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Serum IL8 and Total Sialic Acid as prognostic marker in Patients of Alcoholic Liver Disease with cognitive dysfunction

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Abstract

Alcoholic liver disease is one of the deadliest consequence of excessive alcohol consumption and also leads to cognitive dysfunction. Serum IL-8 levels mediates neutrophilic infiltration, a pivotal process in the pathogenesis of ALD. The synthesis and the catabolism of Sialic acid takes place in the liver thus the status of liver function can influence the serum levels of TSA. **OBJECTIVE** The role of IL8 and TSA as a marker of liver disease and its association with cognitive changes in alcoholic liver disease. **MATERIALS AND METHOD:** 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. Global cognitive functions were assessed periodically with Mini Mental State Examination (MMSE). Serum levels of IL-8 were determined by ELISA provided by Diaclone. 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). The serum IL8 levels (216.4± 31.4 pg/ml) were significantly higher in the alcoholic liver disease than in the healthy controls (20.±6.0 pg/ml). **CONCLUSION** Serum IL-8 can be used as a marker to determine stages of alcoholic liver disease and correlates with cognitive dysfunction and also shows association with poor long term prognosis. Serum TSA can be used as a marker of alcoholic liver disease and may correlate with cognitive dysfunction among ALD patients.

Keywords

Alcoholic liver disease (ALD),Interleukin-8 (IL8),Total Sialic acid (TSA),Mini Mental State Examination(MMSE)

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INTRODUCTION:

Alcoholic liver disease is a major cause of morbidity and mortality worldwide and results in approximately 2.5 million deaths each year.[1] Alcohol consumption is responsible for 4% of the global burden of disease. Alcohol consumption enhances endotoxin production and activates Kupffer cells which produce reactive oxygen species (ROS), cytolytic proteases, pro-inflammatory cytokines and chemokines contributing to alcoholic hepatitis and cirrhosis in humans.[2,3,4,5,6] In some cases this leads to hepatic encephalopathy (HE) resulting in cognitive dysfunction, altered mood and motor in coordination.[7]

Interleukin 8 (IL-8), a chemokine produced by macrophages and other cells like epithelial cells,

airway smooth muscle cells and endothelial cells. Endothelial cells store IL-8 in their storage vesicles known as the Weibel-Palade bodies. Interleukin-8 secretion is known to increased by oxidative stress, which then recruit other inflammatory cells and further increase oxidative stress mediators, making it a key parameter in localized inflammation. Serum IL-8 levels are mediated by endotoxin release and activated IL-8 mediates neutrophil infiltration, a pivotal process in the pathogenesis of ALD.[8, 9, 10] IL-8 levels also reflect the stage and severity of ALD and might serve as a predictor of survival in patients with alcoholic hepatitis. However the role of IL-8 as a marker of liver disease and its association with cognitive changes in Alcoholic liver disease has not been clearly elucidated.

Total 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.[11] In alcoholic subjects, higher sialic acid values have been found both in serum and in saliva thus indicating that TSA can be prove as a valuable biomarker for excessive alcohol consumption.[11,12] 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.[13,14]

From above consideration here in our study we hypothesize that inflammatory marker, specifically IL8 and Total Sialic acid (TSA) can determine stage and severity of alcoholic liver disease and serve as a prognostic marker for the same. We compared the sensitivity of IL-8 and TSA with other traditional liver enzymes and ascertained its role in cognitive changes.

MATERIAL AND METHODS:

Total of 68 adult male patients, consecutively transferred to inpatient detoxification centre were enrolled for the study after confirmation of alcoholic liver disease on the basis of clinical findings and by USG studies. This is a 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. The study was approved by the institutional ethical committee. Informed consent was obtained from all subjects.

The 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 individual.

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.

In IPD, 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. Serum levels of IL-8 were determined with an enzyme-linked immunosorbent assay (ELISA) provided by Diaclone Research. Total Serum Sialic acids (TSA) were measured by using commercially available ELISA kits. Sstatistical 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	--
Quantity (ml/day)	115.8±44.41	--

Table 2. Serum levels of IL8 & TSA in all cases [68] and controls [50].

	Cases (n=68)	Controls (n=50)	P value
IL-8	216.4± 31.4	20±6.0	0.001*
TSA	34.74±11.25	2.21±1.01	0.001***

****** P <0.001 consider statistically significant***

Table 3: Serum levels of IL-8 and TSA in fatty liver, hepatitis, cirrhosis

	Fatty Liver(n-23)	Hepatitis (n-21)	Cirrhosis (n-24)
IL-8 (pg/ ml)	39±21.6* P<0.001	202.7±181*	414.5±364*
TSA (nmol/µl)	35.17±10.9*** P<0.001	31.14±9.69***	36.46±7.66***

**** Denotes statistically significant.***

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

	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 IL8 with MMSE AND AWS

Table 5a: Spearman Rank correlation of TSA, IL8 with MMSE

	MMSE
IL8	-0.84
TSA	0.015*

****significant at 1% Level of significance***

Table 5B: Spearman Rank correlation of TSA, IL8 with AWS

	AWS
IL8	0.75
TSA	-0.134*

****significant at 1% Level of significance***

DISCUSSION

According to WHO, 140 million people suffer from Alcohol dependency worldwide, damaging their lives and economies. One of the major complications of alcoholism is alcoholic liver disease. The Neuropsychiatry conditions accounts for close to 40% of the 58.3 million DALYS.[3] Inflammation plays a crucial role in the pathogenesis of alcoholic liver disease. Release of proinflammatory cytokines, such as tumour necrosis factor (TNF-α) and interleukin-1, and chemo attractant chemokines, such as interleukin-8 have been implicated in the pathogenesis of both alcoholic hepatitis and cirrhosis.

In our study, total 68 males were enrolled. 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. (Table 1) Levels of TSA and IL8 were assessed in all the subject. The levels of both the parameters TSA and IL8 were found to be significantly high among cases with mean of 34.74±11.25, 216.4± 31.4 respectively compared to controls with mean of 2.21±1.01, 20. ±6.0 (Table 2).

It was evident from table 3, TSA levels were highest in Alcoholic cirrhosis followed by alcoholic fatty liver and hepatitis with mean 36.46±7.66, 35.17±10.9 and 31.14±9.69 respectively. The level of serum IL8 and TSA can be used to highlight the starting point of cirrhotic changes in liver. Elevated Serum Sialic acid concentrations may result from significant aberrations in the sialylation of serum glycoproteins in liver diseases.[12] Study by santosh kumar *et al* also indicated that abnormalities of the glycosylation of transferrin occur in the congenital disorders of glycosylation and in chronic alcohol abuse.[15] 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]

IL8 levels were also found to be high in alcoholic cirrhosis followed by alcoholic hepatitis and fatty liver with mean 414.5±364, 202.7±181, 39±21.6 (pg/ml) respectively. Highly elevated IL-8 levels were observed in alcoholic liver cirrhosis with *P* value<0.05. Studies of Swiatkowska-Stodulska R et al, Kawaratani *et al* and Huang YS *et al* in Patients of ALD

showed highly elevated levels of Serum IL-8 in patients with Alcoholic Hepatitis and in contrast IL-8 was only moderately elevated in alcoholic cirrhosis This is linked to neutrophil infiltration.[20,21,22] In our findings significantly high IL-8 levels were observed in alcoholic liver cirrhosis thus indicating that monocytes/macrophages, and not neutrophils, appear to be main responders to IL-8 in liver fibrosis/cirrhosis via CXCR1 which was in accordance to the study by Zimmermann HW *et al* who established that IL-8 serum levels were significantly increased in chronic liver disease patients (CLD) patients, especially in end-stage Cirrhosis.[23] study by Daniluk *et al* strongly suggests that IL8 is a reliable diagnostic parameter for evaluating the severity of liver damage.[24] Fulton showed Cytokines affect the brain and likely contribute to changes in the central nervous System that contribute to long-term changes in behaviour and neurodegeneration.[25] Our results were in accordance with Huang *et al* who demonstrated that higher serum IL8 levels correlated with higher mortality rate in Chinese population.[22]

In our 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)

Statistically significant correlation between IL-8 and MMSE (Table 5a) and AWS (Table 5b) with *P*value <0.001 was observed in our study thus indicating that IL-8 is strongly activated in alcohol related liver cirrhosis and they in turn affect the brain and likely to cause changes in the central nervous system patients or alcoholics. The other parameter TSA also 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.[26] 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 our obtained comparative values of TSA, IL8, MMSE and AWS in different stages of liver diseases it can be concluded that correlation of serum IL8 and serum TSA is parallel to cognitive dysfunction as the changes in liver progresses to cirrhosis. Also high levels of IL-8 were associated with poorer long term prognosis. This is supported by the evidences suggesting correlation between hepatic dysfunction and neuropsychological impairment in alcoholics liver cirrhosis. IL8 and TSA can be used as a prognostic marker for alcoholic liver disease.

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