Research Article

Biochemical assessment of dysfunction of liver and brain in alcoholic liver disease patient

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Abstract

Total Serum Sialic acid has been suggested as a new marker of alcoholic liver disease. The synthesis and the catabolism of Sialic acid takes place in the liver and therefore the status of liver can influence the serum levels of Total Sialic acid (TSA). Increased capacity of transferrin deficient in SA to selectively deposit iron in the hepatocytes might be of significance for the development of hepatic siderosis observed in alcoholism. Estimation of serum sialic acid level may be of help in early diagnosis of alcoholic liver disease and prevents the progression of the disease to terminal stages and complications. However, the role of TSA as a marker of liver disease and its association with cognitive changes has not been clearly elucidated. **Material and methods:** A total of 68 cases and 50 age matched healthy controls were recruited. These patients were further categorized into 3 groups; fatty liver, alcoholic hepatitis and alcoholic cirrhosis. Enrolled patients were followed for 6 months. The study was approved by the institutional ethical committee. Global cognitive functions were assessed periodically with Mini Mental State Examination (MMSE).Serum TSA levels were determined by Biovision's Sialic acid assay kit. **Result:** The serum TSA levels (34.74±11.25 nmol/µl) were significantly higher in the alcoholic liver disease than in the healthy controls (2.21±1.01 nmol/µl). Significantly higher TSA levels were observed in patients with alcoholic cirrhosis (36.46±7.66 nmol/µl, $p<0.001$) compared with alcoholic hepatitis (31.14±9.69 nmol/µl, $p<0.001$) and alcoholic fatty liver (35.17±10.9 nmol/µl, $p<0.001$). MMSE Scores were found to be lowest in alcoholic cirrhosis (10.60± 5.32) followed by hepatitis (12.36 ±5.48) and fatty liver (18.28±3.43). **Conclusion:** Serum TSA is significantly elevated in alcoholic cirrhosis. Serum TSA levels can be used as a marker of alcoholic liver disease and may correlate with cognitive dysfunction among ALD patients.

Keywords: Alcoholic liver disease; TSA; GGT; MMSE; cognitive dysfunction.

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INTRODUCTION

Alcohol abuse is one of the major causes of morbidity and mortality worldwide. Alcohol causes 1.8 million deaths (3.2% of total) and a loss of 58.3 million (4% of total) of total disability adjusted life years (DALYS).[1] The prevalence of alcoholism in India is 21.4%.[2] Chronic prolonged use of ethanol results in reversible redox changes in the liver that are mainly responsible for the accumulation of triglycerides and causing fatty liver in alcoholic patient.[3] Alcohol intake is the major cause of hepatitis that can lead to alcoholic cirrhosis. This further leads to Hepatic encephalopathy (HE) which is a major contributing factor to cognitive dysfunction, altered mood, memory impairment, motor incoordination.[4] Sialic acid refers to a group of N-acyl derivatives of neuraminic acid in biological fluids and in cell membranes as non reducing terminal residues of glycoprotein's and glycolipids. The normal range of total sialic acid (TSA) level in serum/plasma is 1.58 - 2.22 mmol/L.[5] In alcoholic subjects, higher sialic acid values have been found both in serum and in saliva thus indicating that SA can be valuable as a biomarker for excessive alcohol consumption.[5,6] highly metastatic cells have been observed to have significantly elevated amounts of neuraminidase-releasable SA and also an increased degree of sialylation of galactose and N-

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acetylgalactosamine groups compared with non metastatic cells.[7,8] Increased concentrations of SA have been reported in inflammatory processes. The elevations of Sialic acid content in alcoholic liver disease patients indicate the consequence of liver damage resulting in abnormal carbohydrate composition of the fibrinogen in the disease progression.[9,10] We hypothesize that SA levels can determine severity of ALD and may serve as prognostic marker in patients with alcohol related liver damage with cognitive impairment.

METHODS AND MATERIALS

The study was a hospital based case control study conducted in Department of Biochemistry and Department of Psychiatry, Padmashree Dr D.Y Patil Hospital and Research Centre, Nerul Navi Mumbai. A total of 68 adult male patients, consecutively transferred to inpatient detoxification center were recruited for the study after confirmation of alcoholic liver disease on the basis of clinical findings and by USG studies of liver. These 68 patients were further categorized on the basis of clinical findings into 3 groups; fatty liver (Group A), alcoholic hepatitis (Group B) and alcoholic cirrhosis (Group C). Enrolled patients were followed for 6 months. The control group comprising of 50 age matched healthy individuals were recruited from volunteers and healthy persons accompanying the patients in the general outpatient department (OPD). Informed consent was obtained from all subjects before the collection of information. The study was approved by the institutional ethical committee.

Clinical History

Detailed history including amount, duration, type of alcohol consumption in the form of whisky, rum, wine, vodka was taken. Alcohol dependency was enquired in the form of CAGE questionnaire. All 68 patients fulfilled the ICD-10 (The Tenth Revision of the International Classification of Diseases and Health Problems) WHO 1992 criteria for alcohol dependence. Global cognitive functions were assessed periodically with Mini-Mental State Examination (MMSE). A MMSE score of 23 is taken as cut-off as it is the most widely accepted and frequently used. Scores of 23 or lower indicates the presence of cognitive impairment.[11] Of 118 patients enrolled in the study, 66 patients (97.0%) completed the full 6 months of follow-up, 7 patients (10%) died. Alcoholic liver disease was classified by subtype.

Biochemical analysis

On admission of patient ten millilitre of venous blood was collected using all aseptic method. After clotting the samples were immediately centrifuged at 3000 rpm for 10 minutes to Separate serum which was stored at -80°C until further analysis. The samples for GGT were analyzed as follows: Gamma Glutamyl Transferase was determined by IFCC Method using automated laboratory procedures (VITALAB SELECTRA E). The method uses the substrate L-gamma glutamyl- 3-carboxy- 4-nitroanilide with glycylglycine. Total Serum Sialic acid were analysed by ELISA method- Biovision's Sialic acid assay kit(catalog#k566-100) . Biovision's sialic assay kit utilizes an enzyme coupled reaction in which free sialic acid is oxidized resulting in development of the Oxi-Red probe to give fluorescence(EX/Em=535/587nm) and absorbance (OD= 570nm). The kit measures sialic acid in the linear range of 0.1 to 10 nmol with detection sensitivity ~1Assay; M concentration.

Statistical Analysis

All data were fed on excel spreadsheet and statistical analyses were made using SPSS version 17.0.We used the student t test to compare the means. The level of significance was chosen to be $p<0.05$.Categorical data were summarized as frequencies and percentages. Continuous data were summarized as median (minimum, maximum). The relationship between inflammatory markers and outcome measures was determined using Spearman rank correlation. We used *the X^2 test with Yates* correction. Chi square and Fisher's exact test when appropriate and the Odds ratio (OR) along with 95% CI to compare proportions between the groups. We used Mann-Whitney/Wilcoxon Two-Sample Test (Kruskal-Wallis test for two groups).

RESULT

The clinical characteristics of the study group are shown in (Table 1). A total of 68 males were enrolled in the study. The mean age of study population was 41.68±9.84 years. The clinical characteristics of the study group are shown in Table 1. The study groups were age- matched (p .005).

Table 1:

Parameters	Cases 68	Controls 50
Age	41.68±9.84	34.90±8.57
Bmi	22.23±2.91	20.82±1.96
Alcohol consumption type	Country liquor	-------
Duration	5-20 years	-------
Amount	115.8±44.41	-------

All controls were healthy with normal routine laboratory investigations and as was expected serum values of GGT and TSA were found significantly more often in cases than in controls.(Table 2), p< .0001).

Table 2: Serum levels of GGT and TSA in all cases [68] and controls [50].

.	Cases (n68)	Controls (n50)	P value
	Mean	Mean	
GGT	137.62±14.1	25.48±10.8	0.001*
TSA	34.74±11.25	2.21±1.01	0.001*

*Denotes statistically significant

(Table 3) shows the levels of various parameters in different groups of ALD. The TSA levels were significantly highest in Alcoholic cirrhosis followed by alcoholic hepatitis and fatty liver. The GGT levels were highest in Alcoholic hepatitis followed by alcoholic cirrhosis and fatty liver.

Table 3: Serum levels of, GGT and TSA in fatty liver, hepatitis, cirrhosis

Parameters	Fatty liver [N=23]Mean±S.D.	Hepatitis [N=21]Mean±S.D.	Cirrhosis [N=24] Mean±S.D.
GGT (IU/L)	60.99±36.7*	227.08±170.5*	143.74±105.4*
	P<0.001	P<0.001	P<0.001
TSA (nmol/µl)	35.17±10.9***	31.14±9.69***	36.46±7.66***
	P<0.001	P<0.001	P<0.001

* Denotes statistically significant

[N.B.-Results of every parameter of every type of liver disease are compared to that found among controls] The MMSE levels were lowest in Alcoholic cirrhosis followed by alcoholic hepatitis and Fatty liver. (Table 4).

Table 4: Effect of alcohol consumption Gm/Day on MMSE

Alcohol consumption GM/DAY	MMSE Good n (%)	MMSE Bad n (%)	OR (95%CI)	P value*
>115.89 g/day	8(36.4)	14(63.6)	2.45(0.86-6.93)	0.048
<115.89 g/day	28(58.3)	20(41.7)		

* Denotes statistically significant, OR, odds ratio. P <0.05 is significant.

The effect of alcohol consumption on cognitive scales is observed. Significant correlation .occurs between alcohol consumption (g/day) and cognitive dysfunction measured with the help of cognitive scales MMSE (Table3.3.1.). Patients with MMSE values < mean value 14.16 is considered to have bad MMSE scores. It is observed that large group of patients with mean value of alcohol consumption more than 115.89 gram/day(mean value) shows poor MMSE results 14(63.6%) with the (odds ratio [OR] 2.45; 95% confidence interval [CI] 0.86-6.93; *P* = 0.048) compared to patients with low alcohol consumption 8(36.4%) who shows good MMSE.

Table 5: MMSE scores in different stages of ALD

Parameters	Fatty liver mean±S.D.	Median	Range	Hepatitis mean±S.D.	median	Range	Cirrhosis mean±S.D.	median	Range	P value
MMSE	18.28±3.43	18.00	11 -26.	12.36±5.48	11.00	6-25	10.60±5.32	8.00	5-20	0.000*

* Denotes statistically significant.

MMSE Scores were found to be lowest in alcoholic cirrhosis followed by hepatitis and fatty liver.

Table 6: Spearman rank correlation of TSA with GGT and MMSE

GROUPS	GGT	MMSE
Control	0.7	------
Alcoholic fatty liver	-0.1	-0.18
Alcoholic hepatitis	0.00	-0.012
Alcoholic cirrhosis	-0.3	0.18

TSA shows negative correlation with MMSE in alcoholic fatty liver and alcoholic hepatitis and positive correlation in alcoholic cirrhosis.

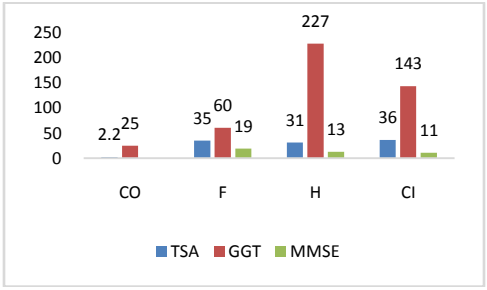

Figure 1: Comparative levels of TSA, GGT and MMSE.

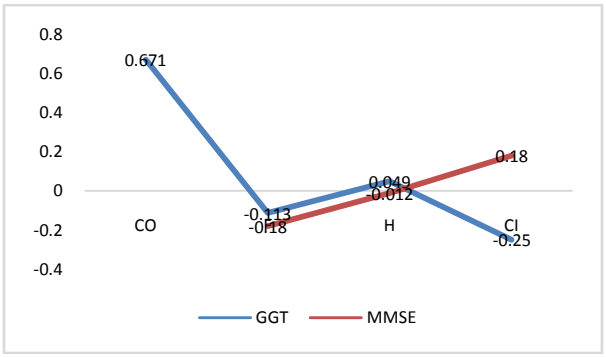

Figure 2: Comparative correlation of TSA with GGT and MMSE

DISCUSSION

One of the major complications of alcoholism is alcoholic liver disease. It is generally believed to be due to the toxic properties of alcohol along with poor nutrition. Studies have showed that ethanol decreases absorption of thiamine in the intestine, affects its hepatic stores and the phosphorylation of thiamine, which converts it to its active form.[12] Patients who drink excessive alcohol tend to consume less amounts of essential nutrients and vitamins and/or exhibit impaired gastrointestinal absorption of these nutrients secondary to the direct effects of alcohol. These relationships make chronic alcoholism a risk factor for thiamine deficiency.[12,13,14] Up to one in five of those who develop alcoholic liver disease will go on to develop cirrhosis. Biochemical mechanisms involving the production of toxic metabolites, called adducts, during the conversion of acetaldehyde to acetate and an immune reaction to liver cells altered by alcohol may be involved in these forms for liver damage.[4] In this study serum GGT concentrations were analyzed and compared with serum TSA levels. We also assessed the association of TSA and cognitive dysfunction and further evaluated its role in predicting long term prognosis of ALD. Our study included the alcoholics who consumed mean alcohol of 115.89 gm/day for duration of minimum 5 to maximum 20 years thus increasing the risk and development of alcoholic liver disease. These findings were supported by Savolainen VT *et al* and Jørgensen *Et al* which established that the incidence of both bridging fibrosis and liver cirrhosis increased significantly only when daily intake of ethanol exceeded 80 g.[15,16] In this study mean GGT concentrations in Alcoholic liver disease is significantly higher as compared to controls and difference in levels between controls and cases is statistically significant (P<0.001). Conigrave *et al* indicated that the GGT levels are typically higher than Aminotransaminase levels in alcohol induced liver damage.[17] which is in accordance with our findings. In the present study Serum GGT levels (from table 3, fig 1) were found to be highest in alcoholic hepatitis followed by cirrhosis and fatty liver. GGT is located in the canaliculi of hepatocyte and epithelial cells lining the biliary ductules.[17] Striking elevations of GGT activity in the serum is observed in patients with excessive and chronic alcohol intake and therefore is commonly used as a screening test for alcoholism. In excessive alcohol consumption, there may be increased release of GGT from the cell membrane. In cases with inflammation and liver cell damage, there may also be cell necrosis with release of the enzyme.[17] Studies have emphasized the value of serum GGT levels in detecting alcohol-induced liver disease. Though serum GGT is considered to be sensitive indicator of hepatobiliary disease that can be detected in most subjects suffering from liver diseases of various types its usefulness is hampered by lack of its specificity. In the present study the mean Sialic acid concentration in patients with alcoholic liver disease is significantly higher as compared to controls whose mean and difference in levels between controls and cases is statistically significant (P<0.001). Though the TSA levels were observed to be high in all the groups of ALD, significantly high levels of TSA (from table3, fig1) were observed those among cirrhosis. Elevated Serum Sialic acid concentrations may result from significant aberrations in the sialylation of serum glycoproteins in liver diseases.[18] another study also indicated that abnormalities of the glycosylation of transferrin occur in the congenital disorders of glycosylation and in chronic alcohol abuse.[19] According to study by Cylwik B, lipid-bound sialic acid (LSA) is higher in patients with alcoholic hepatocellular injury which is in partial accordance with the present study.[20] Kumar *et al* found significant increase in protein bound sialic acid in subjects with ALD compared to control subjects which is in partial accordance with this study.[21] It was found that though diagnostic accuracy of carbohydrate deficient transferrin (CDT) and GGT is highest in detecting problem drinking; however serum SA measurements are of further value when effects of liver pathology and

ethanol drinking need to be differentiated.[22] Recent studies have reported elevation of sialic acid in liver cirrhosis due to massive tissue destruction.[23] Arif and coworkers described the variations of TSA level in liver cirrhosis, fatty liver, acute and chronic hepatitis, liver cancer.[24] They suggested that these results are related to aberration in carbohydrate structure of fibrinogen, which contains 0.6% of Sialic acid, because both, fibrinogen and Sialic acid are the acute-phase reactants; this is in accordance with our study. In the present study all ALD patients were screened for cognitive impairment with MMSE. Impairment of cognitive function is the most disabling symptom of progressive hepatic failure. Studies have established that patients with alcoholic cirrhosis were significantly more impaired in memory and psychomotor speed compared to patients with nonalcoholic cirrhosis. This is attributed to the combined neurotoxic effects of long-term alcohol exposure and the ongoing subacute hepatic encephalopathy.[25] significant correlation occurs between alcohol consumption (g/day) and cognitive dysfunction which was measured with the help of cognitive scales MMSE. The average daily alcohol consumption in the cognitively impaired group was significantly higher. [Mean (SD): 115.82±44.41grams per day]. It is observed that large group of patients with mean value of alcohol consumption more than 115.89 gram/day(mean value) shows poor MMSE results 14(63.6%) compared to patients with low alcohol consumption 8(36.4%) who shows good MMSE . The findings are in agreement with H. Zhou *et al*. and Chan *et al* who established in their studies that heavy alcohol consumption is associated with an increased risk of cognitive impairment while light to moderate alcohol consumption is associated with reduced risk.[26,27] In the present study the MMSE scores were found to be lowest in alcoholic liver cirrhosis ($P < 0.001$) compared to alcoholic hepatitis and alcoholic fatty liver. This study is in accordance with the study which indicated that alcoholic cirrhotic patients had more impaired cognition compared to non alcoholic cirrhotic.[28] and that there is correlation between hepatic dysfunction and cognitive function.[29,30] In the present study TSA showed significant (+ve) correlation with MMSE indicating its role in cognitive impairment in ALD patients. According to comparative values of GGT w. r t. TSA and represented as per correlation presentation (fig 2) it can be concluded that "correlation of serum GGT is parallel to cognitive dysfunction as the condition of liver changes from fatty liver to hepatitis, and opposite correlation with GGT among cirrhosis, w. r. t. serum TSA. this is in accordance with the studies which indicated that biochemical measures of hepatic dysfunction has shown to correlate with neuropsychological dysfunction in alcoholics with cirrhosis.[29] high levels of GGT is associated with neuropsychological deficits in the areas of visuoperceptual and visuoconceptual functioning in alcoholics.[31] Hence we can evaluate "The level of GGT and serum TSA to highlight the starting point of cirrhotic change for maximum cognitive dysfunction i.e. 227(S.D.170) and 31(S.D.9) respectively. Studies have shown that sialic acid (Neu5Ac) is an essential component of brain gangliosides and the polysialic acid chains that modify neural chain adhesion molecules (NCAM). Brain gangliosides and polysialylated NCAM are known to play major role in cell-to-cell interactions, neuronal outgrowth, modifying synaptic connectivity and memory formation. Neu5Ac is known to play critical role in pathogenesis of inflammatory diseases.[32] in the present study though significantly increased mean TSA levels were observed in patients with ALD compared to controls; there were no significant differences in the serum TSA concentration between different groups of ALD. Chronic alcohol abuse alters the concentrations of some sialylated glycoproteins in the sera. The alpha1-antitrypsin, alpha1-acid glycoprotein, and transferrin are the only affected glycoproteins. The serum level of total and free form of sialic acid in the sera of alcoholics depends on the concentration of the most sialylated glycoproteins.[33,34] the sialylation of serum proteins and lipids changes in liver cirrhosis, but only the serum concentrations of FSA are stage-related and reflect the severity of liver disease.[34]

CONCLUSIONS

It is concluded that increase in Total serum sialic acid in the patients with alcohol induced liver disease is an important diagnostic tool in addition to its value in prognosis. This non invasive test may prove beneficial for the patients undergoing treatment for alcoholic cirrhotic liver when it is supported by GGT investigation for cognitive dysfunction. Hence from the present study, Analysis of TSA, along with GGT investigation, is enlighting marker not only for more cognitive dysfunction of brain but also irreversible dysfunction of liver among ALD patients.

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