World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
Published by Atom and Cell Publishers
Available online at: http://www.wjpsonline.org/
Original Article

A Study on levels of Adenosine Deaminase and Sialic Acid in Alcoholic Liver Disease

D. Santha Rao

Department of Biochemistry, Viswabharathi Medical College, Kurnool, Andhra Pradesh, India

Received: 21-10-2015 / Revised: 21-11-2015 / Accepted: 29-11-2015

ABSTRACT

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 disease50 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. 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. To conclude, the present study demonstrates increased adenosine deaminase and protein bound.

Keywords: Adenosine, Deaminase, Sialic Acid, Alcoholic Liver Disease

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].

***Corresponding Author Address:** Dr. D. Santha Rao, Department of Biochemistry, Viswabharathi Medical College, Kurnool, Andhra Pradesh, India*

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

MATERIALS AND METHODS

Subjects: The groups of patients were from Great Eastern Medical College & Hospital, AP 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 form both cases and control, centrifuged and stored at -20°c before biochemical

Measurement of serum ADA activity: Serum ADA activity was determined at 37oC 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.

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.

Measurement of Serum Aspartate aminotransferase, Alanine aminotransferase, and Gamma glutamtyl 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.

Statistical analysis: Statistical analysis was done using r- tool 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.

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)	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 Alcoholic	LiverCirrhosis (N=18)(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)	22.79±1.06*	25.38±2.48*
17.62±0.61		27.97±1.95* η	

Values expressed mean±S.D
*p<0.01 compared to controls η p<0.01 compared to fatty liver

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, sialidiosis 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.

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.

REFERENCES

[1] Liber CS. Alcohol and liver: metabolism of alcohol and its role in hepatic and extra hepatic diseases. J.Med .000; 67:84-94.
[2] Sherlock S. Alcohol and the liver. In: Sherlock S. Diseases of the liver and biliary system. Blackwell publications London, 1995; 6:385-403.
[3] Tome S, Lucey MR. Review article: current management of alcoholic liver disease. Aliment Pharmacol Ther.2004; 19: 707–14.
[4] Bellentani S, Tiribelli C, Saccoccio G, et al. Prevalence of chronic liver disease in the general population of northern Italy: the Dionysos Study. Hepatology.1994; 20: 1442–9.
[5] Safdar K, Schiff ER. Alcohol and hepatitis C. Semin Liver Dis.2004; 24: 305–15.
[6] Mandayam S, Jamal MM, Morgan TR. Epidemiology of alcoholic liver disease.Semin Liver Dis.2004; 24: 217–32.
[7] Sillanaukee P, Ponnio M, Jaaskelainen IP. Occurence of sialic acids in healthy humans and different disorders Eur J Clin Invest. 1999; 29:413-425:.
[8] Kökoğlu E, Sönmez H, Uslu E, Uslu I. Sialic acid levels in various types of cancer Cancer Biochem Biophys. 1992; 13:57-64.
[9] Crook M. Type 2 diabetes mellitus: a disease of the innate immune system?
An update Diabet Med. 2004; 21: 203-207.
[10] Ozben T. Elevated serum and urine sialic acid levels in renal diseases .Ann Clin Biochem.1991; 8: 44-48.
[11] Sillanaukee P, Ponnio M, Seppa K. Sialic acid: New potential marker of alcohol abuse Alcohol. Clin Exp Res. 1999; 23:1039-1043.

[12] Ponnio M, Alho H, Heinala P, Nikkari ST, Sillanaukee P. Serum and saliva levels of sialic acid are elevated in alcoholics. Alcohol Clin Exp Res. 1999; 23: 1060-1064.
[13] Romppanen J, Punnonen K, Petra A, Jakobsson T, Blake J, Niemela O. Serum sialic acid as a marker of alcohol consumption: Effect of liver disease and heavy drinking Alcohol Clin Exp Res. 2002; 26: 1234-1238.
[14] Ponnio M, Sillanaukee P, Franck J. Serum sialic acid levels are increased during relapse to alcohol drinking: A pilot study. Alcohol Clin Exp Res. 2002; 26: 1365-1367.
[15] Fox IH, Kelley WN. The role of adenosine deaminase and 2'- deoxyadenosine in mammalian cells. Ann Rev Biochem.1978; 47: 655-686.
[16] Galanti B, Naddiello S, Russo M, Fiorentino F. Increased lymphocyte adenosine deaminase in typhoid fever. Scand J Infect Dis.1981; 13: 47-50.
[17] Sullivan JL, Osborne WR, Wedgwood RJ. Adenosine deaminase activity in lymphocytes. Br JHaematol.1977; 122: 216-220.
[18] Piras MA, Gakis C, Budroni M, Andreoni G. Immunological studies in Mediterranean spotted fever. Lancet.1982; 8283: 1249.
[19] Giusti G, Galanti B. Colorimetric Method. Adenosine deaminase In: Bergmeyer HU, Methods of enzymatic Analysis, Weinheim: Verlag chemie, 1984; 3:315-23.
[20] Aminoff D. Methods for the quantitative estimation of neuraminic acid and their application to hydrolysates of sialomucoids. Biochem J 1961; 81:384-392
[21] İdiz N,GüvendikG, Boşgelmez İİ, Söylemezoğlu T, Doğan YB, İlhan İ. Serum sialic acid and γ-glutamyltransferase levels in alcohol-dependent individuals. Forensic Sci Int.2004; S67-S70.
[22] Martinez J, Palascak JE, Kwasniak D. Abnormal sialic acid content of dysfibrinogenemia associated with liver disease. The Journal of clinical Investigation. 1978; 61: 535-38.
[23] Carlson J et al. α-1 antitrypsin and other acute phase reactants in liver disease. Acta MedScand. 1980; 207-79.
[24] Cronstein BN. Adenosine, an endogenous anti-inflammatory agent. J Appl Physiol.1994; 76: 5-13.
[25] Adanin S, Yalovetskiy IV, Nardulli BA, Sam AD 2nd, Jonjev ZS, Law WR. Inhibiting adenosine deaminase modulates the systemic inflammatory response syndrome in endotoxemia and sepsis. Am J Physiol Regul Integr Comp Physiol.2002; 282: R1324-1332.