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Serum Mitochondrial Aspartate Transaminase Activity in Alcoholic Liver Disease

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Conflict of interest: Nil

Abstract:

Background: Chronic alcohol consumption affect most of the organs in the body, dose related damage to liver cause alcoholic liver disease. Alcoholic liver disease is a major cause of alcohol related morbidity and mortality .The general marker of alcoholic liver disease used in practice are Aspartate transaminase(AST), Alanine transaminase(ALT), Gamma glutamyltransferase (GGT), some newer markers are carbohydrate deficient transferrin, Mitochondrial AST (m- AST), Glutathione-s-transferase. Mitochondrial AST (m- AST) is an isoenzyme of AST found in mitochondria. In excess alcohol drinking there is an increased expression of this enzyme in the plasma membrane of hepatocytes; following cell necrosis its serum level is elevated. So m-AST in combination with other markers can be used to detect alcoholic liver disease.

Objectives: The objective is to study the activity of serum Mitochondrial Aspartate Transaminase (m-AST) in alcoholic liver disease and compare the results with that of healthy individuals and to calculate m-AST/ total AST ratio. Then find its use as a marker for alcoholic liver disease.

Materials and Methods: This case-control study conducted in our tertiary health care center included 40 healthy volunteers who served as control and 40 cases of alcoholic hepatitis (group I) and 40 cases of alcoholic cirrhosis (group II). The blood samples were analyzed for Serum Mitochondrial Aspartate Transaminase (m-AST), Serum GGT, Serum AST, Serum ALT, Serum Total Bilirubin, Serum Albumin and m-AST/ total AST ratio is calculated.

Results: In this study serum m-AST levels were significantly elevated in group I (19.05±1.60) with p< 0.0001 and group II (13.18±1.17) with p< 0.0001,with respect to controls (2.06±0.13). m-AST/t-AST ratio is increased in group I (18.35±0.52) and group II (18.93±0.53) in comparison to controls(8.28±0.8).The elevated serum m-AST levels seen in group I and group II shows positive correlation with serum GGT values within the group. The Pearson's correlation value obtained in group I (r +0.759) and group II (r+0.8480). Serum GGT is a known marker of alcoholism.

Conclusion: The study shows that m-AST and m-AST/t-AST ratio is elevated in alcoholic liver disease and it correlates with serum GGT a marker of alcoholism. Hence Mitochondrial AST can be used as a marker for chronic alcoholism in combination with other markers.

Keywords: Mitochondrial AST, Aspartate Transaminase, Alcoholic Liver Disease, Gamma glutamyl transferase, Alcohol, Cirrhosis.

Introduction

Chronic alcohol consumption affect most of the organs in the body, dose related damage to liver cause alcoholic liver disease. There is an increased risk of developing alcoholic liver disease when a person drinks alcohol for more than 30-50 g/day and the duration is more than 5-10 years Alcoholic liver disease is a major cause of alcohol related morbidity and mortality. The general marker of alcoholism and alcoholic liver disease used in practice are Aspartate transaminase(AST), Alanine transaminase(ALT), Gamma glutamyltransferase (GGT), Glutamate dehydrogenase (GDH), Mean corpuscular volume(MCV), Serum Bilirubin, Prothrombin time and Albumin, some newer markers introduced are carbohydrate deficient transferrin, Mitochondrial AST (m- AST),

Glutathione-s-transferase. Mitochondrial AST (m-AST) which is measured in this study is an isoenzyme of AST found in mitochondria. In excess alcohol drinking there is an increased expression of this enzyme in the plasma membrane of hepatocytes; following cell necrosis its serum level is elevated. So m-AST in combination with other markers can be used to detect alcoholic liver disease [3].

The aim is to study the activity of serum Mitochondrial Aspartate Transaminase (m-AST) in alcoholic liver disease and compare the results with that of healthy individuals and to calculate m-AST/ total AST ratio. Then find its use as a marker for alcoholic liver disease.

Materials and Methods

Age and sex matched case-control study conducted in our tertiary health care center after getting prior approval from the institutional ethical committee. The study included 40 healthy volunteers who served as control and 40 cases of alcoholic hepatitis (group I) and 40 cases of alcoholic cirrhosis (group II). The cases were recruited from Medical Gastroenterology department.

Inclusion criteria

Patients who are chronic alcoholic and the alcoholic liver disease pathology is confirmed by Ultra sonogram.

Exclusion criteria

1. Non- alcoholic liver disease
2. Patients on enzyme inducing medications like anticonvulsants.
3. Patients with positive serology for Hepatitis B and C.
4. Patients with other coexisting medical or surgical illness like CAD, stroke, diabetes etc.

Sample collection

Random venous blood sample of 5 ml was collected. The serum was separated and analysed for Serum Mitochondrial Aspartate Transaminase (m-AST), Serum gamma glutamyl transferase, Bilirubin, Aspartate transaminase, Alanine transaminase and Albumin. and m-AST/ total AST ratio is calculated.

Measurement of mitochondrial Aspartate Transaminase is done by Differential Kinetic Assay method, by Tom R.C. Boyde based on the principle that Cytoplasmic (c-AST) and Mitochondrial (m-AST) isoenzyme of Aspartate transaminase differ in their kinetic property, so that c-AST is inhibited by adipate and 2-oxoglutarate (substrate) at low pH of 6.0.

As the serum contain both the isoenzyme, two separate measurement is made using two reagents with pH 6.0 (contains adipate) and pH 7.4 (without adipate)

Reaction at pH 6.0 in presence of adipic acid

$$\text{2 Oxoglutarate + L Aspartate} \xrightarrow{\text{m - AST}} \text{L-Glutamate + Oxaloacetate}$$

$$\text{Oxaloacetate + NADH + H}^{+} \xrightarrow{\text{MDH}} \text{L-Malate + NAD}^{+}$$

Reaction at pH 7.4

$$\text{2 Oxoglutarate + L Aspartate} \xrightarrow{\text{c - AST}} \text{L-Glutamate + Oxaloacetate}$$

$$\text{Oxaloacetate + NADH + H}^{+} \xrightarrow{\text{MDH}} \text{L-Malate + NAD}^{+}$$

The rate of change in absorbance of NADH is measured at 340 nm at 37°c. Calculation: For 1cm path length, c- AST activity (U/L) = 2270 X (0.9 V1 - V2), m- AST activity (U/L) = 2270 X (V2 - 0.05 V1), V1- rate of change of absorbance at pH 7.4,V2- rate of change of absorbance at pH 6.

The activity of each isoenzyme is individually measured in two conditions by varying one component assay mixture at time and expressed as a Quotient which gives the activity at pH 6.0 divided by activity at pH 7.4, M for m-AST the value is 0.9 and C for c-AST the value is 0.05, M and C are dimensionless ratio. Upper reference limit of m-AST is 3.2 U/L

Estimation of gamma glutamyl transferase was done by IFCC kinetic method, Total Bilirubin was done by Diazo Method, Aspartate transaminase and Alanine transaminase by Modified IFCC method and serum albumin by Bromocresol green dye binding method.

Results and Statistical Analysis

This study was conducted in a total population of 120 subjects. Out of 120, 40 were controls and 80 were alcoholic liver disease patients divided into two groups, alcoholic hepatitis in group I and alcoholic cirrhosis in group II. Statistical analysis is done using graph pad prism 6 software.

The distribution of age among the controls and group I in Table No.1 and The distribution of age among the controls and group II in Table No.2 found to be statistically not significant.

Mean and standard deviation were estimated for variables in controls, group I and group II and shown in Table No.3 Independent Student‘t’ test was employed to find out ‘p’ value between controls and group I, controls and group II, group I and group II, were shown in Table No.4. Pearson’s correlation analysis was done to assure the relationship of m-AST and GGT shown in Table No.5 and Table No.6, between m-AST and AST,ALT, Total bilirubin, albumin shown in Table No.7 and Table No.8

Table 1: Age Distribution among the Controls and Group I

Groups	No	Mean Age (years)	Standard Deviation	Student t-test
Control	40	43.78	1.816	P= 0.420
Group I	40	41.88	1.486	Not significant

Table 2: Age Distribution among the Controls and Group II

Groups	No	Mean Age (years)	Standard Deviation	Student t-test
Control	40	43.78	1.816	P= 0.744
Group II	40	44.53	1.403	Not significant

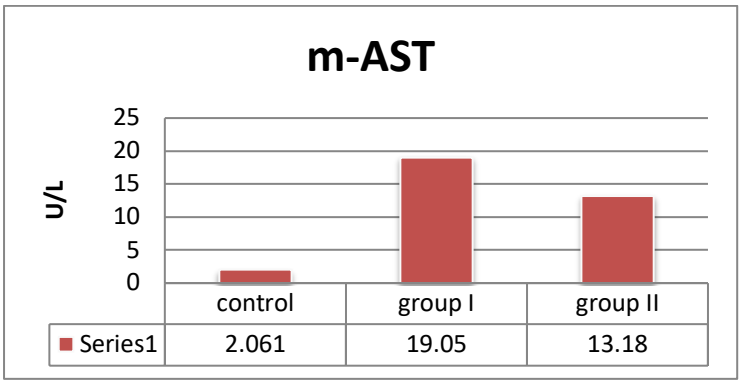

Figure 1:

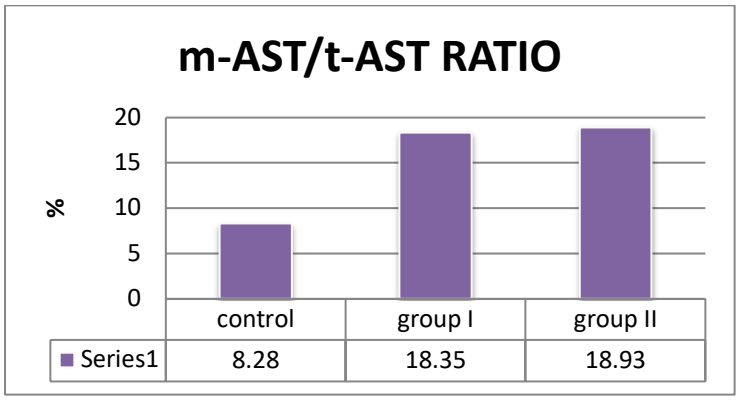

Figure 2:

Table 3: Mean and Standard Deviation of Variables in Groups

S.No	Variables	Control	group I	group II
		mean±S.D	Mean±S.D	Mean±S.D
1	Serum m- AST (U/L)	2.06±0.13	19.05±1.60	13.18±1.17
2	m-AST/t-AST %	8.28±0.8	18.35±0.52	18.93±0.53
3	Serum AST (U/L)	21.60±0.70	106±9.83	69.35±6.74
4	Serum ALT (U/L)	13.20±0.79	40.58±3.04	20.70±2.15
5	Serum GGT (U/L)	17.88±0.84	231.50±38.63	91.73±15.76
6	Serum Total Bilirubin (mg/dl)	0.56±0.03	6.91±1.21	6.96±1.07
7	Serum albumin(g/dl)	5.50±0.08	3.17±0.16	2.55±0.11

Table 4: Comparison between Groups – Student 't'test

S.No	Variables	p-value		
		Control Vs Group I	Control Vs Group II	Group IVs Group II
1	Serum m- AST (U/L)	< 0.0001 Significant	< 0.0001 Significant	0.0043 Significant
2	m-AST/t-AST %	< 0.0001 Significant	< 0.0001 Significant	0.4400 Not significant
3	Serum AST (U/L)	< 0.0001 Significant	< 0.0001 Significant	0.0027 Significant
4	Serum ALT (U/L)	< 0.0001	0.0016	< 0.0001

		Significant	Significant	Significant
5	Serum GGT (U/L)	< 0.0001 Significant	< 0.0001 Significant	0.0013 Significant
6	Serum Total Bilirubin (mg/dl)	< 0.0001 Significant	< 0.0001 Significant	0.9754 Not significant
7	Serum albumin (g/dl)	< 0.0001 Significant	< 0.0001 Significant	0.0021 Significant

P<0.05=significant

Table 5: Pearson's Correlation Between m-AST & GGT in Group I

Variables	Pearson's correlation coefficient (r)	Significance (p)	Interpretation
m-AST Vs GGT	+0.757	<0.0001	Significant and Positive correlation

Correlation (r) always lies between -1 to +1

Table 6: Pearson's Correlation Between m-AST & GGT in Group II

Variables	Pearson's correlation Coefficient (r)	Significance (p)	Interpretation
m-AST Vs GGT	+0.848	< 0.0001	Significant and Positive correlation

Correlation (r) always lies between -1 to +1

Scatter Diagram 1

Scatter Diagram 2

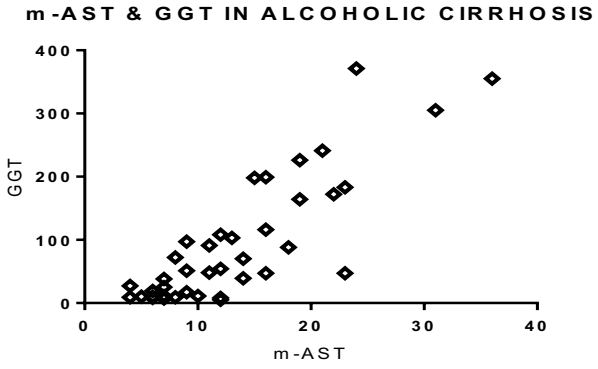

Table 7: Pearson's Correlation Between m-AST & Variables In Group I

S.No	Variables	Pearson's correlation coefficient (r)	Significance (p)	Interpretation
1	m-AST Vs AST	+0.940	< 0.0001	Significant and Positive correlation
2	m-AST Vs ALT	+0.770	< 0.0001	Significant and Positive correlation
3	m-AST Vs Total Bilirubin	+0.279	0.081	Not Significant
4	m-AST Vs Albumin	-0.159	0.324	Not Significant

Correlation (r) always lies between -1 to +1

Table 8: Pearson's Correlation Between m-AST & VARIABLES in Group II

S.No	Variables	Pearson's correlation coefficient (r)	Significance (p)	Interpretation
1	m-AST Vs AST	+0.952	< 0.0001	Significant and Positive correlation
2	m-AST Vs ALT	+0.679	< 0.0001	Significant and Positive correlation
3	m-AST Vs Total Bilirubin	+0.609	< 0.0001	Significant and Positive correlation
4	m-AST Vs Albumin	+0.097	0.550	Not Significant

Correlation (r) always lies between -1 to +1

Discussion

In this study serum m-AST levels were significantly elevated in group I ($p< 0.0001$) and group II ($p< 0.0001$) with respect to controls. m-AST/t-AST ratio is increased in group I ($p< 0.0001$) and group II($p< 0.0001$) in comparison to controls. Between group I and group II there is no significant difference in m-AST/t-ASTratio (p 0.4400), this shows m-AST/t-AST ratio is increased in chronic alcoholics irrespective of their liver disease stage.

The serum m-AST elevated levels seen in group I and group II shows positive correlation with serum GGT values within the group. The Pearson's correlation value obtained in group I(r +0.759) and group II(r+0.8480). Serum GGT is a known marker of alcoholism in use with detection limit of 34-85%.

The mean value of m-AST in controls (2.06 ±0.13) group I (19.05±1.60) group II (13.18±1.17) with the range of 1-4 U/L in controls and 4-41U/L in alcoholic liver disease patients. The range obtained by lanKwoh-GaI et al is 2.10-37.49 U/L in alcoholic subjects.

The mean value of m-AST/t-ASTratio in controls (8.28±0.81) group I (18.35±0.52) group II (18.93±0.53). The median value in alcoholic liver disease patients obtained in this study was 18.85% and in controls was 7.3%.The median obtained by lan Kwoh-GaI et al is 11.52% in alcoholic subjects.

The differential kinetic method used in this study to measure mitochondrial AST is cheap to perform in comparison with the immunochemical methods, further study in this methodology can be done by replacing the phosphate buffer used in the method with organic buffers. The further study which can be done are, the changes in the serum m-AST activity and m-AST/t-ASTratio values with respect to abstinence from alcohol intake for varying duration of time in alcoholic liver disease patients and re drinking after abstinence. Serum m-AST and m-AST/t-ASTratio can be compared with newer markers of alcoholism like carbohydrate deficient transferrin (CDT) and alpha glutathione -s-transferase.

Conclusion

The study shows that m-AST and m-AST/t-AST ratio is elevated in alcoholic liver disease and it correlates with serum GGT a marker of alcoholism. Hence Mitochondrial AST can be used as a marker for chronic alcoholism in combination with other markers**.**

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