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ASSESSMENT OF INSULIN RESISTANCE AND LIPID BOUND SIALIC ACID IN ALCOHOLIC LIVER DISEASE

C.Selvakumar*[1] and M.G. Sridhar[2]

*[1]Assistant Professor, Department of Biochemistry, Meenakshi Medical College and Research Institute, Enathur, Kanchipuram, Tamilnadu-631512 ,India.

[2]Professor, Department of biochemistry, Jawaharlal Nehru Institute of Postgraduate Medical Education and Research, Puducherry- 605 006.

Email:drselvakumarc@yahoo.com

ABSTRACT

Background: Even though alcoholic liver disease is common disease in India, there are only limited reports about insulin resistance and no reports about lipid bound sialic acid in these cases. The present study was designed to evaluate insulin resistance and lipid bound sialic acid levels in patients with alcoholic liver disease

Materials and methods: 50 alcohol liver disease cases groups [Fatty liver (n=18), alcoholic hepatitis (n=19) and cirrhosis (n=13)] and 38 controls were enrolled in the study. Fasting insulin, lipid bound sialic acid and liver function test parameters were analyzed in both the groups

Results: Insulin, homeostasis model of insulin resistance and lipid bound sialic acid were significantly increased in alcohol liver disease cases compared to controls. Both insulin and insulin resistance were higher in alcoholic hepatitis and cirrhosis group compared to fatty liver group and controls

Conclusion: To conclude, the present study demonstrates increased insulin resistance and lipid bound sialic acid in patients with ALD

Key words: Alcohol liver disease, insulin resistance, lipid bound sialic acid

INTRODUCTION

Alcoholic liver disease (ALD) is a major health and economic problem in the world. Recent data indicate that the 5yr survival rate for alcoholic cirrhosis is between 23% and 50% [1]. It has been reported that in India the prevalence rate is higher owing to consumption of illicit liquor. Narawane NM et al have reported that liver disease is more common in subjects with the habits of daily drinking, volume of consumption > 200 ml per day, and duration of drinking > 14 years [2].

Insulin resistance is the hall mark of various pathological conditions including liver disease. It has been reported that chronic alcoholics have endogenous insulin resistance and associated damage to exocrine pancreas and decomposition of beta cell function [3]. Experimental studies with rats have established insulin resistance in alcohol liver disease [4]. However the relationship between alcohol consumption and insulin resistance [5] is still controversial.

Sialic acid (SA) is a derivative of neuraminic acid attached to the carbohydrate chains of glycoproteins and glycolipids. The terminal position in carbohydrate chains of proteins and lipids constituents of plasma lipoproteinsis occupied by sialic acid[6] .This location predisposes its to the participation in cellular andmolecular interactions and this way sialic acid plays a significant role in lipoproteins and lipids metabolism[7,8]. Glycosylation and sialylation of lipids and proteins takes place in the liver. There are evidences that the changes in glycosylation or sialylation of proteins and lipids have an important role inthe pathogenesis and progression of various liver disease [9,10].

The group of sialic acid-containing constituents of lipoproteins are glycolipids[11] .The most characteristic of their representative in the blood are gangliosides[12]. In liver diseases of different etiologies, the concentration, pattern and distribution of gangliosides in lipoproteins are altered [13]. The content of sialic acid associated with lipid part of lipoproteins can be determined as a lipid-bound sialic acid (LSA) [14]. Previous studies have reported increased total and free sialic acid levels in patients with Alcoholic liver disease [15]. Recent studies by Cylwik B have shown that sialic acid levels can be used to differentiate alcoholic cirrhosis from patients with non alcoholic liver disease [16]. However to the best of our knowledge, the level of lipid bound sialic acid levels in ALD have not been reported till date.

The present study was designed to evaluate insulin resistance and lipid bound sialic acid levels in patients with Alcoholic liver disease

MATERIALS AND METHODS

The present study was conducted in dept. of Biochemistry and Medicine, JIPMER. 50 ALD cases and 38 controls were enrolled in the study. Further the alcoholic liver disease patients were divided in to three groups [Fatty liver (n=18), alcoholic hepatitis (n=19) and cirrhosis (n=13)] based on the Ultrasonagram and clinical presentation with laboratory parameters. .Patients clinically diagnosed with viral hepatitis, sclerosing cholangitis, autoimmune hepatitis, other systemic disorders and those who are on any kind of medications were excluded from the study. Written consent was obtained from all the subjects. This study was approved by Institute Human Ethical Committee.

5 ml of fasting venous blood samples were collected and centrifuged at 2500g for 5 min. Fasting glucose and liver function test parameters were estimated immediately and rest of the sample was stored at -20^{0}C for the analysis of insulin and lipid bound sialic acid.

Fasting plasma insulin was estimated using human ELISA kit (United biotech Inc, USA) adapted to ELISA reader. Fasting plasma glucose, serum proteins and liver function test (Serum bilirubin, ALT, AST, GGT, Total protein) parameters were estimated using the reagent kits from Agappe Diagnostics (Kerala, India) adapted to 550 Express plus Batch Auto Analyser (CIBA CORNING, BAYER DIAGNOSTICS). Serum Lipid bound sialic acid concentration was measured according to the method described by Katopodis and Stock [14]. Homeostatic model of insulin resistance (HOMO-IR) was calculated using the formula

HOMO-IR=fasting insulin (μU/ml) ×fasting glucose (mM/L) /22.5 [17]

Statiscal analysis

Statistical analysis was done using SPSS.13 package. Results were expressed as MEAN±S.D. Insulin, Glucose, Sialic acid and 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, bilirubin and PT were significantly higher in ALD patients as compared to controls. Whereas, total protein and albumin was lower in ALD patients than controls.

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

VARIABLE	CONTROL (n=38)	ALD PATIENTS (n=50)
AGE	42.5±6.54	44.92±6.5
BMI	20.97±1.97	20.2±2.1
TOTAL PROTEIN g/dl	6.12±1.0	5.18±0.77*
ALUBIMIN g/dl	4.44±0.8	2.7±0.6*
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**
BILIRUBIN mg/dl	0.59±0.142	5.93±3.5**
PT (seconds)	12.47±2.02	18.96±7.7*

*P<0.05 and ** p < 0.01 compared to controls

Table 2 shows mean and S.D of insulin, HOMA IR and lipid bound sialic acid in controls and ALD cases. Insulin, HOMA IR and lipid bound sialic acid were significantly higher in ALD patients as compared to controls.

Table 2: Mean and S.D of insulin, HOMA IR and lipid bound sialic acid in controls and ALD cases

VARIABLE	CONTROL (n=38)	ALD PATIENTS (n=50)
FASTING INSULIN µU/ml	18.33±6.5	40.39±22.8*
FASTING GLUCOSE mg/dl	5.08±0.6	5.15±0.73
HOMA IR	4.17±1.7	9.25±5.2*
LIPID BOUND SIALIC ACID mg/dl	23.1±16.3	29.6±15.5*

* p < 0.05 compared to controls

Table 3 shows mean and S.D of insulin, HOMA IR and lipid bound sialic acid in controls and different stages of ALD cases. Insulin, HOMA-IR and lipid bound sialic acid were significantly increased in Alcoholic hepatitis and alcoholic cirrhosis cases compared to controls. Also insulin and HOMA-IR were significantly increased in alcoholic hepatitis cases compared to fatty liver patients.

Table 3: Mean and S.D of insulin, HOMA IR and lipid bound sialic acid in controls and different stages of ALD cases

VARIABLE	CONTROL n=38	FATTY LIVER n=18	ALCOHOLIC HEPATITIS n=19	CIRRHOSIS n=13
AGE	42.5±6.54	43.8±6.43	44.3±6.95	45.1±6.89
BMI	20.9±1.97	20.6±2.70	19.83±1.25	20.2±2.27
FASTING INSULIN µU/ml	18.3±6.50	29.2±8.15	49.8±27.04* [a]	42.4±25.33*
FASTING GLUCOSE mg/dl	91.6±12.40	96.4±12.8	99.2±11.98	88.0±12.12
HOMA IR	3.4±1.27	6.97±2.08*	11.7±6.28* [a]	9.2±5.42*
LIPID BOUND SIALIC ACID mg/dl	23.1±16.3	26.5±15.2*	27.4±15.2*	28.6±16.7*
TOTAL PROTEIN g/dl	6.12±1.02	5.29±0.87*	5.17±0.87*	5.02±0.61*

[a] p<0.01 compared to fatty liver and *p<0.01 compared to controls

ISCUSSION

Chronic ethanol consumption disrupts glucose homeostasis and is associated with the development of insulin resistance. Previous studies have reported the presence of insulin resistance in ALD [18]. In the present study, we found a significant increase in fasting insulin and HOMA – IR in subject with ALD compared to control. Among ALD group, both insulin and HOMA-IR were found to be increased in hepatitis group compared to fatty liver and cirrhosis group. Although the mechanism of insulin resistance in ALD is unclear, experimental studies have demonstrated that ethanol exposure causes insulin resistance by altered expression of IGF, oxidative stress and inflammation which may reduce binding of insulin with its receptor [19].

Most recently the variations of serum total sialic acid (TSA) level in liver cirrhosis, fatty liver, liver cancer and acute and chronic hepatitis have been described [20]. Because the synthesis (sialylation too), degradation (also desialylation) and storage the lipids and lipoproteins are attributed to the liver, we can suspect that the liver diseases affect not only the serum level of lipids and lipoproteins, but also the level of sialic acid bounded with these compounds, that means LSA.

Even though various studies have reported increased total and free sialic acid levels in ALD patients [21], there are no reports regarding lipid bound sialic acid in such cases. In the present study we found a significant increase in lipid bound sialic acid in subjects with ALD compared to control subjects. Though we did not find a significant difference in lipid bound sialic acid levels between different clinical groups of ALD, subjects with fatty liver were found to have increased lipid bound sialic acid levels when compared with hepatitis and cirrhosis.

To conclude, the present study demonstrates increased insulin resistance and lipid bound sialic acid in patients with ALD. The main limitation of the study was small sample size because of which we didn't get any significant association between insulin and lipid bound sialic acid. Further studies are warranted to explore the potential role of alcohol included inflammation in the pathogenesis of insulin resistance in ALD.

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