver injuries.[4, 5] Furthermore, the liver is the only site for synthesis of albumin, one of the major plasma proteins, production of which decreases in case of chronic liver disease which results into a decreased albumin to globulin ratio (AGR).[6]

Since, an association of AGR with liver enzymes have not been studied in our settings, therefore, this study aims to establish an association of AGR with liver enzymes as well as with De Ritis ratio among ALD patients.

METHODS

A cross-sectional study was conducted among the patients visiting General Medicine Department of Kathmandu Medical College Teaching Hospital (KMCTH) from November 2022 to September 2023 and the samples were collected from its central laboratory. Ethical approval was taken from the Institutional Review Committee of Kathmandu Medical College and Teaching Hospital (Ref. 12082022/04).

The present study included 148 participants belonging to both sexes, with their age ranging from 30 to 60 years. Non-probability sampling method and convenience sampling technique was used to enroll the participants who were informed regarding the nature of the study and written consent was taken. ALD was diagnosed by clinician on the basis of physical examination and patients' history who were included as cases whereas nonalcoholic, healthy individuals without any known liver diseases were enrolled as controls.

Blood sample collection was done under aseptic condition, by veni-puncture from cubital vein, collected in a vial and serum

was separated after centrifugation which was followed by specific tests with the use of automated analyzer.

Liver enzyme activities, and the levels of bilirubin, total protein and albumin in serum were estimated by Selectra pro S fully automated analyzer using Elitech reagent. Globulin was calculated by subtracting the value of albumin from the value of total protein. Similarly, AST to ALT ratio was calculated by dividing the results of AST by ALT and AGR was calculated by dividing the results of Albumin by globulin.

Sample size was calculated according to the study conducted by Rizal S et al.[7] using the following formula: Z^2pq/d^2. A 90% confidence level is employed to accept a slightly lower level of confidence in exchange for smaller sample sizes since within a time frame of this study, lesser number of cases were diagnosed to be alcoholic liver disease. Therefore, we narrowed the confidence interval. Since, most of the studies have conducted prevalence types of research, on the basis of which sample size has been calculated for this study. However, AGR and its association with liver enzymes have not been studied by other authors, therefore, to meet the research objective, we compared mean values of dependent variables.
$= (1.64)^2X0.5 \text{ X } 0.5/(0.1)^2 = 67.24$
Where
Z= statistic for a level of confidence. (For the level of confidence of 90%, which is conventional, Z value is 1.64).
p= 50%, q= (1-p)
e= absolute error or precision.(considered 0.1). Since, the precision can be affected due to the availability of resources, such as time frame, budget as well as the availability of sample. In addition, for precise estimation, a higher precision level (such as 0.1) is used. Adding 10% to address the non-response rate, sample size estimated would be
= 67.24+6.724
= 73.96
Therefore, a total of 74 cases and the same number of controls were recruited for the study.

After obtaining the laboratory results, analysis was done by using Statistical Package for the Social Sciences (SPSS) version 16. Descriptive statistics was represented as Mean ± Standard deviation (SD) with 95% confidence intervals for continuous data (age, liver enzymes, bilirubin, total protein and albumin) and the frequency as well as percentage were used to depict the categorical data (gender, De Ritis ratio and AGR). Student T test was applied for comparison of case and control in which some data accepted while some other rejected the null hypothesis. For measuring the correlation between two variables, Pearson's coefficient of correlation 'r' was used which assessed the relation between AGR vs liver enzymes and AGR vs De Ritis ratio. Statistical significance was assumed at $p<0.05$.

RESULTS

This study found a male dominance with 81.1% of males and only 18.9% of females in ALD. The mean age of the patients of ALD was 46.70 years and that of control group was 45.59 years, which is shown in table 1.

Table 1: Gender and age wise comparison of study participants

Parameters	ALD		Control	
Gender	Male	Female	Male	Female
Frequency (%)	60 (81.1%)	14 (18.9%)	52 (70.3%)	22 (29.7%)
Age (mean±SD) year	46.70±8.4		45.59 ±12.77	

Serum levels of liver panel, such as AST, ALT, GGT, ALP, total and conjugated bilirubin, as well as AST to ALT ratio were statistically significantly raised in ALD compared to that in control. Serum globulin level was raised but statistically insignificant in ALD compared to that in control. However, total protein and albumin as well as the Albumin to Globulin ratio (AGR) were significantly decreased in ALD compared to that in control, which is shown in table 2.
More than 70% of the patients with ALD have AST to ALT (De-ritis) ratio greater than or equal to two whereas the ratio is less than two in almost all (98.60%) control group. Similarly, two thirds of the ALD patients have AGR less than one whereas, it is more than one in 89.2% of the control group which is depicted in table 3.

Table 2: Comparison of Biochemical parameters in Alcoholic liver disease and control

Parameters	ALD (Mean±SD)	Control (Mean±SD)	P value
AST	164.77±54.48	26.12± 5.90	<0.001
ALT	77.68±23.55	25.47± 6.78	<0.001
AST/ALT	2.11±0.35	1.06±0.26	<0.001
GGT	247.28±75.14	36.14±12.86	<0.001
ALP	101.60± 21.12	82.11± 14.99	<0.001
Total Bilirubin	3.83± 1.57	0.70± 0.25	<0.001
Direct Bilirubin	1.87± 1.01	0.20± 0.15	<0.001
Total protein	6.56±0.85	7.10±0.66	<0.001
Albumin	3.11±0.50	3.98±0.52	<0.001
Globulin	3.45±0.68	3.13±0.50	0.02
Albumin: Globulin	0.93±0.25	1.31±0.35	<0.001

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Table 3: Distribution of De-ritis ratio and Albumin to Globulin ratio in ALD and control group

Parameters	ALD		Control	
AST: ALT	<2	≥2	<2	≥2
	20 (27.00%)	54 (73.0%)	73 (98.60%)	1 (1.4%)
Albumin: Globulin	<1	≥1	<1	≥1
	50 (67.60%)	24 (32.40%)	8 (10.8%)	66 (89.2%)

This study found a negative correlation of AGR with all the liver enzyme panel as well as with the De Ritis ratio, which were statistically insignificant and is depicted in table 4.

Table 4: Correlation of liver enzymes with AGR

Variables	Correlation coefficient (r value)	p value
AGR vs AST	-0.056	0.63
AGR vs ALT	-0.038	0.74
AGR vs ALP	-0.075	0.52
AGR vs GGT	-0.191	0.10
AGR vs AST:ALT	-0.092	0.43

DISCUSSION

This study is intended to evaluate serum levels of liver panel as well as comparing De Ritis ratio, AGR and an association of latter with the liver enzyme panel in ALD and in control group.

In the present study, activities of serum aminotransferases, the AST and ALT, ALP, GGT, total bilirubin and conjugated bilirubin were significantly raised in ALD compared to that in control. Similarly, De Ritis ratio is also significantly increased in ALD compared to that in control. This finding is supported by a number of studies[7,8]. Serum activities of ALT and AST, are two of the most useful measures of liver cell injury. Any types of diseases that primarily affect hepatocytes, will cause an elevation of the ALT and AST levels in plasma. Increased levels of these enzymes on plasma indicate damage to hepatocytes, and thus, these are released from hepatocytes to plasma. In spite of a rise in serum activities of both the enzymes, a rise in AST activity was far greater than the rise in ALT activity in ALD in this study.

ALT is mainly present in the cytoplasm whereas AST is present in both the compartments- the cytoplasm and the mitochondria of hepatocytes and are known as- cytosolic AST (cAST) and mitochondrial AST (mAST) respectively where the latter is the more prevalent isoenzyme in human being.[9]

Alcohol as well as its metabolite, the acetaldehyde is injurious to hepatocytes as these increase oxidative stress in Kupffer cells, damage DNA and cause lipid peroxidation in the hepatic cells, which thus leads to liver cell injury[10,11] and finally resulting into the leakage of mAST on the plasma.[9]

In addition to release of ALT and AST from hepatic injuries, an elevation in AST can also be caused by extrahepatic disorders, such as in the disorders of cardiac muscle, skeletal muscle, kidneys, brain, pancreas, lungs as well as in leucocytes, and erythrocytes. Therefore, an elevation in AST is not considered as sensitive or specific for the liver injuries as that of ALT.[12]

Since, ALT is released mainly from the cytosol of the hepatocytes on plasma making its lesser activity compared to that of AST activity. Other possibility is, for the transamination reaction, pyridoxal phosphate (PLP), an active form of vitamin B6 is required but in alcoholics, metabolite of alcohol, the acetaldehyde enhances the degradation of intracellular PLP, hence, due to deficiency in PLP, ALT activity is compromised.[13] Finally, decreased activity of ALT compared to that of AST, makes the De Ritis ratio high in ALD. This study also showed statistically significantly increased De Ritis ratio (2.11±0.35) in ALD compared to that in control (1.06±0.26) group. Further, more than 70% of the patients had De Ritis ratio greater than or equal to two which is in accordance with other similar types of studies,[14, 15] however, some authors have reported a slightly lesser value of De Ritis ratio i.e. less than two in ALD[16] and this ratio is less than one in patients with viral hepatitis.[17]

The present study showed a small rise in serum ALP in ALD compared to that in control group which is in accordance with the study by Arthi M et al.[18] Unlike the findings of present study, a recently conducted case study by Axley et al. found a very high levels of ALP (805 U/L) in alcoholic hepatitis.[19] ALP is present in various tissue sources including liver, bone, small intestine, placenta and kidney. In case of liver disease, especially in hepatic obstruction, increased activity of ALP in plasma is due to raised synthesis and release of the enzyme by cells lining the bile canaliculi, usually in response to intrahepatic or extrahepatic obstruction.[20] Therefore, an increased activity of ALP in plasma may either be due to leakage from bile canaliculi into the hepatic sinusoids or due to failure of liver to excrete ALP which is present in liver as well as in other sources.

This study had shown a statistically significantly raised levels of GGT in ALD compared to that in control which is in accordance with the study of Atul P et al.[21] Since, GGT is an inducible enzyme, alcohol induces the expression of protein for the increased production of GGT[22] and a raised value of it indicates alcohol consumption. Since, alcohol or acetaldehyde increases oxidative stress which leads to increased oxidized glutathione that induces the production of GGT; which in turn converts oxidized glutathione to reduced glutathione. Glutamine reduces the degree of oxidative stress and improves hepatic steatosis in the liver of rats with non-alcoholic fatty liver disease.[23] Thus, hepatic GGT is thought to defend against liver damage caused by oxidative stress. Besides, alcohol abuse causes damage to the liver cell causing release of GGT from microsomes of the hepatocytes on the blood. Therefore, raised

levels of GGT can be used as a marker for alcohol consumption and the predictor of ALD.[21, 24]

Present study also showed a significantly raised levels of total and conjugated bilirubin in ALD compared to that in control which is supported by other studies. [8, 25] Total bilirubin includes unconjugated and conjugated fractions of bilirubin. A rise in unconjugated bilirubin in blood indicates a damage to hepatocytes due to which either unconjugated bilirubin is not taken up normally by hepatocytes, i.e. it remains in blood, or even if it is taken up by hepatocytes, it may not be conjugated due to defect in conjugating system of the liver, hence, the unconjugated bilirubin is regurgitated in blood resulting into its raised value. Also in alcoholic liver disease, transport of conjugated bilirubin may be impaired due to intrahepatic cholestasis which thus leads to conjugated hyperbilirubinemia. Because of increased levels of both the type of bilirubin in blood, there is hyperbilirubinemia.[26]

However, the present study found a decreased in total protein and albumin with a drop in albumin to globulin ratio in ALD compared to that in control group which is in consistent with some other studies. [8, 25] Contradictory to the findings of present study, a study conducted few years back has shown a slightly higher level of albumin than that of globulin making the AGR a bit higher than one in ALD.[18] Albumin is referred to be a negative acute phase reactant because its concentration is diminished in almost all disease states. A decreased concentration of albumin in serum is either due to decreased production or increased catabolism of albumin or both.[27] Evenmore, serum concentration of albumin is lowered due to organ dysfunction, malnutrition or as a result of capillary escape of albumin in case of increased endothelial permeability secondary to the systemic inflammation.[28]

Present study showed a negative correlation of AGR with liver enzyme activities as well as with De Ritis ratio. A correlation of AGR with AST, ALT, ALP and GGT as well as with De Ritis ratio was found to be negative but statistically insignificant. This suggests, a drop in AGR is either due to decrease in albumin or an increase in globulin. Since, a decreased production of albumin is due to inflammatory conditions related to chronic disease or poor lifestyle, such as smoking or alcoholism.[29] On the one hand, a damage to hepatic function leads to a decreased production of albumin resulting into a lowered AGR. On the other hand, serum immunoglobulins are elevated in specific liver diseases, such as- immunoglobulin G (IgG) is elevated in autoimmune hepatitis, immunoglobulin M (IgM) is raised in primary biliary cirrhosis and the immunoglobulin A (IgA) is increased in ALD and is associated with more advanced liver fibrosis.[30] This increase in globulins contributes to a lower AGR suggesting a damage or destruction to hepatic cells in ALD leading to the release of more AST and ALT from hepatic cells making their increased activity in plasma. In this study correlation of AGR with De Ritis ratio was negative and statistically insignificant suggesting hepatic injury leads to decreased AGR with increased activity of AST compared to that of ALT though both the aminotransferases are raised significantly in ALD.

This study found a raised level of serum ALP in ALD and showed a negative but statistically insignificant correlation of AGR with ALP which could be due to the involvement of bile duct.

Similarly, GGT was raised in ALD which was used as marker for liver disease, particularly when there is alcohol involvement. [21, 24] Elevated GGT levels indicates continuing damage to the liver and is associated with decreased albumin levels. Hence increased GGT is indicative of liver damage and inflammation as well as explanatory to the severity of ALD. The negative correlation between the AGR and hepatic enzymes can signify the severity of ALD. The more damage to the hepatocytes, the lesser would be the plasma levels of albumin suggesting increased inflammation to the hepatocytes, resulting into a lower AGR but higher hepatic enzyme activities.

CONCLUSION

An elevated activity of serum aminotransferases, in which AST activity was higher than that of ALT activity was found in ALD patients which in turn leads to an increased De Ritis ratio with greater than two. However, Albumin was found to be decreased in ALD patients with a small rise in globulin making AGR less than one which is negatively correlated with liver enzymes though the correlation was found to be statistically insignificant. Therefore, instead of estimating a number of liver function panel, calculation of AGR can support for the diagnosis of ALD and can also be implemented to identify the prognosis in ALD.

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