Efficacy if De Retis ratio in diagnosing liver diseases in Puducherry population.

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ABSTRACT

The estimation of aminotransferases which include Aspartate Aminotransferase (AST) and Alanine Aminotransferase (ALT), in serum, is an established liver function test which helps in the differential diagnosis of hepatobiliary diseases. Though both the enzymes are present in the liver, ALT is more specific for the liver. There are contradictory reports on the usefulness of De Ritis ratio (AST/ALT) in differentiating the various types of liver diseases. This prospective study was undertaken primarily to compare the De Ritis ratio in three groups of patients, namely viral hepatitis, non viral hepatitis and alcoholic liver disease (ALD). In this prospective study we included 110 patients, in the age group of 25 to 40, and with abnormal liver function. Patients were classified into 3 groups, namely, those with viral hepatitis (n=40), non viral hepatitis (n=30) and ALD (n=40). Patients with ischemic heart disease, skeletal muscle disorders, and patients with cirrhosis as depicted by ultrasonography were excluded from the study. Serum ALT levels were significantly high (p=0.004) in viral hepatitis group as compared to ALD and non-viral hepatitis groups. When we compared De Ritis ratio among the three groups, ALD group showed significantly high values compared to the other two groups (p= 0.000). The De Ritis ratios were lesser than 1, greater than 1 but lesser than 2 and greater than 2 in viral hepatitis, non viral hepatitis and ALD respectively. We did not find any significant differences in serum AST levels among the three groups. De Ritis ratio can be a simple reliable and economically viable biochemical indicator for the diagnosis of alcoholic liver diseases.
**Key words:** Alcoholic liver disease, De Ritis ratio, Transaminases, Aspartate Aminotransferase, Alanine Aminotransferase

**INTRODUCTION**

Aspartate aminotransferase (AST) and Alanine aminotransferase (ALT) are intracellular enzymes that belong to the family of Transaminases or aminotransferases. These intracellular enzymes leak out into the extracellular fluid in tissue injury and hence are of diagnostic relevance. ALT is found maximally in the liver and is more specific to the liver injury. When the cells are damaged, these enzymes leak out into the extracellular fluid, wherein their quantitation serves as an indicator of cell damage/death. With reference to liver diseases, maximum elevations of ALT are observed in viral and drug induced hepatitis, whereas enhanced levels of AST is observed in liver necrosis. Although serum levels of both AST and ALT become elevated as and when the disease process affect the liver cells, ALT is the more specific liver enzyme. Moreover, enhancements in ALT activity persist longer than that of AST activity. The inclusion of ALT and AST (aminotransferases) as part of the routine chemistry profile has been in vogue since several decades. In general, serum ALT levels increase with great rapidity when the liver is damaged. The general causes include hepatitis, hepatic cirrhosis, liver malignancies, post hepatic jaundice and hepatotoxic drugs. The ratio of AST/ALT is referred to as De Ritis ratio. The evaluation of aminotransferase activities in serum is improved by the ratio of AST: ALT (De Ritis ratio). The ratio had been implicated in the past in the differential diagnosis of liver diseases. In viral hepatitis and other liver diseases associated with hepatocellular necrosis, levels of AST and ALT, as determined in the serum are elevated much prior to the appearance of clinical signs and symptoms. The common diseases associated with the liver where the determination of serum aminotransferases would be of value include viral hepatitis, non viral hepatitis, toxic hepatitis and alcoholic liver disease (ALD). In viral hepatitis, the peak levels of both aminotransferases are observed between the 7th and 12th days and decline to normal levels in 3 to 5 weeks provided an uneventful recovery takes place. Very high levels of aminotransferases are observed in ALD also. In all types of liver diseases, the De Ritis ratio gets altered, secondary to changes in the levels of aminotransferases. However, since the last decade certain contradictory reports have been appearing regularly in the literature. These point out to the decreased efficacy of employing De Ritis ratio in the diagnosis of liver diseases. Furthermore, not many published reports are currently available pertaining to the Indian population and especially those comparing the means of AST and ALT in different liver diseases. In addition and more significant is the fact that very few reports are available with respect to the south Indian population as also concerning the Union territory of Puducherry. Mention must be made of the fact that Puducherry and the adjoining areas record a high percentage of alcoholics and hence the need arises for undertaking a detailed study on the efficacy of simple biochemical parameters such as De Ritis ratio in predicting or diagnosing liver diseases, in general and ALD in particular. In this study, the three liver diseases viz., viral hepatitis, non viral hepatitis and ALD had been selected for probable application of De Ritis ratio in the differential diagnosis and also to evaluate the efficacy of the latter (De Ritis ratio), in the...
light of the differential diagnosis of liver diseases, besides deciphering ambiguity if any with reference to its clinical utility. De Ritis ratio would enable us to determine whether the hepatic or extrahepatic tissue has been damaged. Furthermore, there are reports suggesting that the ratio could help determine whether liver damage is related to alcohol dependence. With this background we planned our study and the following are aims and objectives

To compute the numerical value of De Ritis (AST/ALT) ratio in different types of liver diseases (viral hepatitis, ALD and non viral hepatitis).
To determine the utility and efficacy of De Ritis ratio in enabling the differential diagnosis of liver diseases.
To check for the possible ambiguity, with reference to the previous reports, in the assessment of liver diseases, based on the De Ritis ratio.

MATERIALS AND METHODS

This was a hospital based prospective study, which was conducted in Mahatma Gandhi Medical College and Research Institute, Puducherry between July 2009 and September 2009. We included 110 patients in the age group of 25 to 40 and with abnormal liver function tests who had attended our clinics. Having made a provisional diagnosis of hepatic diseases, the patients were classified into 3 groups, namely, those with viral hepatitis (n=40), non viral hepatitis (n=30) and ALD (n=40). The subjects were explained in detail about the procedure and purpose of the study, in their native language and their consent was obtained. 2 ml of blood samples were collected from patients for performing the necessary biochemical investigations. The following biochemical parameters were evaluated

Aspartate aminotransferase [AST]
Alanine aminotransferase [ALT]
De Ritis ratio [AST/ALT] was computed based on the values obtained for AST and ALT.
Serological tests to confirm/rule out viral hepatitis were also undertaken. The reports from De Ritis ratio in all the three groups were compared as follows:
ALD vs. viral hepatitis
Viral vs. non viral hepatitis
ALD vs. non viral hepatitis

Since ALT levels are maximally elevated in viral hepatitis (based on previous literature), De Ritis ratio were compared between viral and non viral hepatitis; since the present study has been focused on the use of De Ritis ratio in the differential diagnosis of ALD, the De Ritis ratio was also compared between viral hepatitis and ALD as also non viral hepatitis and ALD. The comparison of the mean levels of AST and ALT were also undertaken for the three groups.

Exclusion criteria: Patients on acetaminophen, antibiotics and statin therapy and other drugs which are known to affect the parameters we studied, were excluded from the study. Patients with ischemic heart disease and skeletal muscle disorders were also excluded. Patients with cirrhosis as depicted by ultrasonography were also excluded from the study. Patients with diabetes mellitus (types 1 and 2), hypertension and any other metabolic or endocrine disorders were also excluded from our study.
Quantitative estimation of all the biochemical parameters were performed utilizing established assay procedures approved by the International Federation of Clinical Chemistry and Laboratory medicine (IFCC). Commercially available kits were employed for all quantitative determination and fully automated chemistry analyzer Hitachi 902 was used in all determinations. The results of all analyses were validated in the light of the internal quality control effected using the QC sera provided by M/S Biorad USA and the External Quality Assessment was monitored by the Clinical Biochemistry Laboratory of Christian Medical College (CMC & H), Vellore, India. Statistical analysis was done using descriptive statistics and testing of hypothesis. The data was analyzed using Excel 2007, R 2.8.0 Statistical Package for the Social Sciences (SPSS) for Windows Version 17.0 (SPSS Inc; Chicago, IL, USA) and the EPI Info 3.5.1 Windows Version. A p-value of less than 0.05 was used to establish statistical significance.

RESULTS

The age & sex distribution of the cases enrolled in our study is depicted in the table 1. Majority of the cases were males aged more than 30 years.

Table 1: Age and sex distribution of cases among the three groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Alcoholic Liver Disease</th>
<th>Viral hepatitis</th>
<th>Non-viral hepatitis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Male</td>
<td>34</td>
<td>33</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>Age group (years)</td>
<td>0-30</td>
<td>12</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>31-100</td>
<td>28</td>
<td>25</td>
</tr>
</tbody>
</table>

The mean values of AST, ALT, De Ritis ratio were compared among the three groups namely, Alcoholic liver disease, viral hepatitis and non-viral hepatitis (Table 2). The means of AST of all three groups were compared simultaneously using the one way analysis of variance [ANOVA]. The comparison was not significant (p= 0.342; Table 2). The means of ALT of all three groups were compared simultaneously using the one way ANOVA. The comparison was found to be significant (p=0.004; Table 2). The pair--wise comparison was performed using the Post Hoc Test, based on the Least Significant Difference test (Tukeys HSD test). The comparison of mean ALT between group1 and 2 was significant (p= 0.006), between group 2 and 3 was also significant (p= 0.028) but it was not significant between group 1 and 3 (p= 0.772). The comparison of mean De Ritis ratio was performed between the groups using one way ANOVA. The comparison was found to be significant (p= 0.000).The pair-wise comparison was carried out using post hoc test. The mean of De Ritis ratio of group 1 and 2 were compared and was found to be significant (p= 0.000), that of group 2 and 3 was not found to be significant (p= 0.056), but that of group 1 and 3 was found to be insignificant (p= 0.00).
De Ritis ratio in diagnosing liver diseases

Table 2: Comparison of mean levels of AST, ALT and de Ritis ratio among the three groups

<table>
<thead>
<tr>
<th>Variables</th>
<th>Alcoholic liver disease (n=40)</th>
<th>Viral hepatitis (n=40)</th>
<th>Non-Viral hepatitis (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>AST</td>
<td>205± 30</td>
<td>212± 25</td>
<td>129 ±15</td>
</tr>
<tr>
<td>ALT</td>
<td>115± 23*</td>
<td>343 ± 47*#</td>
<td>137 ± 14#$</td>
</tr>
<tr>
<td>De Ritis ratio</td>
<td>2.34± 1.3*$#$</td>
<td>0.75 ± 0.31$</td>
<td>1.19 ± 0.86#$@</td>
</tr>
</tbody>
</table>

Comparison was done by one way ANOVA with Tukeys HSD as post-hoc test. SD = Standard Deviation.
* indicates significant difference with p value 0.006 between ALD and viral hepatitis.
# indicates significant difference with p value 0.028 between viral hepatitis and non-viral hepatitis.
$ indicates significant difference with p value 0.000 between ALD and viral hepatitis.
@ indicates significant difference with p value 0.000 between ALD and non-viral hepatitis.

We also compared other tests which are now commonly used to assess the hepato-biliary functions, among them serum ALP was significantly different between groups ALD and viral hepatitis with p value 0.004. Direct bilirubin was significantly higher in patients with viral hepatitis when compared with group non-viral hepatitis with p values of 0.000 (Table 3)

Table 3. Comparison of other Biochemical parameters among the three groups.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Alcoholic liver disease (n=40)</th>
<th>Viral hepatitis (n=40)</th>
<th>Non-Viral hepatitis (n=30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin</td>
<td>3.3 ± 0.6</td>
<td>3.35 ±1.0</td>
<td>3.4 ± 0.59</td>
</tr>
<tr>
<td>Globulin</td>
<td>3.1± 0.6</td>
<td>5.70 ±1.3</td>
<td>2.9 ± 0.46</td>
</tr>
<tr>
<td>ALP</td>
<td>212.8 ± 90*</td>
<td>325.5 ± 93*</td>
<td>251.1 ± 99.5</td>
</tr>
<tr>
<td>Indirect bilirubin</td>
<td>2.2 ± 0.5</td>
<td>4.5 ±1.2</td>
<td>1.20 ± 0.38</td>
</tr>
<tr>
<td>Direct Bilirubin</td>
<td>2.3 ± 0.3</td>
<td>3.4±0.3$</td>
<td>0.8± 0.2#</td>
</tr>
</tbody>
</table>

Comparison was done by one way ANOVA with Tukeys HSD as post-hoc test. SD = Standard Deviation.
* indicates significant difference with p value 0.004 between ALD and viral hepatitis.
# indicates significant difference with p value 0.000 between viral hepatitis and non-viral hepatitis.
DISCUSSION

AST is present in several tissues like liver, erythrocytes, heart, muscle, kidney, brain and lungs. Two isoenzyme forms of AST are available - AST1, a cytosolic isoenzyme which is mainly derived from the heart and erythrocytes & AST2, which is of mitochondrial origin, is mainly present in the liver. AST2 also known as ASTm is of value in the diagnosis of liver diseases and is used in association with other enzymes. The ease with which the aminotransferases could be estimated in the Clinical Biochemistry Laboratory as also the economic viability of this test in comparison to the battery of Liver Function tests (LFT) enables the computation of De Ritis ratio and offers pragmatic approach in facilitating the diagnosis of liver diseases, in general and ALD in particular.

The ratio had been indicated as a predictor of liver diseases.\textsuperscript{1, 12, 13, 15, 18} However, certain reports are available which project the equivocal nature of the ratio and its uncertainty as a diagnostic indicator. Therefore, in the light of these reports and also in view of the high percentage of alcoholics in Puducherry and the adjoining areas the study was undertaken. Earlier reports cite the usefulness of the ratio, when used in association with other parameters of LFT. However, our study indicates that the De Ritis ratio could be used as a single and a reliable indicator of liver diseases, despite the lack of pronounced changes in the other parameters. Studies demonstrated that De Ritis ratio could be used as an effective index to differentiate Non Alcoholic Steatohepatitis (NASH) from ALD.\textsuperscript{19} They suggest that a ratio of \(<1\) implies NASH whereas a ratio of \(\geq 2\) implies ALD. Studies undertaken by Nyblam \textit{et al} point to the fact that a high De Ritis Ratio might indicate advanced ALD rather than heavy drinking.\textsuperscript{20} Cohen and Kaplan evaluated the usefulness of the De Ritis ratio and upheld its diagnostic value as an indicator of ALD.\textsuperscript{21, 22} Our study also clearly suggests that De Ritis ratio is the maximum in ALD and hence could clearly delineate ALD from the other liver diseases that are commonly encountered (viral and non viral hepatitis). In ALD, pyridoxal phosphate (PLP) which is the coenzyme for aminotransferases is deficient. Since ALT is more dependent on PLP, AST/ALT ratio is increased. AST is present as a cytosolic component and a mitochondrial component (ASTc & ALTm). ASTm is present in the perivenular area in the liver, around the central vein. In ALD, since more perivenular hepatocytes are damaged ASTm is elevated and hence the De Ritis ratio is elevated. This promotes the efficacy of De Ritis ratio, as related to the differential diagnosis of ALD. Moreover, several earlier reports projected the usefulness of this ratio only in the presence of other diagnostic parameters. Our study clearly suggests that De Ritis ratio could be used as a cost-effective indicator of liver diseases, in general and ALD in particular.

Patients suffering from ALD are diagnosed by a group of physical manifestations and laboratory findings. Among the various biochemical parameters that are evaluated to monitor liver function, De Ritis ratio (AST/ALT) assumes relevance as a sensitive indicator during any phase of the disease.\textsuperscript{23} The ratio is based on the quantitation of aminotransferases in the serum, namely AST and ALT. The ratio affords an easy and reliable biochemical indicator to assess ALD, besides diagnosing other liver diseases.\textsuperscript{23} The estimation of De Ritis ratio is also useful for the rational understanding of the extent of damage in alcoholic liver disease.\textsuperscript{24}
We also suggest that since biochemical quantitation of aminotransferases in serum is comparatively quicker, easier and cost-effective compared to other sophisticated tests which are more sensitive but available only in the tertiary care centres. Hence, De Ritis ratio could be routinely used in primary and secondary health care centres for screening alcoholic liver disease and also to assess the extent of hepatocellular damage.

CONCLUSION

1. The De Ritis ratio is lesser than 1 in viral hepatitis, greater than 1 and lesser than 2 in non viral hepatitis and greater than 2 in ALD.
2. De Ritis ratio could be used as a cost-effective biochemical marker in clearly differentiating ALD from other diseases of the liver, namely viral and non viral hepatitis. There is no ambiguity involved in the use of De Ritis ratio pertaining to the differential diagnosis of liver diseases.
3. De Ritis ratio can be a useful test to screen population for liver diseases, especially pertaining to less-affluent socio-economic groups who cannot afford to pay for the full battery of LFTs.

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