Is Serum Sialic Acid a Marker of Alcohol Abuse?

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ABSTRACT

A comparative study of serum sialic acid concentration and traditional biochemical markers such as serum gamma glutamyl transferase (GGT), aspartate amino transferase (AST), alanine amino transferase (ALT), and AST/ALT ratio, as well as prognostic markers such as serum total protein and serum albumin in alcohol abuse was done. A total of 70 male subjects aged between 20 and 60 years, with 40 cases diagnosed as alcoholics, and 30 healthy controls were included in the study.

Serum GGT, AST, ALT, total protein and serum albumin were estimated using auto analyzer Dade Behring clinical chemistry system. Serum sialic acid was estimated manually by modified Warren's colourimetric method.

An increased concentration of serum sialic acid and other traditional biochemical markers GGT, AST, ALT was observed in cases compared to that of controls. Overall, GGT demonstrated good sensitivity and specificity. The other traditional markers used in alcohol abuse varied considerably in their specificities and sensitivities. The AST/ALT ratio was increased in cases compared to controls, but not high enough to confirm alcoholic liver disease. The albumin concentration in cases was decreased when compared to controls, while total protein concentration did not show any statistical significance in both the groups indicating that it has negligible effect.

In this study, sialic acid proved to be a reliable test with sensitivity of 70% and specificity of 90%, with a diagnostic accuracy of 80%, showing that it can be used as a biochemical marker in alcohol abuse, where secondary effects of liver disease hamper the use of traditional markers.

Key Words: Sialic acid; Alcohol; Gamma glutamyl transferase (GGT); Aspartate amino transferase (AST); Alanine amino transferase (ALT)

Introduction

Alcohol is among the commonest drugs of abuse worldwide, and unfortunately causes considerable morbidity, mortality and social disruption. It is said to cause 4% of total disability adjusted life years (DALYs), and alcohol use disorders account for 1.4% of total burden of disease.[1] In Southern India, the prevalence of alcohol use varies between 33% and 50%, with higher prevalence among lesser-educated and lower socio-economic groups.[2] The all-India prevalence of alcohol intake is reported to be 5-20%.[3] Thus alcohol abuse plays a major role in public, family and health-related problems, with impairment of social, legal, interpersonal, and occupational functioning.

Biochemical markers play an important role in assessing liver function to identify alcohol abuse. Gamma glutamyl transferase (GGT), even though considered as a sensitive marker for alcohol abuse, actually reflects alcohol-related liver disease.[4] In recent times, sialic acid has been suggested as a marker of excessive alcohol consumption, with diagnostic efficiency as good as other common markers.[5] It is being increasingly touted as a valuable marker for detecting and monitoring alcohol abuse.[6] This

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study was therefore undertaken to find out whether sialic acid is more sensitive and specific than GGT and other traditional markers that are commonly used in alcohol abuse.

Materials and Methods

This study was conducted at St John's Medical College, Bangalore, over a period of one year from May 2006 to April 2007. The study was approved by the ethics committee of the institute, and all subjects signed an informed consent form indicating their willingness to take part in the study. Seventy male subjects in the age group of 20-60 yrs were taken for the study. The sample size comprised of 40 cases (group I) and 30 controls (group II).

Group I comprised of patients diagnosed to have alcohol dependency, who consumed more than 375 ml of whisky and/or rum (alcohol concentration of 40% to 50% v/v) daily, or intermittently with abstinence of two to three days for more than five years. Controls comprised subjects with absolutely no history of alcoholism or any other medical illness. Clinical history was obtained from both cases and controls.

Four ml of random blood from each of the mentioned subjects was collected from median cubital vein. Serum was separated by centrifugation at 3000 rpm for 3 minutes. It was aliquoted and kept at -20°C until analysis was performed. Biochemical analysis was carried out on the serum samples. Subjects with history of diabetes mellitus, cardiac disease, hepatitis, carcinomas, meningitis and current use of hepatotoxic drugs were excluded from the study. The samples were analysed by automated method for total protein, albumin, AST, ALT and GGT using Dade Behring max analyzer. Serum sialic acid was determined manually by modified Warren's calorimetric method.

Results and Discussion

Among the 40 cases, 70% demonstrated sialic acid level of more than 2.63 mmol/L, when compared to controls, among whom only 6.7% demonstrated such levels, the difference in proportion being statistically significant with OR (Odd Ratio) 32.67. This indicates that elevated sialic acid (>2.63 mmol/L) is 32.67 times more likely to be in cases as compared to controls. There were 87.5% cases who had GGT >50 U/L, while this was true only of 16.7% controls, the difference in proportion being statistically significant with OR 31.00, indicating that elevated GGT >50 U/L is associated with 31.00 times more likelihood in cases when compared to controls. There were 77.5% cases who had AST >37 U/L, while this was true only of 6.7% controls, the difference in proportion being statistically significant with OR 48.2, indicating that elevated

Table 1 Comparison of Study Parameters Between Control and Study Groups [Results are presented in Mean ± SD (Min-Max)]

Study Parameter	Case (Group I)	Control (Group II)	p value	Effect Size
Total protein (gm/dl)	7.74±0.75 (6.60-9.50)	7.72±0.38 (7.00-8.50)	0.907	0.03 (N)
Albumin (gm/dl)	3.84±0.32 (3.10-4.30)	4.28±0.22 (3.70-4.70)	<0.001	1.54 (VL)
AST (U/L)	93.38±76.74 (5.0-366.0)	24.75±7.59 (13.0-47.0)	<0.001	1.16 (L)
ALT (U/L)	93.68±70.71 (14.0-299.0)	49.77±23.20 (18.0-120.0)	0.002	0.75 (M)
GGT (U/L)	288.80±406.55 (15.0-2127.0)	39.67±20.24 (18.0-105.0)	0.001	0.80 (L)
Sialic acid (mmol/L)	2.95±0.64 (2.0-4.50)	1.89±0.46 (0.28-2.70)	<0.001	0.84 (L)
AST/ALT	1.13±0.92 (0.08-5.64)	0.56±0.23 (0.29-1.33)	0.002	0.79 (L)

N: Negligible; M: Moderate; L: Large; VL: Very Large

AST >37 U/L is 48.2 times more likely to be in cases when compared to controls. There were 52.5% cases who had ALT >65U/L, while this was true only of 20.0% controls, the difference in proportion being statistically significant with OR 4.42, indicating that elevated ALT >65 U/L is 4.42 times more likely to be in cases when compared to controls. There were 42.5% cases who had AST/ ALT >1, while this was true only of 6.7% controls, the difference in proportion being statistically significant with OR 10.34, indicating elevated AST/ALT is 10.34 times more likely to be in cases when compared to controls. There were 17.5% cases who had albumin levels < 3.5g/dl, which was lower than the normal levels.

Sialic acid (SA) is an N-O-acyl derivative of neuraminic acid which is a nine-carbon sugar, an epimer of glucosamine with a pyruvate attached by an alpha-glycosidic linkage to the non-reducing residues of carbohydrate chains of glycoprotein and glycolipids. The mean sialic acid concentration in alcohol-dependent subjects was higher when compared to controls, and the difference in levels between controls and cases was statistically significant ($p<0.001$). The diagnostic accuracy of Serum SA in our study was similar to that of the study by Jarkko R et al.[5] However the sensitivities of SA by Jarkko R et al was found to be much higher when compared to that of specificity. The difference in sensitivities between our study and their study could be because of the methodology that was used. High Performance Liquid Chromatography (HPLC) system with pulsed amperometric detection (HPAE-PAED) was used in the former, but in our study, a modified Warren's manual colourimetric estimation of serum SA (which calculates SA levels against the standards) was used.

Our study on SA showed a high specificity, which was in accordance with Sillanuakee P et al.[7] The increase in serum sialic acid concentration in alcohol abusers in our present study is in accordance with the studies conducted by other investigators also.[8,9] The increase in sialic acid concentration in alcohol abusers is because excessive alcohol intake has been found to affect the glycosylated proteins such as transferrin. Chronic alcoholism causes desialylation of transferrin and other glycoproteins. Ethanol decreases the activities of sialyl transferase in Golgi apparatus, and increases the activities of sialidase in cytosol and plasma membrane.[5] Ethanol-induced effect on glycoproteins is not due to a simple mechanism, but rather a multistep process.

In conclusion, our study suggests that sialic acid levels rise in alcohol abusers when compared to controls. Traditional markers differed from each other with respect to sensitivity and specificity. The albumin concentration in cases was decreased when compared to controls, while total protein concentrations did not show any statistical significance in both the groups, indicating it had a negligible effect. GGT, AST, ALT and AST/ALT ratio increase when there is hepatocellular damage. AST/ALT ratio also helps in identifying whether an alcoholic has progressed to a stage of active liver disease. In our study however, while it was observed that AST/ALT ratio was increased in cases as compared to controls, none of them had active liver disease. Overall, GGT demonstrated good

Table 2 Comparison of Elevated Study Parameters Between Controls and Cases

Study Parameter	Case (Group I)(n=40)	Control (GroupII)(n=30)	p value	OR (95% CI)
AST (>37 U/L)	31 (77.5%)	2 (6.7%)	<0.001	48.20 (9.58-242.48)
ALT (>65 U/L)	21 (52.5%)	6 (20.0%)	0.006	4.42 (1.49-13.13)
GGT (>50 U/L)	35 (87.5%)	5 (16.7%)	<0.001	31.00 (9.15-133.88)
Sialic acid (>2.63 mmol/L)	28 (70.0%)	2 (6.7%)	<0.001	32.67 (6.69-159.54)
Albumin (<3.5 gm/dl)	7 (17.5%)	—	0.017	—
AST/ALT (>1.0)	17 (42.5%))	2 (6.7%)	0.001	10.34 (2.16-49.52)

sensitivity and specificity. The specificity of SA was almost similar to that of AST. But both of them lacked in being highly sensitive. SA may be useful in assessing drinking problems in which secondary effects of liver disease hamper the use of traditional markers.

Further studies are needed on larger samples including people with alcoholic and non-alcoholic liver disease, so as to more precisely assess the diagnostic accuracy of serum sialic acid in alcoholism.

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