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Research Article

ASSESSMENT OF SIALIC ACID, ADENOSINE DEAMINASE AND C-REACTIVE PROTEIN IN ALCOHOLIC LIVER DISEASE

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ABSTRACT
Alcohol consumption is associated with a number of changes in hepatic cell functions as liver is the major site of its metabolism. Acetaldehyde metabolite formed during metabolism of ethanol is very reactive compound and is indicator of tissue and organ damage. The relationship of serum sialic acid, adenosine deaminase and C-reactive protein in patients of alcohol abuse is unknown so the current study was planned. The aim of this study was to evaluate the effect of alcoholic liver disease on the serum level of Sialic acid, adenosine deaminase and C-reactive protein and can be used as non invasive prognostic tool which may be helpful in the management of patients before they develop the complications of the disease. We determined serum Sialic acid, adenosine deaminase and C-reactive protein in 50 alcoholics and 50 healthy controls by manual kit methods and immunoturbidimetric method. The (mean +/- SD) levels of Sialic Acid, adenosine deaminase and C-reactive protein were significantly higher in the alcoholics than in the healthy controls. On comparing their levels in patients of liver cirrhosis, fatty liver and hepatitis the correlations were found to be insignificant. The estimation of protein bound sialic acid, adenosine deaminase and C-reactive protein in serum is an important non invasive prognostic tool which may be helpful in the management of patients before they develop the complications of the disease.

Keywords: *Protein Bound Sialic Acid, Adenosine Deaminase, C-Reactive Protein, Alcoholic Liver Disease*

INTRODUCTION
Alcoholic liver disease includes the hepatic manifestations of alcohol overconsumption including fatty liver, alcoholic hepatitis and chronic hepatitis with hepatic fibrosis or cirrhosis (O'Shea *et al.,* 2010). Fatty liver is present in >90% of binge and chronic drinkers. A much smaller percentage of heavy drinkers will progress to alcoholic hepatitis, thought to be a precursor to cirrhosis. The prognosis of severe alcoholic liver disease is dismal; the mortality of patients with alcoholic hepatitis concurrent with cirrhosis is nearly 60% at 4 years, although alcohol is considered a direct hepato-toxin, only between 10 and 20% of alcoholics will develop alcoholic hepatitis. The explanation for this apparent paradox is unclear but involves the complex interaction of facilitating and co-morbid factors such as gender, heredity and immunity (Harrison *et al.,* 2014).
Glycosylation and sialyation of lipids and proteins takes place in the liver. There are evidences that the changes in glycosylation or sialylation of proteins and lipids have important role in the pathogenesis and progression of various liver disease (Blomme *et al.,* 2009). Sialic acids are either N or O-acetyl derivatives of 9-carbon sugar neuraminic acid - an aldol condensation product of mannosamine and pyruvic acid. Sialic acids are terminal sugar components of the oligosaccharide chains of glycoproteins and glycolipids and sialic acid is localized at the end chain of many acute phase proteins. The majority of sialic acids are found in either protein (PBSA) or lipid – bounded (LBSA) forms, while little amounts is in the free forms (Nayak and Roberts, 2006). Increased levels of total SA and/or lipid associated SA have been observed in various diseases including several types of cancer, diabetes, and renal disease. It has been previously reported that SA levels may be increased in biological fluids of alcoholics, and it has been suggested that SA can be valuable as a biomarker for excessive alcohol consumption. Previous studies have reported increased total and free sialic acid levels in patients with Alcoholic liver disease.

Adenosine deaminase (ADA) is an essential zinc-metallo enzyme and widely distributed in human tissue and blood. It is involved in the catabolism of purine bases capable of catalysing the deamination of adenosine, forming inosine in the process. It is essential for the differentiation of lymphoid cells, particularly T-cells and plays a role in the maturation of monocytes to macrophages and hence considered an indicator of cell-mediated immunity (Sullivan *et al.*, 1997). It was reported that high serum ADA activities were observed in patients with acute hepatitis, chronic active hepatitis, liver cirrhosis and hepatoma (Sherlock, 1995).

C-reactive protein belongs to the pentraxin family of calcium-dependent ligand-binding plasma proteins (Thompson *et al.*, 1999). CRP is named for its capacity to precipitate the somatic C-polysaccharide of Streptococcus pneumoniae and is a sensitive systemic marker of inflammation and tissue damage (Pepys and Baltz, 1983). Plasma CRP is produced only by hepatocytes, predominantly under transcriptional control by the cytokine IL-6, although other sites of local CRP synthesis and secretion also occur. The plasma half-life of CRP is about 19 hours and is constant under all conditions of health and disease, so the sole determinant of circulating CRP concentration is the synthesis rate, which thus directly reflects the intensity of the pathological process stimulating its production.

The CRP concentration is thus a very useful biochemical marker of inflammation, measurement of which contributes importantly to screening for organic disease, monitoring of the response to treatment of inflammation, infection and detection of inter current infection in immune-compromised individuals (Imhof *et al.*, 2001).

Hence, the present study was done to find out the serum protein bound sialic acid adenosine deaminase and CRP levels in alcoholic liver disease.

MATERIALS AND METHODS

The present study was conducted in the department of Biochemistry in collaboration with Department of Medicine, Pt. B.D. SHARMA, P.G.I.M.S, Rohtak. Subjects were 50 males clinically diagnosed cases of alcoholic liver disease in the age group of 25-60 years. As a control group, 50 healthy individual aged 25-60 years from the same area were recruited. Ethical consents were obtained from all participants of this study. Clinical diagnosis of patients was confirmed by serological tests, ultra sonogram and other clinical findings. 10 ml of venous blood was collected from both cases and control, centrifuged and stored at -20°c before biochemical analysis.

Special Investigations

Estimation of Protein Bound Sialic Acid

The protein bound sialic acid of serum proteins was measured by modified Aminoff's method (Aminoff, 1961). The bound sialic acid is released by sulfuric acid and reacts with thiobarbituric acid (TBA) to form TBA-sialic acid complex. On boiling in water bath, this gives a pink colour. This colour is further extracted using acid–butanol mixture and then measured at 549nm spectrophotometrically.

Estimation of Adenosine Deaminase

Serum ADA activity was determined at 37ºC by a method described by Giusti and Galanti (1984). That was based on the Bertholet reaction Enzyme adenosine deaminase catalyses the hydrolytic cleavage of adenosine to inosine and ammonia. Ammonia forms intense blue coloured iondphenols with sodium hypochlorite and phenol in alkaline solution. Sodium nitruprusside is the catalyst. The ammonia concentration is directly proportional to absorbance of indophenols, which is measured by spectrometry at 620nm against water. One unit of ADA is defined as the amount of enzyme required to release 1μmol of ammonia/min from adenosine at standard assay conditions. Results were expressed as international unit (IU) of enzyme activity of serum.

Estimation of C-Reactive Protein

The estimation of C-reactive protein was done by Immuno-turbidimetric method (Hokama and Nakamura, 1987). Latex particles coated with anti-human C reactive protein (CRP) antibodies agglutinate when mixed with sample containing CRP resulting into insoluble antigen-antibody complex. These insoluble complexes increase the turbidity which is measured at 550 nm. Increase in turbidity is directly

proportional to concentration of CRP in the sample that can be quantified by comparison with a calibrator of known CRP concentration.

Measurement of Serum Aspartate Aminotransferase (SGOT), Alanine Aminotransferase (SGPT) and Alkaline Phosphatase (ALP)

Enzymes required to evaluate the liver function tests were performed by using enzymatic kit assay systems (Randox diagnostics) in autoanalyser system and performed as described by manufacturers (Schumann *et al.*, 2002, 2002; Teitz's *et al.*, 1983).

Statistical Analysis

Statistical analysis was done using SPSS 16 package. Results were expressed as mean±S.D. ADA, Sialic acid, CRP and Liver Function Test (LFT) parameters of cases were compared with controls by student's t test. Comparison of parameters for different stages of alcoholic liver disease was done using One -Way ANOVA test.

RESULTS AND DISCUSSION

Results

Table 1 shows mean and S.D of protein bound sialic acid, ADA and CRP between control and alcoholic liver disease patients (cases). Their levels were significantly higher in Alcoholic Liver Disease patients (cases) as compared to controls.

Table 1: Mean and S.D of Protein Bound Sialic Acid and ADA in Cases and Controls

Investigation	Cases (n=50)	Control(n=50)	P Value
Protein Bound Sialic Acid (mg/dL)	4.90±1.01	1.46±0.51	<0.001 (Highly Significant)
ADA (U/L)	66.55±30.89	6.14±2.56	<0.001 (Highly Significant)
C-Reactive Protein (mg/L)	20.48±5.29	6.79±0.50	p <0.001 (Highly Significant)

Table 2 shows number of cases in different stages of alcoholic liver disease classified based on ultra sonographic findings and majority of patients suffered from hepatitis followed by cirrhosis. Only 8 patients were found to be suffering from fatty liver disease.

Table 2: Number of Patients (Cases) in Different Stages of Alcoholic Liver Disease

USG Findings	No. of Patients n (%)
Cirrhosis	20 (40%)
Fatty Liver	8 (16%)
Hepatitis	22 (44%)

Table 3 shows mean and S.D of protein bound sialic acid, ADA and CRP in different stages of ALD cases

Table 3: Levels of Protein Bound Sialic Acid, ADA and C-Reactive Protein in Patients of Liver Cirrhosis, Fatty Liver and Hepatitis

USG Findings	No. of Patients	Protein Bound Sialic Acid (mg/dL)	ADA (U/L)	CRP (mg/L)
Cirrhosis	20 (40%)	4.62±0.93	78.89±37.12	19.93±6.03
Fatty liver	8 (16%)	4.87±1.09	58.32±15.35	21.92±4.43
Hepatitis	22 (44%)	5.17±1.02	58.32±25.68	20.46±4.98

Table 4 shows correlation of sialic acid, adenosine deaminase and C-reactive protein with each other using Pearson's Correlation of Coefficient test (`r' value). A positive correlation was observed between

bound sialic acid and ADA and correlation between C-reactive protein and bound sialic acid as well as C-reactive protein and ADA was found negative. All correlations were found to be insignificant.

Table 4: Correlation of Specific Investigations in Study Group (Cases)

Specific Investigations	Correlation Coefficient `r' Value	P Value
ADA and Sialic Acid	0.042	0.772
C-Reactive Protein and Sialic Acid	-0.134	0.351
C-Reactive Protein and ADA	-0.036	0.803

Table 5 shows mean and S.D values of liver function test parameters between control and alcoholic liver disease patients. Serum bilirubin, SGOT, SGPT and ALP were significantly higher in ALD patients as compared to controls whereas serum proteins and A: G ratio was low.

Table 5: Liver Function Test Parameters between Control and Alcoholic Liver Disease Patients

Investigations	Cases (n=50)	Control(n=50)	P Value
S. Bilirubin (mg/dL)	6.2±7.32	0.37±0.13	<0.001 Highly Significant
SGOT (IU/L)	79.38±35.77	27.9±7.02	<0.001 Highly Significant
SGPT (IU/L)	55.5±24.52	27.5±5.65	<0.01 Significant
S. ALP (U/L)	142.78±77.67	77±17.75	<0.001 Highly Significant
S. Proteins (g/dL)	6.30±0.94	7.03±0.53	<0.001 Highly Significant
A:G ratio	0.85±0.34	1.36±0.20	<0.001 Highly Significant

Discussion

Alcoholic liver disease (ALD) is one of the major medical complications of alcohol abuse and serious health issue with major socio-economic consequences. Significant morbidity is related to chronic heavy alcohol use and alcoholics seek advice only when a complication of drinking sets in. The diagnosis is often based on patients self – reporting of alcohol consumption which is unreliable and requires high degree of clinical suspicion (Arumalla *et al.*, 2012).

Liver is the most biochemically complex organ which plays a vital role in the metabolism. Alcohol is the most common cause of hepatic injury. Acetaldehyde metabolite formed during metabolism of ethanol is very reactive compound and is indicator of tissue and organ damage (Sreedevi and Chakrapani, 2014). In present study among 50 cases 44%, 16% and 40% had hepatitis, fatty liver and cirrhosis respectively.

In our study we have measured the serum concentrations of inflammatory markers like protein bound sialic acid, Adenosine deaminase and C –reactive protein in patients of alcoholic liver disease and compared their levels in healthy controls. Generally, we detected increased levels of protein bound sialic acid in all ALD subjects when compared with controls but we did not find any significant difference in protein bound sialic acid levels between different clinical sub groups. Similar findings were reported by Kumar and Kalaivani, (2011). The elevation in sialic acid was due to decrease in sialyltransferase and increase in sialidase activities by chronic alcohol consumption.

Selva Kumar and coworkers (2012) also showed Lipid bound sialic acid in ALD patients was higher in cases in comparison to controls. Their study also suspected that the liver disease affect not only the serum level of lipids and lipoproteins but also the level of sialic acid bounded with these compounds. The

elevated sialic acid suggested that due to alcohol liver damage lead to abnormal carbohydrate composition of the fibrinogen as it contains 0.6% sialic acid (Kaniak *et al.,* 1980).

The increased Protein bound sialic acid levels in alcoholic liver disease indicated that the deformation has been occurred in the hepatic cells and an amount of sialic acid containing glycolipid or glycoprotein (mainly acute phase proteins, such as alpha acid glycoprotein and alpha antitrypsin) were released from vascular cells into blood stream (Carlson, 1980).

Further various studies suggested that inflammatory neutrophils undergo a interleukin-8- inducible recruitment of intracellular sialidase to the cell surface, where the release of bound sialic acid from surface molecules in the surrounding environment is responsible for raised sialic acid concentration (Romppanen, 2003).

As reported in previous studies, the present study also shows significant increase in AST, ALT, and ALP in Alcoholic liver disease. Though, we did not find a significant difference in protein bound sialic acid levels between different clinical groups of ALD and correlation of sialic acid, adenosine deaminase and C-reactive protein with each other was found to be insignificant.

Further in our study, we have found significant increase in adenosine deaminase level in ALD patients. However the difference in ADA level between all the groups was not found significant.

Similar findings were reported by Kumar and coworkers (2011) in their study where ADA level in alcoholic liver disease patients was higher than in controls. He also reported ADA levels in different stages of ALD patients. As ADA level is raised in inflammatory conditions and this statement is supported by report of Kaya and coworkers.

They found raised ADA level in patients of hepatitis B & C when compared to healthy controls (Selcuk *et al.,* 2007). Elevated serum ADA activity in patients with hepatitis is proposed to reflect the amplified phagocytic activity of macrophages and may provide useful diagnostic information on the pathogenesis of alcoholic liver disease (Barnes *et al.,* 1995). Thus, clearing the role of ADA in maintaining and performing immunological response in inflammatory conditions.

Alcohol can increase extacellular adenosine levels both by inhibiting adenosine uptake into the cells and by increasing adenosine production throughout the body as a result of alcohol metabolism in the liver (Donald *et al.,* 1996). Adenosine has been suggested to be critical regulator of inflammation and increased adenosine release could be utilized to diminish inflammation. The increased ADA activity may be related to inflammatory process in alcoholic liver disease (Donald *et al.,* 1996).

In our study the CRP level was significantly elevated in cases as compared to controls. Elevated levels of CRP were also reported by Jagiello and coworkers also. CRP has a long half – life, affording stability of its level with no observable circadian variation and has proved to be a very useful marker of inflammation in clinical studies (Pepys and Berger, 2001; Meier *et al.,* 2001). CRP is capable of stimulating IL-6 and TNF-α production by monocytes Ballou SP and Lozanski, (1991) and reactive oxygen species formation as well (Wang *et al.,* 2003).

Liver has one of the largest resident population of macrophages (Kupffer cells), which are key components of the innate immune systems. Hepatic macrophages generate various inflammatory mediators and cytokines which have an important role not only in stimulating the CRP production but also in inhibiting albumin synthesis in the liver (Black *et al.,* 2004). So it can be concluded that due to hepatic cell injury induced by alcohol the inflammatory markers like CRP rises.

Alcohol consumption is associated with a number of changes in hepatic cell functions. In the present study, we have analyzed various biochemical parameters in alcoholic liver disease patients and healthy controls.

It is concluded that the estimation of protein bound sialic acid, adenosine deaminase and CRP in serum is an important non invasive prognostic tool which may be helpful in the management of patients before they develop the complications of the disease. Nature of alteration in the sialic acid, adenosine deaminase and CRP may provide a basis for better understanding of pathogenesis and mechanism responsible in the patients of alcoholic liver disease.

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