



Diagnostic Utility of Total Serum Sialic Acid in Alcoholic Liver Disease Patients with Cognitive Impairment

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Abstract

Alcohol induced liver disease plays an important role in precipitating the cognitive impairment encountered in alcohol dependent patients and compounds the alcohol's neurotoxic effects. Total serum level of Sialic acid (TSA) has been demonstrated to be the novel marker of Alcoholic liver disease. The metabolism of Sialic acid takes place in the liver and therefore the status of liver can influence the TSA levels. **OBJECTIVE:** The role of TSA as a marker of liver disease and its association with cognitive changes in alcohol induced liver disease. **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. TSA levels were determined by Bio Vision's Sialic acid assay kit. Result: The serum TSA levels ($34.74 \pm 11.25 \text{ nmol}/\mu\text{l}$) were significantly higher in the alcoholic liver disease than in the healthy controls ($2.21 \pm 1.01 \text{ nmol}/\mu\text{l}$) significantly higher TSA levels were observed in patients with alcoholic cirrhosis ($36.46 \pm 7.66 \text{ nmol}/\mu\text{l}$, $P < 0.001$) compared with alcoholic hepatitis ($31.14 \pm 9.69 \text{ nmol}/\mu\text{l}$, $P < 0.001$) and alcoholic fatty liver ($35.17 \pm 10.9 \text{ nmol}/\mu\text{l}$, $P < 0.001$). **CONCLUSION:** Significantly elevated levels of TSA were observed in Alcoholic liver cirrhosis.

Keywords

Alcoholic liver disease, TSA, AST, MMSE, Cognitive dysfunction.

INTRODUCTION:

Alcohol abuse is one of the major cause of morbidity and mortality worldwide. Consumption of alcohol is associated with health and social consequences like intoxication, dependency etc. Estimated figures state that 2 billion people consume alcoholic beverages and 76.3 million have diagnosable alcoholic disorders. ^[1] Since 1970, 35% of developed countries and 47% of developing countries had reported increased consumption of absolute alcohol per adult: ^[2] 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). ^[3]

The effects of alcohol (Ethanol) on various tissues depend on blood alcohol concentration [BAC]. Harmful levels of consumption are > 50 units (400 g) of alcohol per week for men and are associated with increasing risk of various diseases like alcoholic hepatitis, cirrhosis, pancreatitis and dementia. ^[4] A growing body of evidence suggests that alcohol induced liver disease plays an important role in precipitating the cognitive impairment encountered in alcohol dependent patients which compounds the neurotoxic effects.

Sialic acids (SA) are considered to be one of the most important molecules involved in many biological and pathological phenomena. ^[5] It refers to a group of N-acyl derivatives of neuraminic acid in biological fluids and cell membranes as non-reducing terminal residues of glycoproteins and glycolipids. ^[5] Higher levels of Sialic acid were found both in serum and in saliva in alcoholic subjects. ^{[6][7]} Increased SA concentrations have been reported in other inflammatory diseases, probably due to increased levels of sialylated acute-phase glycoproteins. ^[7] Recently Sialic acid has been suggested as a marker of chronic alcohol use. However, the role of Sialic acid as a marker of liver disease and its association with cognitive changes in Alcoholic liver disease has not been clearly elucidated.

In this study, we hypothesize that Sialic acid levels can determine the stage and severity of alcoholic liver diseases (alcohol induced fatty liver, alcohol induced hepatitis and Alcoholic cirrhosis) and ascertained its role in cognitive changes in subjects

by assessing Mini mental scale examination (MMSE) and Alcohol withdrawal scale(AWS).

METHODS & MATERIALS

This was the case control study conducted in Department of Biochemistry and Department of Psychiatry at 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. The study was approved by the institutional ethical committee.

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 comprised of 50 age matched healthy individuals. Informed consent was obtained from all subjects.

Clinical History:

Detailed history including amount, duration, type of alcohol consumption was taken. Alcohol dependency was enquired in the form of CAGE questionnaire. All 68 patients fulfilled the ICD-10 WHO 1992 criteria for alcohol dependence. The alcohol withdrawal syndrome was assessed with the Alcohol Withdrawal Scale (AWS). Global cognitive functions were assessed periodically with Mini-Mental State Examination (MMSE). Scores of 23 or lower considered as an indicator of cognitive impairment through MMSE. ^[8] All 68 patients which were enrolled in the study completed the full 6 months of follow-up. After admission, patient's 10 ml of venous blood was collected under aseptic conditions. The samples were centrifuged at 3000 rpm for 15 minutes to separate serum which was stored at -80°C for further analysis. The Aspartate Aminotransferase is estimated by Kinetic method. Total Serum Sialic acids (TSA) were measured by using commercially available ELISA kits.

Statistical Analysis:

All data were fed on excel spread sheet and statistical analyses were made using SPSS version 17.0

RESULT

Table 1: The clinical characteristics of the Study and Control group

Parameters	Cases (68)	Controls (50)
Age	41.68±9.84	34.90±8.57
BMI	22.23±2.91	20.82±1.96
ALCOHOL	Country liquor	-----
DURATION	5-20 years	-----
AMOUNT (ml/day)	115.8±44.41	-----

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

	Cases (n68)	Controls (n50)	P value
AST	118.51±79.9	20.3±5.22	0.001***
TSA	34.74±11.25	2.21±1.01	0.001***

*** $P < 0.001$ is considers strong statistically significant.

Table 3 Serum levels of AST & TSA in fatty liver, hepatitis, cirrhosis.

Parameters	Fatty liver [N=23]	Hepatitis [N=21]	Cirrhosis [N=24]
AST IU/L	95.73±61.1 NS	130.7±66.35*** $P < 0.001$	135.8±85.8*** $P < 0.001$
TSA nmol/ μ l	35.17±10.9*** $P < 0.001$	31.14±9.69*** $P < 0.001$	36.46±7.66*** $P < 0.001$

* Denotes statistically significant.

[N.B.-Results of every parameter of every type of liver disease are compared to that found among controls]

Table 4: MMSE & AWS scores in different stages of ALD.

Parameters	Fatty liver	Hepatitis	Cirrhosis	P value
MMSE	18.28±3.43	12.36±5.48	10.60±5.32	0.001*
AWS	11.42±2.99	14.15±3.77	15.47±2.35	0.000*

* Denotes statistically significant.

Table 5: Spearman rank correlation of TSA and AST with MMSE AND AWS

Table 5A

Spearman Rank co-relation of TSA, AST with MMSE

	MMSE
AST	-0.21
TSA	0.015*

*significant at 1% Level of significance

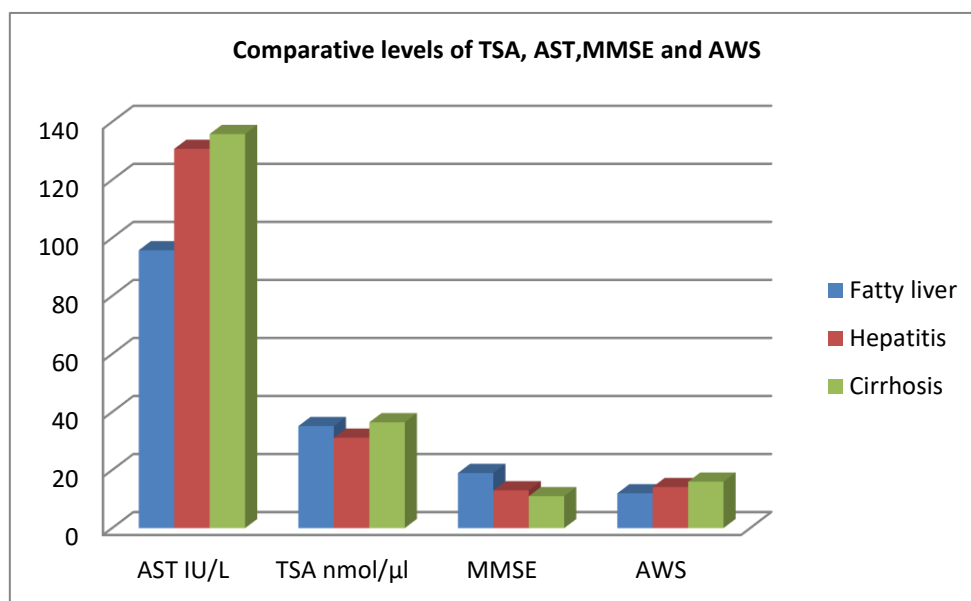
Table 5B

Spearman Rank co-relation of TSA, AST with AWS

	AWS
AST	0.06
TSA	-0.134*

*significant at 1% Level of significance

Fig 1: Comparative levels of TSA, AST, MMSE and AWS.



DISCUSSION:

Alcohol is the world's largest risk factor for disease burden. Consumption of Alcohol results in 2.5 million deaths each year. World health organisation (WHO) estimated 140 million people suffer from Alcohol dependency worldwide, damaging their lives and economies. According to India spend analysis of 2013 National Crime Record Bureau (NCRB) Data, 15 people die every day because of effects of Alcohol consumption.^[9] Also, the Neuropsychiatry conditions accounts for close to 40% of the 58.3 million DALYS. Mortality of Alcoholic liver diseases is increasing in India.^[3]

Hence, we had studied serum TSA levels, also compared and correlated it with cognitive impairment in patients suffering from different stages of Alcoholic liver disease.

Total of 68 males were enrolled in our study. The mean age of study population was 41.68±9.84 years. Most of the study population were found to consume Country liquor for the duration of 5 to 20 years. Average intake of Alcohol was found to be 115.8 ml/day every day in the patients enrolled for the study. (Table 1)

Levels of TSA and AST were assessed in all the subjects. It was observed that levels of both the parameters, TSA and AST were significantly high among cases with mean 34.74±11.25, 118.51±79.9 compared to controls 2.21±1.01, 20.3±5.22 respectively (Table 2). In liver diseases, disturbance of the carbohydrate content of several of the plasma glycoproteins were observed and this alteration of the carbohydrate moiety in some cases may be

responsible for a functional defect of the protein. High levels of AST is known indicator for hepatocellular injury.^[10,11] In alcoholic liver disease, AST is elevated more than ALT indicating partly as a reflection of alcohol induced skeletal damage. This is reverse of the pattern observed in hepatic diseases like acute viral hepatitis where ALT is elevated compared to AST.^[11]

Table 3 shows the levels of TSA and AST in different stages of ALD. The TSA levels were highest in Alcoholic cirrhosis followed by alcoholic fatty liver and hepatitis with mean 36.46±7.66, 35.17±10.9, 31.14±9.69 respectively. Elevated Serum Sialic acid concentrations may result from significant aberrations in the sialylation of serum glycoproteins in liver diseases.^[14] Another study also indicated that abnormalities of the glycosylation of transferrin occur in the congenital disorders of glycosylation and in chronic alcohol abuse.^[15]

The proposed mechanism for the elevation of serum Sialic acid concentration is that ethanol can depress the activities of sialyl transferases in the Golgi apparatus and synaptosomes and can increase the activities of sialidase in the cytosol and plasma membranes.^[16] According Cylwik B, lipid-bound Sialic acid (LSA) is higher in patients with alcoholic hepatocellular injury which is in partial accordance with the present study.^[16] Kumar *et al* found significant increase in protein bound Sialic acid in subjects with ALD compared to control subjects which is also in partial accordance with our study.^[17] Arif and coworkers described the variations of TSA level in liver cirrhosis, fatty liver, acute and chronic

hepatitis, liver cancer.^[18] In contrast Matsuzaki *et al.* observed higher serum TSA levels in hepatoma and metastatic cancer than in control group and cirrhosis group.^[19]

In present study, Serum AST levels were found to be highest in alcoholic cirrhosis followed by alcoholic hepatitis and fatty liver with mean of 135.8 ± 85.8 , 130.7 ± 66.35 and 95.73 ± 61.1 respectively. Our findings were in contrast to the findings of Conigrave *et al.*, which showed minimally elevated levels of aminotransferases in Fatty liver and level may rise to 500 U/L in alcoholic hepatitis.^[12] In our study, serum AST levels were found to be higher than serum ALT levels in ALD which is in accordance with the study by Harinasuta *et al.* who was the first to report the predominance of AST over ALT in ALD.^[13] It was noted that AST is considered to be more sensitive in chronic and infiltrative lesions.

The level of serum AST and TSA can be used to highlight the starting point of cirrhotic changes in liver i.e. 135.8 ± 85.8 & 36.46 ± 7.66 respectively. Thus, Sialic acid levels in patients of ALD can play an important role in diagnosis and prognosis in patients undergoing treatment.

In this study, all ALD patients were screened for cognitive impairment with MMSE and severity of alcohol withdrawal was assessed with AWS. It was found that the MMSE scores were found to be lowest in alcoholic liver cirrhosis compared to alcoholic hepatitis and alcoholic fatty liver with mean of 10.60 ± 5.32 , 12.36 ± 5.48 and 18.28 ± 3.43 respectively. (Table 4)

We found that TSA showed significant positive correlation with MMSE indicating its role in cognitive impairment in ALD patients (Table 5a). Alcoholic cirrhotic patients had more impaired cognition compared to non-alcoholic cirrhotic.^[20] Also, the AWS scores were found to be highest in alcoholic liver cirrhosis and hepatitis compared to fatty liver (15.47 ± 2.35 , 14.15 ± 3.77 , 11.42 ± 2.99). (Table 4)

From comparative values of TSA, AST, MMSE and AWS in different stages of liver diseases (figure 1, Table 4), it can be concluded that correlation of serum TSA is parallel to cognitive dysfunction as the changes in liver progresses to cirrhosis. This is supported by the evidences suggesting correlation between hepatic dysfunction and neuropsychological impairment in alcoholic's liver cirrhosis.^[21]

CONCLUSION:

It is concluded that increase in Total Serum Sialic acid in the patients with alcohol induced liver disease is an important diagnostic and prognostic tool. Thus,

analysis of TSA, along with MMSE and AWS score investigation, is an enlighting marker not only for irreversible dysfunction of liver but also for cognitive impairment among ALD patients.

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