A study on probable correlation of AST/ALT (De Ritis) ratio with the advancement of underlying severity of alcoholic liver disease in the North East Indian population

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ABSTRACT

Background: Alcohol consumption has been linked with one of the most common causes of liver disease, and the AST/ALT (De Ritis) ratio has emerged as a useful indicator for its diagnosis. Our study is on patients of alcoholic liver disease, graded as per Child-Pugh classification, and an effort was made to examine whether AST/ALT ratio can indicate the underlying severity of liver damage caused by alcohol abuse.
Materials and methods: A sample of 142 patients of alcoholic liver disease undergoing treatment in Satribari Christian Hospital, Guwahati, India from December 2014 to February 2016 was selected for the study. Blood samples were collected and AST/ALT ratio and Child-Pugh score of all the patients were calculated from the biochemical and clinical parameters.
Results: The mean age of the subjects was 49 years and there were more males (97.89%) than female patients, who were diagnosed with alcoholic liver disease. Using Child-Pugh score, subjects were grouped into A, B and C. Estimation of AST/ALT ratio shows that the mean values of the ratio were 1.7350, 2.0751 and 2.7608 respectively for A, B and C classes and there exists statistical difference among mean values of the ratio across class groups. Further, this study highlights that there exist a positive correlation (r= 0.362 & p=0.000) between Child-Pugh score and the AST/ALT ratio. In fact, this was found to be statistically significant.
Conclusion: On the basis of results obtained, it can be said that the value of AST/ALT ratio has a positive correlation with the advancement of the underlying liver damage.

Key words: Alcoholic liver disease; AST/ALT (De Ritis) ratio; Child-Pugh score, North East Indian population.

Introduction

Measurement of hepatic transaminase levels, such as alanine transaminase (ALT) and aspartate transaminase (AST), are an effective modality to detect hepatic dysfunction, and their values can be helpful in the differential diagnosis of the hepatobiliary diseases. ALT and AST catalyze the transfer of α-amino groups from alanine and aspartate respectively, to alpha–ketoglutarate [1]. ALT is present mainly in the cytosol of liver, and present much less in the other tissues, is more specific for liver disease [2]. AST can be fractionated into cytosolic and mitochondrial forms, and is less specific for liver disease, as it is found, in addition to the liver, in the heart, skeletal muscle, kidneys, brain, pancreas, lungs, leukocytes, and erythrocytes [1].Although estimation of a single enzyme, *per se*, has low specificity and sensitivity, the use of test combinations can considerably augment the information received with single serum enzyme estimations. The pattern of the transaminases elevation can be helpful diagnostically; particularly the ratio of AST to ALT has much clinical utility in detecting the cause of injury to the liver. The AST/ALT ratio is also known as the De Ritis ratio and it was named after De Ritis, who had performed several pioneering studies on

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serum transaminases [3]. An elevated serum AST in relation to serum ALT has been proposed as an indicator that alcohol has induced the hepatic damage [4]. The De Ritis ratio has a diagnostic relevance, as an elevated ratio of >1.5 is highly suggestive that alcohol abuse is the cause of injury to the patient's liver [5], and in an alcoholic background, if the ratio exceeds 2, it is almost diagnostic [6]. Alcoholic liver disease may represent a spectrum of clinical manifestations and morphological changes that range from fatty liver to hepatic inflammation and necrosis (alcoholic hepatitis) to progressive fibrosis (alcoholic cirrhosis) [7]. Alcohol abuse is known to be very high among the North East Indian population as it has been an integral part of socio-cultural life of many ethnic tribes of the region. In one study conducted in the North East Indian population, 72.2% cirrhosis is caused by alcohol consumption [8]. A high De Ritis ratio can be a vital biochemical clue in these alcoholic patients and also can be a sensitive indicator of the phase of disease. Cirrhosis of liver is a major health problem in the North East Indian population and affects mostly males in the most productive years [8].In this study, the Child-Pugh score is used to assess the severity of the liver disease in the subject group. The score is still considered as the cornerstone in prognostic evaluation in chronic liver diseases, although it was originally used to predict mortality during surgery. Thus, this study was carried out to assess if an increased value of AST/ALT (De Ritis) ratio has any correlation with the severity of liver damage induced by alcohol consumption, measured by Child-Pugh classification.

Materials and Methods

Subjects: The study was carried out on in-patients of the Satribari Christian Hospital, Guwahati, Assam, India, admitted for treatment of alcoholic liver diseases, over a period of 15 months i.e., from December 2014 to February 2016. Clinical history and blood chemistry reports of all the subjects were evaluated. A total of 142 subjects, who were diagnosed with alcoholic liver disease and treated in the hospital, were included in the study. Informed consent was obtained from the subjects included in the study. The subjects selected for the study were above the age of 18 years and were diagnosed with alcoholic liver disease. However, patient diagnosed with alcoholic liver disease, but had coexistent myopathy, congestive heart failure, right sided heart failure or other causes of congestive/ischemic hepatitis, overt hypothyroidism or hyperthyroidism, history of consumption of hepatotoxic drugs in recent past, or had been admitted for treatment for malaria, dengue fever or viral hepatitis, or had other co-morbid conditions which can by themselves derange liver enzyme levels, were excluded from the study.

Sample collection: Blood samples were collected in vials containing clot activators as well as vials containing 3.2% sodium citrate from each subject in study group. Serum and plasma were separated by centrifugation at 2500 rpm for 15 minutes in a Remi Laboratory Centrifuge machine (R-8C).

Analytic methods: Quantitative estimation of serum AST and ALT activity were done based on the IFCC method [9-10]. Serum bilirubin and serum albumin were measured based on the principle of Modified Jendrassik & Grof method [11] and Bromocresol green [12] respectively. The reagents for bilirubin, ALT and AST were manufactured by Avantor India Ltd. (Dehradun, India), and that for albumin were manufactured by Trivitron Healthcare (Chennai, India). The tests were carried out in Photometer 5010 (Robert Riele, GmbH & Co KG, Berlin). Prothrombin time was measured in automated coagulation analyser, KC1 Delta (Trinity Biotech Plc, Ireland) and International Normalized Ratio (INR) were calculated. The thromboplastin reagent (Uniplastin) for prothrombin time determination was manufactured by Tulips Diagnostics Ltd. (Goa, India)

Child-Pugh classification of subjects: In order to assess the severity of liver damage of the subjects, Child-Pugh score was calculated from the given clinical and biochemical parameters of all the subjects. The Child-Pugh classification employs five clinical measures of liver disease and each of the measure/parameter is scored 1-3, with 3 indicating most severe derangement of liver function [13]. The same is shown in the table below –

Table 1: The Measures/Parameters used for Child-Pugh classification

Measures/Parameters	1 point	2 points	3 points
Total bilirubin (mg/dl)	< 2.0	2.0 to 3.0	> 3.0
Serum albumin (g/dl)	> 3.5	2.8 to 3.5	< 2.8
International Normalized Ratio	< 1.7	1.71 to 2.20	> 2.20

Ascites	None	Suppressed with medication	Refractory
Hepatic encephalopathy	None	Grade I-II (or suppressed with medication)	Grade III-IV (or refractory)

Source: Pugh RN, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R.(1973) [13]
Based on the total score obtained by adding the individual scores against 5 measures/parameters (as mentioned under Table 1), the subjects are classified into Child-Pugh class – A, B and C, by employing the added score from above.

Table 2: The Child-Pugh classification

Total Score/Points	Child-Pugh Class
5 to 6	A
7 to 9	B
10 to15	C

AST/ALT (De Ritis) Ratio: The serum AST and ALT values of all the subjects were obtained and then the AST/ALT ratio was calculated.
Statistical tools: The mean and standard deviation of AST/ALT ratio were calculated for all the subjects classified under the three class groups as per Child-Pugh classification. The mean values of AST/ALT ratio of the subjects under the three class groups were then compared to know whether there exists any significant difference across the groups. This was carried out by using One-Way ANOVA test. Thereafter, effort was also made to know the extent of correlation between Child-Pugh score and the AST/ALT ratio and the test was carried out at 95% confidence level. SPSS 20.0 was used for data analysis.

Results

Demographic profile of the subjects

The subjects considered in this study were above the age of 18 years and the mean age of the subjects was 49 years. Majority of them (i.e., 97.89%) were males and only 3 were females (i.e., 2.11%). All the subjects were residents of the North Eastern States of India and were found to be diagnosed with alcoholic liver disease.

Child-Pugh classification and AST/ALT (De Ritis) ratio

After classifying the subjects into three classes namely A, B & C as per the measures for Child-Pugh classification, the AST/ALT ratio for all the subjects was calculated. The mean and SD values of AST/ALT ratio of the subjects that were obtained in relation to Child-Pugh score is shown in table 3 below –

Table 3: Mean & SD values of AST/ALT Ratio in relation to Child-Pugh classification

Group Statistics			
Child-Pugh Class	**N**	**Mean**	**Std. Deviation**
A	20	1.7350	0.39846
B	74	2.0751	0.88536
C	48	2.7608	1.18475
Total	**142**		

Source: Author's Calculation

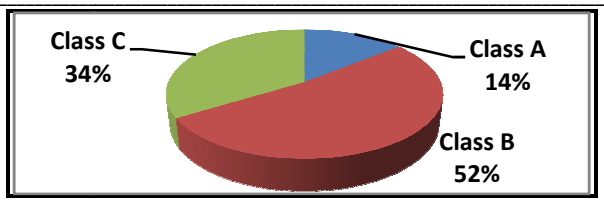

Fig. 1: Child-Pugh Classification of Subjects

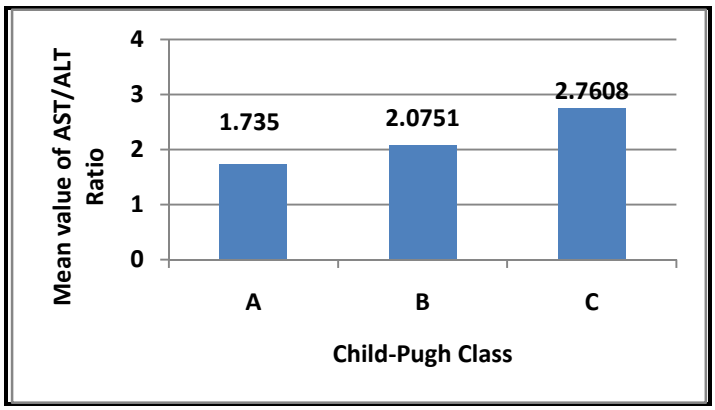

Fig. 2: AST/ALT Ratio in relation to Child-Pugh Class

In order to know whether the mean value of AST/ALT Ratio of the subjects differ significantly across the class groups, One-Way ANOVA Test was performed. The result of One-Way ANOVA Test is shown in Table 4 below –

Table 4: One-Way ANOVA Test

ANOVA					
AST_by_ALT_Ratio					
	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	20.081	2	10.041	11.058	.000
Within Groups	126.210	139	.908		
Total	146.291	141			

Source: Output of SPSS 20.0

The values of F and p – obtained from One-Way ANOVA Test – are 11.058 and 0.000 respectively. As $p < \alpha$ (=0.05), it can be inferred that the difference of mean values across the class groups is statistically significant.

Correlation between Child-Pugh score and AST/ALT (De Ritis) ratio

Further, attempt was made to know whether there exists any correlation between Child-Pugh score and AST/ALT Ratio. The Pearson correlation result is shown in Table 5 below –

Table 5: Correlation between Child-Pugh Grading and AST/ALT (De Ritis) ratio

Correlations			
		Child_Pugh_Class	AST_by_ALT
Child_Pugh_Classification	Pearson Correlation	1	.362**
	Sig. (2-tailed)		.000
	N	142	142
AST_by_ALT	Pearson	.362**	1

	Correlation		
	Sig. (2-tailed)	.000	
	N	142	142
**. Correlation is significant at the 0.01 level (2-tailed).			

Source: Output of SPSS 20.0

Pearson Correlation test shows that there exist a positive correlation between Child-Pugh score and AST/ALT Ratio. With r = 0.362 and p = 0.000, the correlation was found to be statistically significant. Thus, it can be inferred that the AST/ALT ratio increases with increase in Child-Pugh score.

Discussion

The present study was carried out on 142 patients diagnosed with alcoholic liver disease. Alcohol consumption has been an integral part of socio-cultural life of many tribes of the North East part of India, and has been linked with one of the most common causes of liver disease in this population group. It is worth mentioning that the predominance of AST over ALT in alcohol-related liver disease was first reported by Harinasuta et al. in 1967 [5]. However, it became more widely recognised only in 1979 with the research publication of Cohen and Kaplan [4]. Interestingly, analysis of data by using One-Way ANOVA test reveals that the mean values of AST/ALT ratio increases with increased Child-Pugh score. In view of this, it can be said that the AST/ALT ratio increases in progressive impairment of the hepatic function, and our study supports the finding of the study conducted by - Gurung et al (2013) [14], where they have shown that the mean of AST/ALT ratio appears progressively rising as the Child-Pugh score of liver damage increases. Also in this study, it was seen that the mean values of AST/ALT ratio differ significantly across the Child-Pugh class groups.Several research points out that as the AST/ALT ratio elevates, the severity of underlying liver disease also increases. The probable explanation for high AST is increased leakage of mitochondrial AST in the plasma due to toxicity of the metabolites and/or oxidative stress in the hepatocytes due to ingested ethanol. Injury to the hepatocytes has been found to be dependent on a major metabolic product of ethanol oxidation, acetaldehyde, which account for the most of the functional derangements associated with alcohol abuse. Along with the toxic metabolites, the generation of reactive oxygen species (ROS) due to induction of cytochrome P450 2E1 (CYP450 2E1) by ethanol, leads to increased oxidative stress, cell membrane permeability, cell necrosis and leakage of mitochondrial AST into the blood [15]. In cirrhotic patients, high AST may also possibly be due to its reduced plasma clearance, secondary to impaired function of the sinusoidal cells [16].Further, a lowered plasma level of pyridoxal 5'-phosphate (PLP), the coenzyme form of vitamin B_6 is frequently encountered in individuals with chronic alcohol abuse. The liver being the primary source of this coenzyme in plasma and also the principal organ responsible for oxidation of ethanol, its oxidation product - acetaldehyde, may impair the synthesis of PLP in these cells by enhancing its hydrolysis [17]. ALT and AST both use pyridoxine as a coenzyme, but the synthesis of ALT is more strongly inhibited by pyridoxine deficiency than the synthesis of AST. Our study had also examined the correlation of Child-Pugh score and the AST/ALT ratio. It was found that there exist a positive correlation between Child-Pugh score and AST/ALT ratio and this correlation was found to be statistically significant with Pearson Correlation (r) = 0.362 and p = 0.000. Thus, it can be inferred that with increased Child-Pugh score, the AST/ALT ratio gets elevated.

Conclusion

The different patterns of elevation of serum transaminase levels can reflect the different causes of injury to the liver and can be used as a guide to direct further evaluation of diseases that affect the organ. The AST/ALT (De Ritis) ratio can be a sensitive, reliable and economically feasible biochemical indicator for the diagnosis of alcoholic liver diseases. In this study, it was found that a high AST to ALT ratio is not only suggestive of liver damage due to ethanol abuse, but a rising value of the ratio has a positive correlation with the advancement of the underlying liver damage. Further studies are needed with a larger population group to confirm the findings of this study.

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