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Biochemical Markers in Alcoholic Liver Cirrhosis

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Abstract

Objective: To study De Ritis ratio and Gamma Glutamyl Transferase (γ-GT) along with Total Sialic Acid (TSA) in alcoholic liver cirrhosis and to correlate the utility of these parameters as a diagnostic indicator in liver function.

Material and Methods: A case control study was carried out in 100 subjects , out of which 50 cases were with chronic alcoholism of 31-50 years of age admitted in medicine ward of tertiary care hospital with clinical and ultrasonographic evidence of cirrhosis of liver without hepatic encephalopathy and G.I. bleed and 50 cases were of age matched healthy controls without any disease visited for routine check up at our OPD. The AST, ALT and γ-GT were determined by routine methods and TSA was determined by Pluncinsky M.C. *et al*. method.

Results: Serum levels of De Ritis ratio(2.069±0.308), activity of Gamma Glutamyl Transpeptidase(109.278±60.747) and Total Sialic Acid (71.460±14.458) in liver cirrhosis subjects were higher than the healthy controls (De Ritis ratio 1.037±0.338,γ-GT 25.980±11.653, TSA 52.940±7.017) and were statistically significant ($p<0.001$).

Conclusion: Our observed results show elevated in the levels of serum De Ritis ratio , activity of Gamma Glutamyl Transferase and Total Sialic Acid pointing their role in the diagnosis of alcoholic liver cirrhosis. De Ritis ratio was found to be positively correlated with serum γGT and serum TSA respectively. In combination assessment of De-Ritis ratio , γGT and Total Sialic Acid is a sensitive means of detecting severity of alcohol induced liver damage.

INTRODUCTION

The Alcohol (ethanol) has been produced and consumed by humans for millennia, in the form of fermented and distilled alcoholic beverages.[1]

Alcohol related liver disease is a major cause of morbidity and mortality rate. Alcoholic liver disease encompasses a histological spectrum, including fatty liver, alcoholic hepatitis and alcoholic cirrhosis. Fatty liver is a benign and reversible condition but progression to alcoholic hepatitis and cirrhosis is life threatening. Alcoholic hepatitis is diagnosed predominantly on the basis of clinical history, physical examination and laboratory investigation, although liver biopsy is often necessary to secure the diagnosis.[2]

About half of all cirrhosis in the world are alcohol induced and about 10–20 % of all alcoholics are cirrhotic. Alcoholic cirrhosis has 5 years mortality of 77 % and at 10 years, survival rate only 7 %. In men, ethanol intake of 40–80 g/day produces fatty liver and that of 160 g/day for 10–20 years causes hepatitis or cirrhosis. Only 15 % of alcoholics develop ALD (Alcoholic Liver Disease). The threshold for developing ALD in men is an intake of 60–80 g/day of alcohol for 10 years. Social, nutritional, immunologic and host factors also play important role in development of pathogenic process.[3]

A large number of existing biochemical parameters such as Transaminases ,Gamma glutamyl transferase (γGT) etc. have been proposed for the detection of alcoholism and associated liver diseases and they show highly changeable values but are not specific since in some of the stages / conditions of liver diseases these parameters show altered activities these, γGT, AST, ALT are commonly used as screening test.[4]

Sialic acid is the name for a series of acyl derivaties of neuraminic acids that occur as non-reducing terminal residues of glycoproteins or glycolipids in biological fluids and cell membranes. Sialic acid level is noted to be elevated by high alcohol consumption and reduced during abstinence, thus, Sialic acid seems to be an interesting marker that needs further evaluation as a diagnostic tool for alcohol induced hepatic disorders.[5]

De Ritis Ratio is the ratio between AST and ALT (AST/ALT) and is useful in differential diagnosis and classification of hepatic disorders. For normal individual De Ritis Ratio vary from 0.7-1.4.

Thus, the clinical sensitivity of GGT, AST, ALT and other parameters for alcoholic liver disease is satisfactory but not specific enough; therefore attempt is being made to evaluate biochemical parameters such as De Ritis ratio (AST/ALT), glutamate dehydrogenase and Total Sialic Acid(TSA) etc. to offer their advantages over conventional biomarkers.

MATERIAL AND METHODS

The present study is a hospital based case-control study and total 100 subjects were enrolled in this study. Fifty male patients diagnosed as having Alcoholic Liver Cirrhosis (ALC) admitted to hospital ward of , were selected as study group subjects. The diagnosis of patients with alcoholic liver cirrhosis was done by the clinicians on the basis of radiological screening by ultraonography, detailed clinical history and other relevant pathological/ biochemical investigations.

Control population consisted of 50 healthy males matched for age, attending the routine health check-up in our outpatient department. Controls were selected on the basis of a medical history**,** no participants had habit smoking habit of used caffeine and had history of thyroid disease, diabetes mellitus and hypertension.

EXCLUSION CRITERIA

Patients with administered drugs for treatment of alcoholic liver disease, cirrhosis following viral hepatitis (B and C), cirrhosis following biliary obstruction (primary and secondary biliary cirrhosis) , drug induced cirrhosis (toxic hepatitis), alcoholic cirrhosis with hepatic encephalopathy and G.I bleed, alcoholic cirrhosis with history of thyroid disease, diabetes, cardiac disease, metabolic disease and alcoholic fatty liver were excluded from the study.

Sample collection-

Blood samples were obtained from antecubital veins of study group subjects and control group. Fasting venous blood samples were collected in the morning from cases group immediately after the diagnosis and before giving any medication and from controls at their routine visits. The blood was allowed to clot at room temperature in plain bulb and serum was separated by centrifugation at 1500 rpm for 10 minutes. The serum was collected and transferred into clean and dry tubes and as per necessity stored at -20^0 C.

Parameters: Activity of Gamma glutamyl transferase By Gamma GT (S.L) Kit.[7] whereas of AST & ALT Level (De Ritis Ratio) By Reitman & Frankel method.[6] TSA was determined by Pluncinsky M.C. *et al*. method.[8] All the biochemical parameters measured in study group subjects were statistically compared with those estimated in controls. Biochemical parameters were expressed as mean±SD for each group.

RESULTS

In present study, the activities of AST and ALT were increased in alcoholic liver cirrhosis when compared with healthy controls **(Table No.1)** which is highly significant **(p < 0.001).**

Table No.1: Biochemical parameters in healthy controls and alcoholic liver cirrhosis patients.

Parameter	**Control**	**Cases**	**p value**
AST (IU/L)	24.260±10.374	105.360±41.324	< 0.001
ALT (IU/L)	23.900±8.809	51.160±21.189	< 0.001
De Ritis ratio	1.037±0.338	2.069±0.308	< 0.001
γGlutamyl Transpeptidase (IU/L)	25.980±11.653	109.278±60.747	< 0.001
Total Sialic acid (mg/dl)	52.940±7.017	71.460±14.458	< 0.001

The ratio of De Ritis was also elevated in alcoholic cirrhosis subjects as compared to control subjects which was > **2** (**Table No.2**). The De Ritis ratio in alcoholic cirrhosis patients was increased significantly as compared to controls **(p < 0.001)**.

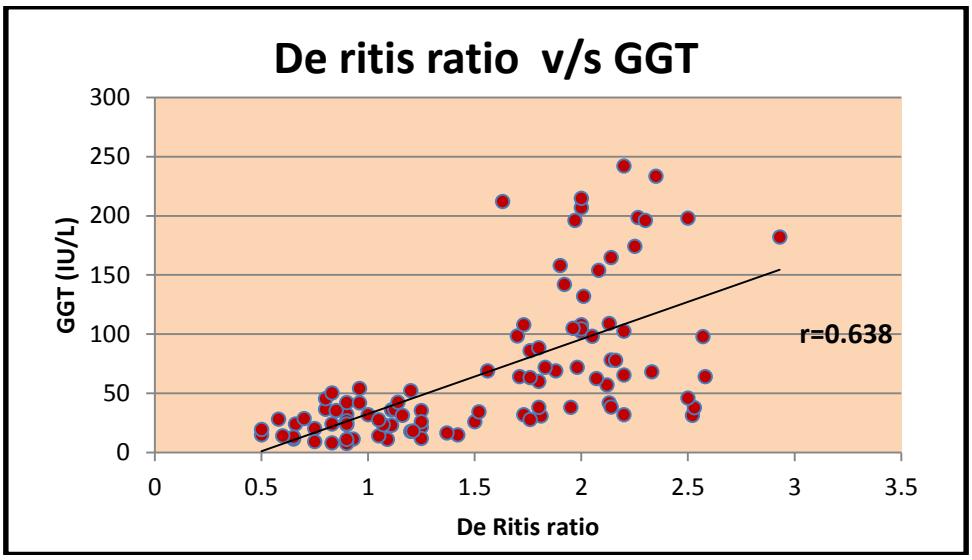
Table No.2: The De Ritis ratio and number of cases.

No.	**De Ritis ratio**	**Alcoholic liver cirrhosis**
1.	**<2**	**12**
2.	**>2**	**38**

In present study, serum γGT was estimated both in healthy controls and in alcoholic liver cirrhosis patients assuming a known conventional parameter for this study subjects. The activity of serum γGT in alcoholic cirrhotic patients was upsurged very significantly as compared to controls **(Table No. 1)**.

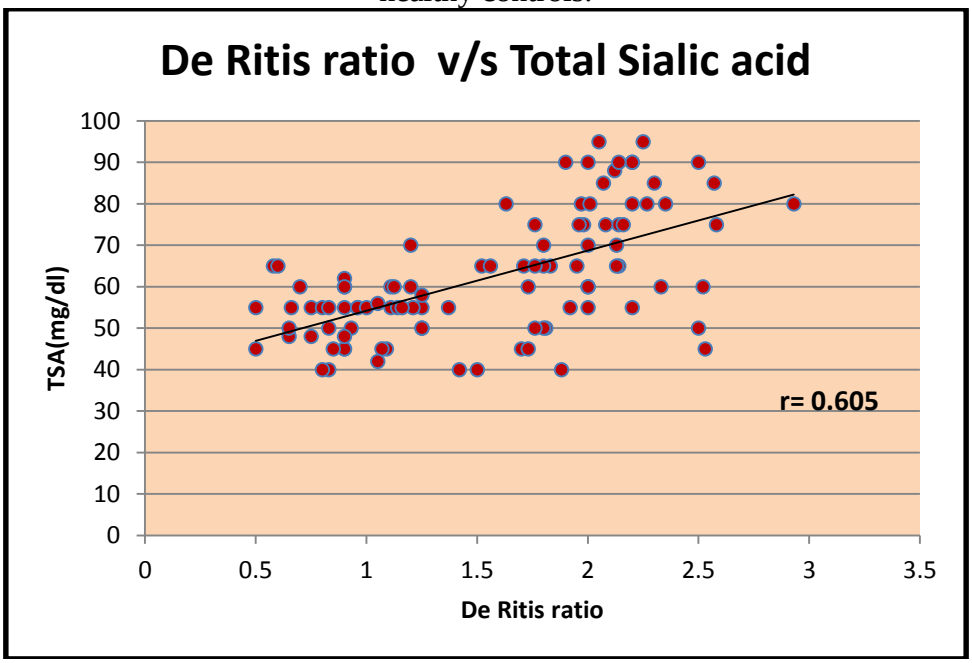
The increase in the activity of serum γGT was highly significant in patients of alcoholic liver cirrhosis as compred to controls **(p < 0.001).** De Ritis Ratio was correlated with γGT and a positive correlation was found between De Ritis ratio and γGT (**r = 0.638, Graph A**).

Graph A: The correlation between De Ritis ratio and activity of Gamma Glutamyl Transferase in ALC and healthy controls.



De Ritis ratio is significantly **positively correlated** with activity of GGT.
r =0.638, p value= < 0.001- Highly significant.

Graph B: The correlation between De Ritis ratio and Total sialic acid in ALC and healthy controls.



De Ritis ratio is significantly **positively correlated** with TSA.
r= 0.605 , p value<0.001- Highly significant.

In this study, mean of TSA concentration was significantly increased **(p value< 0.001)** in alcoholic liver cirrhosis as compared to healthy controls **(Table No. 1)** and also observed positive correlation between De Ritis ratio and TSA **(r= 0.605, Graph B)**.

STATISTICS

The results obtained in the study were evaluated using MYSTAT STATISTICAL PACKAGE at 95% confidence interval and at a significance level of $p<0.05$.

DISCUSSION

In recent years, conventional biochemical markers and potential ones have aroused the interest of researchers to study the damages caused by ethanol in liver. Alanine transaminase (ALT) , Aspartate transaminase (AST), Glutamate dehydrogenase (GLDH) and Gamma glutamyl transpetidase (γGT) are predominant in reflecting the damage to hepatocytes but they are not sensitive or specific enough for use as single test. Physicians have long sought an accurate and inexpensive means of identifying persons who are suffering from alcoholic liver cirrhosis. No study however, has formally assessed the utility of serum De Ritis ratio and γGT to differentiate alcoholic from non-ALD.

Our study suggests that these biochemical parameters may be used prior to invasive and expensive investigations like biopsy and sonography to support the clinical diagnosis so that early diagnosis and treatment can be done to prevent the further complications. Overall, there are poor chances of recovery in patients of ALD, timely intervention in the form of alcohol abstinence and supportive treatment should be emphasized to halt the disease process.

In the present study we made an attempt to correlate De Ritis ratio with conventional biochemical parameter gamma glutamyl transferase (γGT) and with other parameter such as Serum Total Sialic Acid(TSA) in alcoholic liver cirrhosis.

In the present study, the activities of AST and ALT were increased in alcoholic liver cirrhosis when compared with healthy controls which is highly significant ($p < 0.001$).The ratio of De Ritis ratio was elevated in alcoholic cirrhosis subjects as compared to control subjects which was > 2 and this ratio in alcoholic cirrhosis patients was increased significantly as compared to controls ($p < 0.001$).

The determination of the De ritis ratio in alcoholic liver cirrhosis patients can be considered as a dependable marker, which has also been proved by international data. Some interrelated reasons have been reported for the high AST/ALT ratio in alcoholic liver cirrhosis: i) A decreased hepatic ALT activity ii) Pyridoxal 5' phosphate depletion in the liver of alcoholics iii) Mitochondrial damage leading to an increase in

the serum activity of mitochondrial aspartate in patients with high alcohol consumption.

There may also be some contribution of the direct toxic effect of alcohol on the De Ritis ratio. In addition, the estimation of the De ritis ratio is essential for the rational understanding of the extent of damage in alcohol liver cirrhosis. Hence, this ratio can be considered as a reliable marker of alcoholic liver cirrhosis.[9]

Several studies assigned the elevated activities of AST and ALT in most advanced stage of alcoholic liver cirrhosis and ratio always more than 2 as compared to controls indicating that the activities were directly related to alcohol consumption and extent of the liver damage.[9]

Niemela O (2007) stated that in alcoholic hepatitis patients, De Ritis ratio is greater than 2 as compared to the control subjects and AST and ALT levels were almost always less than 500 IU/L.[10]

Pujar S and Kashinakunti S V (2010) observed the raised activities of AST and ALT in patients with liver cirrhosis and increase in De Ritis ratio was more in alcoholic liver cirrhosis as compared to controls and finally concluded that the estimated De ritis ratio was essential for the rational understanding of the extent of damage in alcoholic liver cirrhosis, hence, the De Ritis ratio considered as a reliable marker of alcoholic liver cirrhosis.[9]

Dr. G. Vijaya Benerji and M. Farid Babu (2013) confirmed that in alcoholic liver cirrhosis AST and ALT activities were elevated with the active alcohol consumption and AST activity had reported to be greater than ALT and usually did not exceed 300 IU/L and De Ritis ratio was greater than 2 because of existing mitochondrial damage.[11]

Serum γ Glutamyl Transferase (γGT):-

The activity of serum γGT in alcoholic cirrhotic patients was upsurged very significantly as compared to controls. Serum γGT activities were increased highly significantly in patients of alcoholic liver cirrhosis **($p < 0.001$).** De Ritis ratio was also correlated with γGT and found positive correlation between AST/ALT and γGT (**$r = 0.638$, Graph A**).

We perceived elevation in γGT is unique to alcoholic liver cirrhosis; Several studies have also reported elevations in the circulating activity of γGT in cirrhosis[12-14] , this has provided us with a rationale for using serum γGT as possible marker for detection of alcoholic liver cirrhosis.

Das S.K. *et al.* (2008) stated γGT was induced by alcohol plus the serum activities rise in response to the acute hepatocellular damage and activities were especially high in patients with severe alcoholic liver cirrhosis, these were more likely to be elevated

in the regular rather than the episodic drinkers as compared to nonalcoholic healthy controls.[12]

Rekha M *et al*. (2011) reported their observation as, γGT can be used to monitor abstinence and reabuse in alcoholics furthermore its elevation reverts to normal with abstention and this test can be of sensitive to detect liver involvement at an early and reversible stage of alcoholic cirrhosis. γGT remains the best of simple laboratory screening test for alcoholic liver cirrhosis.[13]

γ Glutamyl Transferase (γGT) is widely used as a biologic marker of alcohol consumption in early detection of heavy drinkers and in monitoring the treatment success among problem drinkers. As a screening test for alcoholism and alcohol abuse, its sensitivity has been considered to be acceptable, but its specificity is poor. In contrast to this, γGT has been found to have reasonably specific but low sensitive to changes in alcohol consumption. γGT also correlates poorly with long-term average alcohol intake measured with daily diaries among nonalcoholic males. However, serum γGT is an enzyme having special relation with alcoholic liver diseases and several studies also showed that serum γGT is the most sensitive, moderately specific and most widely employed marker of alcohol consumption; furthermore measurement of its activity in serum has been found useful in screening alcohol abuse. A sudden increase in the serum γGT activity in chronic alcoholics is suggestive of a recent bout in alcohol drinking.[15]

In this present study, mean of TSA concentration was significantly increased **(p value< 0.001)** in alcoholic liver cirrhosis as compared to controls**.** We perceived positive correlation between De Ritis ratio and TSA **(r= 0.605, Graph B)**.
According to study by **Ramppanen J. *et al*. (2002)** who found that serum SA concentrations are significantly higher in alcohol abusers than in healthy controls. **Ramppanen J. *et al* (2002) and Sillanaukee *et al*. (1999)** both concluded that the serum TSA level has been shown to be a recognized marker of excessive alcohol consumption.[16, 17]

The reasons of the increased TSA concentration in the sera of alcoholics are unknown. The rationale for their study was the explanation of elevated levels of TSA in the sera of alcoholics.[18]

Chrostek *et al*. (2007) have shown in alcohol abusers the positive correlation between elevated level of TSA and rich sialylated glycoproteins, especially a1-antitrypsin and a1-acid glycoprotein, therefore they suggested that the increased serum TSA concentration can be attributed (at least partly) to an elevated level of sialylated glycoproteins, in fact to increase PBSA. Evidence of this was the positive correlation of elevated TSA levels with the concentration of sialylated glycoproteins in the sera of alcoholic patients. [19]

Pönniö *et al.* and Sillanaukee *et al*. (2002) concluded that SA concentration in serum may be increased in alcoholics. These results are in accordance with earlier findings by **Sillanaukee *et al.*** [20]

The TSA levels in our study were somewhat lower than those reported previously, which may be due to different methods and differences in standardization of assays.

In studies on the markers of alcohol consumption, most studies have not distinguished between the effects of alcohol consumption and the secondary effects of liver disease as the underlying mechanism for increased marker values. When compared with the traditional alcohol markers (AST, ALT, γGT and Bilirubin), one study indicated that serum TSA has a higher efficiency than serum AST, although it is lower than that of serum γGT, for detecting alcohol abuse in heavy drinkers. [20]

The presence of liver disease increases serum activity of ALT, AST and γGT concentrations and decreases the specificity of these markers, especially in hospitalized patients. Serum CDT (Carbohydrate deficient transferring), which has recently been widely used in alcohol screening programs, was also found to be influenced by liver status. In contrast, serum Sialic Acid seemed to be less dependent on liver status in these comparisons.[20] Our results showed that the studied biochemical variables viz. De Ritis ratio, γGT and TSA were significantly increased and correlate well with the progression of disease condition i.e. alcoholic cirrhosis.

Limitation of our study is that evaluated sample size is small and moreover these parameters mentioned may lack specificity and sensitivity when used singly.

CONCLUSION

In brief, our results suggested that Serum levels of AST , ALT, De Ritis Ratio, activities of γGT, and concentration of TSA in alcoholic liver cirrhosis patients were highly significantly increased than in controls ($p < 0.001$). Also correlation graphs showed positive correlation between the De Ritis Ratio and Serum levels of TSA and activity of GGT, in patients which could add up the diagnostic predictability of alcoholic liver cirrhosis.

The combined increase of De Ritis ratio, γGT, TSA, would be of immense help in using them as biochemical markers and in increasing the accuracy of clinical diagnosis. Thus, the results of present study revealed that the estimation of De Ritis ratio, Bilirubin, γGT, and TSA can be of clinical importance in using them as biochemical markers in assisting diagnosis of alcoholic liver cirrhosis. Further studies from different areas like sensitivity, specificity and predictive values involving large scale sample size are needed to elucidate and to confirm the findings of present study.

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