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Study of Adenosine Deaminase and Serum Protein Bound Sialic Acid Levels in Alcoholic Liver Disease

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ABSTRACT

Background: Even though Alcoholic liver disease (ALD) is common disease in India, there are only limited reports about adenosine deaminase activity and no reports about protein bound sialic acid in these cases. The present study was designed to evaluate adenosine deaminase activity and protein bound sialic acid levels in patients with alcoholic liver disease. **Materials and methods:** 50 alcohol liver disease patients grouped in to three cases groups [Fatty liver (n=18), alcoholic hepatitis (n=19) and cirrhosis (n=13)] and 50 controls were enrolled in the study. Adenosine deaminase, protein bound sialic acid and liver function test parameters were analyzed in both the groups. **Results:** Adenosine deaminase and protein bound sialic acid were significantly increased in alcohol liver disease cases compared to controls. Both adenosine deaminase and protein bound sialic acid were higher in alcoholic hepatitis and cirrhosis group compared to fatty liver group and controls. **Conclusion:** To conclude, the present study demonstrates increased adenosine deaminase and protein bound

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1. Introduction

Alcoholic liver disease (ALD) refers to alcohol induced disease of the hepatobiliary system with genetic, psycho-social and environmental factors influencing its development and having liver specific and systemic manifestations [1]. The disease is often progressive and is considered to be a major cause of morbidity and mortality [2]. Alcoholic liver disease represents a spectrum of clinical illness and morphological changes that range from fatty liver to hepatic inflammation and necrosis (alcoholic hepatitis) to progressive fibrosis (alcoholic cirrhosis) [3]. Furthermore, sustained excessive alcohol intake favours the progression of other liver diseases, such as virus-related chronic hepatitis, also increasing the risk of hepatocellular carcinoma [4–6].

Sialic acid (SA) is the generic term given to a family of acetylated derivatives of neuraminic acid which occur mainly at terminal positions of glycoprotein and glycolipid oligosaccharide side-chains. Several biological functions have been suggested for SA, such as stabilizing the conformation of glycoproteins and cellular membranes, assisting in cell-cell recognition and interaction,

contributing to membrane transport, providing binding sites for ligands for the membrane receptor functions, and affecting the function, stability and survival of glycoproteins in blood circulation [7]. Increased levels of total SA and/or lipid associated SA have been observed in various diseases including several types of cancer [8], diabetes [9], and renal disease [10]. 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 [11–14].

Adenosine deaminase (ADA) is an enzyme involved in the catabolism of purine bases, capable of catalysing the deamination of adenosine, forming inosine in the process [15]. ADA activity is widely distributed in human tissues and is higher in lymphoid tissues, and principal biological activity of ADA is detected in T lymphocytes [16]. Its main physiologic activity is related to lymphocytic proliferation and differentiation. As a marker of cellular immunity, its plasma activity is found to be elevated in diseases in which there is a cell-mediated immune response [17]. It was reported that high serum ADA activities were observed in patients with acute hepatitis, chronic active hepatitis, liver cirrhosis and hepatoma [18].

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

2. Materials and Methods

2.1. Subjects

The groups of patients were from Sri Manakula Vinayagar Medical College & Hospital, Pondicherry where alcohol consumption is very common in this territory. Subjects were 50 male patients in the age group of 20-50 years divided in to three groups based on their diagnosis as fatty liver (n=18), alcoholic hepatitis (n=19), and cirrhosis (n=13). As a control group, 50 healthy individual aged 20-50 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. 5ml of blood was collected from both cases and control, centrifuged and stored at -20°C before biochemical

2.2. Measurement of serum ADA activity

Serum ADA activity was determined at 37°C by a method described by Giusti and Galanti [19] that was based on the Bertholet reaction. In brief, the formation of colored indophenols complex from ammonia liberated from adenosine and quantified spectrophotometrically. 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.

2.3. Estimation of protein bound sialic acid

The protein bound sialic acid of serum proteins was measured following by modified Aminoff's method [20]. Trichloroacetic acid precipitates the proteins present in serum. The protein 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.

2.4. Measurement of Serum Aspartate aminotransferase, Alanine aminotransferase, and Gamma glutamyl transferase

Enzymes required to evaluate the liver function tests were performed by using colorimetric techniques with kit assay systems (Randox diagnostics) in Randox Imola autoanalyser system and performed as described by manufacturers.

2.5. Statistical analysis

Statistical analysis was done using SPSS.13 package. Results were expressed as mean±S.D. ADA, Sialic acid 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 followed by tukey's test.

3. Results

Table 1 shows mean and S.D of age, BMI and liver function test parameters between control and alcoholic liver disease patients. AST, ALT and GGT were significantly higher in ALD patients as compared to controls.

Table 1: Mean ± S.D of age, BMI and liver function test parameters between control and alcoholic liver disease patients

| Variable | Control (n=50) | ALDPatients (n=50) |
|--------------------------|----------------|--------------------|
| AGE (Years) | 42.5±6.54 | 44.92±6.5 |
| BMI (kg/m ²) | 20.97±1.97 | 20.2±2.1 |
| AST (U/L) | 33.67±14.6 | 146.68±51.47* |
| ALT (U/L) | 22.63±7.09 | 100.08±51.04* |
| GGT (U/L) | 23.2±6.34 | 117.58±46.5* |

Values expressed mean±S.D P<0.01 is considered as significant

Table 2 shows mean and S.D of protein bound sialic acid and ADA in controls and different stages of ALD cases. Adenosine deaminase and sialic acid levels were significantly elevated in all stages of ALD compared to controls. Also ADA was significantly increased in cirrhosis cases compared to fatty liver patients

Table 2: Mean and S.D of protein bound sialic acid and ADA in controls and different stages of ALD cases

| Variable | Control (n=50) | Fatty Liver (N=18) | Alcoholic Hepatitis (N=19) | Cirrhosis (N=13) |
|-----------------------------------|----------------|--------------------|----------------------------|------------------|
| Protein bound Sialic acid (mg/dl) | 2.8±1.06 | 5.07±1.07* | 4.87±1.59* | 4.97±1.36* |
| Adenosine Deaminase (iu/l) | 17.62±0.61 | 22.79±1.06* | 25.38±2.48* | 27.97±1.95* η |

Values expressed mean±S.D

*p<0.01 compared to controls η p<0.01 compared to fatty liver

4. Discussion

Alcoholic liver disease (ALD) is a common type of liver disease in India especially in Pondicherry. It comprises of three well established entities namely fatty liver, hepatitis and cirrhosis. In the present study, out of 50 cases 36%, 38%, and 26% had fatty liver, hepatitis and cirrhosis respectively. Although we did not find significant age difference between the 3 groups, we found patients with cirrhosis were older compared to others.

Sialic acid is an acute phase protein, which increases in inflammation. It is known to rise in conditions like cancer, diabetes, sialidosis and inflammatory diseases. In ALD increased total and free sialic acid levels have been reported previously [21]. However to the best of our knowledge, the level of protein bound sialic acid levels in ALD have not been reported. For the first time, in the presently study we found a significant increase in protein bound sialic acid in subjects with ALD compared to control subjects.

Though, we did not find a significant difference in protein bound sialic acid levels between different clinical groups of ALD, subjects with fatty liver were found to have increased protein bound sialic acid levels when compared with hepatitis and cirrhosis. Though the

mechanism by which protein bound sialic acid levels are increased in alcoholic liver disease is not known, previous studies have show that chronic alcohol consumption stimulates the hepatic synthesis of acute phase proteins like sialic acid [22]. Other possible reason could be the inflammatory response due to alcohol related liver injury and transferase activity. Biochemical alteration of sialic acid in various liver diseases has been studied from time to time. It has been proved that plasma glycoproteins show an alteration in the carbohydrate content in hepatic damage [23]. This disturbance in the carbohydrate moiety may in some cases be responsible for a functional defect of the protein. As reported in previous studies, the present study also shows significant increase in AST, ALT, and GGT in Alcoholic liver disease.

In our study, we have found significant increase in adenosine deaminase levels in all stages of alcoholic liver disease. The finding suggests that there may be an interaction between alcohol intake and inflammation. In addition, increased ADA activity may be related to inflammatory process in alcoholic liver disease. In the literature, there were limited studies encountered on serum ADA activity in alcoholic liver disease. Adenosine has been suggested to be critical regulator of inflammation and increased adenosine release could be utilized to diminish inflammation [24]. The Adenosine deaminase catalyzes the deamination of adenosine to inosine contributing to the regulation of intracellular and extracellular concentrations of adenosine, and probably modulates energy metabolism. Systemic administration of an Adenosine deaminase inhibitor produce clear anti-inflammatory effects [25]. Our study shows that serum protein bound sialic acid levels and serum adenosine deaminase activity were increased in alcoholic liver disease both of which are directly related with alcohol induced inflammation.

5. Conclusion

The estimation of serum protein bound sialic acid and adenosine deaminase level in the patients of alcoholic liver disease are an important non invasive prognostic tool which may be helpful in the management of patients, before they develop the complications of the disease. Further investigations into the nature of alterations in the sialic acid content of glycoproteins in future may provide a basis for a better understanding of pathogenic mechanism responsible for it in the patients of alcoholic liver disease which may give a clue to its treatment before the disease advances to fatal level.

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