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Assessment of Serum Enzymes Gamma Glutamyl Trasnferase, Aspartate Transaminase and Alanine Transaminase in Liver Diseases

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Abstract
Back Ground: Liver diseases are common disorders encountered in clinical practices. Investigations in liver diseases are used to detect hepatic abnormality, to measure its severity, to define it structural effect on the liver, to look for specific causes of liver diseases, assess prognosis and to evaluate therapy.
Aim and Objectives: To study and compare the altered serum levels of enzymes GGT, AST and ALT in various liver diseases and also to assess their usefulness in differential diagnosis of liver diseases.
Methods: The patients admitted to Chigateri General Hospital and Bapuji Hospital Davangere were selected for the present study. Total number of 124 subjects were chosen which include 50 controls and 74 cases of liver diseases. The liver diseases were further grouped into acute hepatitis, chronic hepatitis and cirrhosis of liver.
Abbreviation: GGT-Gamma glutamyl transferase, AST- Aspartate transaminase,ALT-Alanine transaminase.
Results: The mean levels of GGT was increased in acute liver hepatitis, chronic hepatitis and cirrhosis of liver (non alcoholic and alcoholic) with P value highly significant $p < 0.001$ as compared to control subjects. The mean levels of AST and ALT were increased in acute hepatitis, chronic hepatitis and cirrhosis of liver (non alcoholic and alcoholic) with p value is highly significant $p < 0.001$ as compared to control subjects. Statistical significance is observed in non alcoholics and alcoholic cirrhosis for AST but no statistical significance found for ALT in non alcoholic and alcoholic cirrhosis and duration wise comparison in alcoholic cirrhosis.
Conclusion: Liver function tests can be of value prognostically in screening for liver disease and are vital in monitoring of liver disease in response to treatment.
Key Words: GGT, AST, ALT, Hepatitis, Cirrhosis

1. INTRODUCTION

The evaluation of modern medicine is attributed to the development of various investigative procedures in order to attain accurate diagnosis. The liver is the most important complex organ concerned with various metabolic activities of human body. It is known for its multiplicity of function with great reserve capacity and regenerating ability.

It is important to recognize that no one liver function tests enable the clinician to assess accurately the total functional capacity of liver. In order to increase both the sensitivity and specificity of lab tests in the detection of liver disease, it is helpful to use them as a battery. Selection of appropriate tests depends on particular clinical problem and on an understanding of the use and limitation of application of each test.

Investigations of liver diseases are used to detect hepatic abnormality to measure its severity, to define its structural effect on the liver, to look for specific causes of liver diseases, to assess prognosis and to evaluate therapy.

Hans and co-workers in 1950 described GGT in sheep kidney later in 1960 works in Poland and US demonstrated the presence of GGT in normal human serum[11]. GGT is a glycoprotein involved in the transfer of gamma glutamyl residue from the gamma glutamyl peptides to amino acids and other small peptides. GGT has an important role in peptide and protein synthesis, regulation of tissue glutathione level and transport of amino acids across the cell membrane GGT is present in kidney, erythrocytes, small intestine, choroid plexus of brain, prostate, pancreas and liver. Normal value of GGT in serum being for male – 6-45IU/L and female 5-30 IU/L, GGT is synthesized as a single polypeptide comprising a hydrophobic leads peptide with a molecular weight 78,600 daltons GGT is one of the sensitive enzyme assay in hepato biliary disorders. It is also elevated in pancreatitis, (acute and chronic), alcoholism, Myocardial Infarction, congestive Cardiac Failure, Diabetes Mellitus, renal disorder, hypothyroidism, obesity, stroke, epilepsy and meningitis.

AST and ALT are aminotransferase that catalyze the inter-conversion of amino acids and alpha-oxo-acids by transfer of amino group. AST is present in the cytoplasm of hepatic cell as C-AST and that which is present in the mitochondria is known as m-AST. But ALT is located in hepatic cell cytoplasm.

AST and ALT are present in liver, heart, skeletal muscle, kidney have usually high activity. AST is a dimer of 2 identical subunits. M-AST is a dimer with a molecular mass of 90,400 dalton. AST and ALT peak activity occur at ages 30-40 for men and ages 50-60 for women. Obese men and women have higher value due to fatty liver. Half life of ALT in serum is 50 hours and AST is 12 hours.

The transaminase serve as markers of liver function tests. If the serum transaminase are abnormal, liver disease must be a part of differential diagnosis.

Both AST and ALT are useful in establishing the presence of liver cell injury of any cause. ALT is more sensitive test in acute and obstruction of liver disease whereas AST is more sensitive in chronic and infiltrative lesions. In acute hepatocellular injury, AST and ALT activity is 40 times the upper reference limit of normal. The serum amino transferase levels gradually rise for 1-2 weeks before the patient becomes jaundiced.

Elevation of aminotranserase in patients suffering from cirrhosis only occur if viral activity is persistant or active or with active alcohol abuse or with persistant chemical or drug hepatocellular injury.

Serum AST is the first enzyme to be elevated in myocardial infarction. Other conditions where AST is elevated are myocarditis, CSF skeletal muscle disorder etc.

2. MATERIALS AND METHODS

The present study was composed of 124 subjects. Out of which 50 were healthy controls, 21 were suffering from Acute Viral Hepatitis, 23 patients were suffering from Chronic Hepatitis and 30 patients with cirrhosis. The controls and diseased subjects were in the age group of 15-65 years.

The controls and diseased subjects were taken from the local population in and around Davangere. Informed consent was taken and approved by the ethical committee of J.J.M. Medical College. Patients and controls voluntarily participated for this study.

A total number of 50 control subjects who were healthy, non alcoholics, non smokers, no past history of liver disease, no history of consumption of any medications and no history of any heart, renal, pancreatic and neurological diseases or thyroid disorders or diabetes mellitus or obesity were excluded from our study.

The present study of estimation of GGT, AST, ALT in controls and subjects with Acute Viral Hepatitis, Chronic Hepatitis and Cirrhosis of liver was conducted on in patients admitted for treatment in Chigateri General Hospital and Bapuji Hospital, Davangere (which are teaching Hospitals for J.J.M. Medical College,Davangere).

A careful history and thorough clinical examination was done in all the study groups, according to the proforma prepared for the purpose of study. The cases were diagnosed by clinical signs and symptoms and confirmed by ultrasound and liver biopsy.

Following admission, 6ml of plain blood was drawn with aseptic precaution from large peripheral vein into a sterile bulb before treatment was started. Serum was separated by centrifugation and the test were carried out by standard biochemical procedures and chemicals and reagents used for the procedure were pure high grade analytical type. The laboratory parameters were recorded and subjected to standard statistical analysis. The observations were tabulated and conclusion obtained from the biochemical data.

Estimation of GGT/GAMMA activity was carried out by optimized Szasz method with the principle being.

L Gamma – Glutamyl 3 carboxy – 4 nitro anilide + glycylglycine

GGT

L Gamma glutamyl – glycylglycine + 5 amino – 2 – nitro benzole

Determination of SGOT/ AST and SGPT/ALT by Reitman and Frenkel method and the principle being that the pyruvate produced by transamination by GPT reacts with 2,4 dinitrophenyl hydrazine (DNPH) to give a brown coloured hydrazine. Which is measured in the calorimeter at 510nm. The oxalate formed in the reaction with GOT decarboxylates spontaneously to pyruvate which is again measured by hydrozone formation.

$$\text{AST} = \frac{\text{T - C}}{\text{S - B}} X\, 67 \mu\ mol$$

$$\text{ALT} = \frac{\text{T - C}}{\text{S - B}} X 133 \mu\ mol$$

This is converted into international units per litre by reference table

3. RESULTS

It is evident from the table 1 that there is increase in levels of GGT, AST, ALT in subjects with Acute Viral Hepatitis when compared to healthy controls. The p-value is highly significant for GGT, AST and ALT.

Also it is evident from the Table 1 that there is increase in the levels of GGT, AST and ALT in subjects with chronic hepatitis when compared to healthy controls and the p value is highly significant for GGT, AST and ALT.

From table 1, we observe that there is increase in the levels of GGT, AST and ALT in subjects with nonalcoholic cirrhosis when compared to healthy controls and the p value being highly significant for GGT, AST, ALT. Also we observe from the table 1, that there is increase in the levels of GGT, AST, and ALT in subjects with alcoholic cirrhosis when compared to healthy controls. The p value is highly significant for AST, ALT and GGT. It is evident from the table 1, that there is increase in the level of GGT, AST and ALT in subjects with alcoholic cirrhosis < 10 years alcohol abuse when compared to healthy subjects where p value is highly significant for GGT, AST and ALT. Also from Table 1 we observe that the there is increase in the level of GGT, AST and ALT in subjects with alcoholic cirrhosis > 10 years alcohol abuse when compared to healthy controls, where, p value is highly significant for GGT, AST and ALT.

It is evident from the Table 2 that there is increase in the levels of GGT, AST and ALT in patients with alcoholic cirrhosis when compared to non alcoholic cirrhosis subjects. The p value is highly significant for GGT, significance for AST and p value is non significant for ALT.

It is evident from Table 3 that there is increase in the levels of GGT, AST and ALT in subjects with alcoholic cirrhosis (with alcohol abuse of > 10 years) when compared to subjects suffering form alcoholic cirrhosis (with alcoholic abuse of <10yrs)

P value is insignificant for ALT and AST but highly significant for GGT.

Also it is evident from table 4 that the estimated levels of GGT, AST and ALT in acute viral hepatitis is increased when compared to cirrhosis and p value is not significant for GGT but highly significant for AST and ALT.

Also this Table 4 shows that the estimated levels of GGT AST and ALT in chronic hepatitis is increased when compared to cirrhosis. Where p value is non significant for GGT and ALT but significant for AST.

The Table 5 shows that there is increased levels of GGT, AST and ALT in chronic hepatitis when compared to non alcoholic cirrhosis. Where p value is significant for GGT but highly significant for AST and ALT.

According to Table 5 the estimated levels of AST, ALT in chronic hepatitis is increased when compared to alcoholic cirrhosis. But the levels of GGT is decreased in chronic hepatitis when compared to alcoholic cirrhosis. The p value is not significant for GGT and AST but significant for ALT.

4. DISCUSSION

Measurement of enzyme activities in serum are useful for diagnostic assessment of Hepatobiliary disease. Both the concentration and pattern of enzyme activities gives valuable information regarding the type, extent and severity of liver disease. Several workers have conducted many studies to asses the usefulness of serum enzymes estimation in the diagnosis

and prognosis of various liver disease. In our present study estimated levels of GGT, AST and ALT in liver diseases are discussed to correlate with other studies. There is no correlation with age and sex wise in the liver disease studied.

The mean GGT levels in all the liver diseases subjects were higher significantly with p value < 0.001 as compared to their respective controls. Also in our study the mean level of GGT increased in acute viral hepatitis subjects compared to healthy control subjects where p value being significantly high ($p < 0.001$). Thus the mean levels of GGT is elevated 3-4 times the upper limit of normal in this study. The cause for increased GGT is due to the increase rate of synthesis of the enzymes as an adaptive response to inflammation has been postulated. In our study the mean levels of GGT is increased in chronic hepatitis compared to controls. Pvalue being highly significant (<0.001) in chronic Hepatitis GGT has diagnostic specificity and sensitivity. GGT estimation is a useful marker in evaluating the course of acute hepatitis and in detecting the development of chronic hepatitis wherein GGT is the most markedly elevated parameter. It is evident from our study that the mean value of GGT is increased in cirrhosis compared to control. P value being highly significant < 0.001. Mean values of GGT is increased in non alcoholic cirrhosis compared to controls p value is highly significant < 0.001.

Also in alcoholic cirrhosis, in our study the mean level of GGT is increased when compared to controls. P value is highly significant < 0.001. Mean levels of GGT are increased both the alcoholic cirrhosis with alcohol abuse of < 10 years duration and with alcoholic abuse of > 10 years duration. GGT is an objective indicator of alcoholism. It is well documented that increased levels of GGT in alcoholics is due to the microsomal injury as a result of alcohol toxicity which results in induction of GGT and also microsomal location of GGT in the liver. GGT is a sensitive and highly specific test of liver cell injury in suspected alcoholics and is superior to transaminase determination. Increased GGT is seen in heavy drinkers even without clinical evidence of liver disease. If liver disease is present the concentration of GGT is 3 fold increased over alkaline phosphatase, alcohol should be considered as the possible cause.

GGT is a sensitive indicator and used to detect occult alcoholism. It is used to monitor abstinence and reabuse in alcoholics. GGT elevation reverts to normal with abstention and this test can be of sensitivity to detect liver involvement at an early and reversible stage. GGT remains the best of simple lab screening test and depending on population studied the sensitivity is in the order of 50% and specificity about 85%.

The mean levels of AST and ALT was higher significantly $p < 0.001$ in all liver diseases in our study when compared to healthy controls. The mean levels of AST and ALT in acute viral hepatitis is increased with p value being highly significant $p < 0.001$ when compared to healthy controls. AST and ALT is elevated by 4-6 times the upper limit of normal in our study. The higher levels of AST and ALT above normal indicate acute hepatocellular injury. The amino transferases begins to increase late in the prodrome of viral hepatitis and often decreases when Jaundice appears[14]. The mean levels of AST and ALT are increased in chronic hepatitis when compared to controls in our study about 2-4 times the upper limit of normal. Increase in serum enzyme levels in liver is due to response to injuries to the hepatocytes[15].

Also the mean levels of AST and ALT are increased in non alcoholic cirrhosis of liver when compared to controls with p value being highly significant $p < 0.001$. In alcoholic cirrhosis, the mean levels of AST and ALT are increased when compared to controls in our study with p value < 0.001 highly significant. Therefore in alcoholic cirrhosis AST and ALT are moderately elevated 2-4 times the upper limit of normal and p value being highly significant.

The mean levels of AST and ALT in alcoholic cirrhosis of < 10yrs alcohol abuse and alcoholic cirrhosis of > 10yrs alcohol abuse are increased compared to controls p value is significant highly $p < 0.001$.

In alcoholic cirrohosis of < 10 years of alcohol abuse, AST, ALT are moderately elevated by 3-4 times the upper limit of normal where p value is highly significant. Therefore AST and ALT are objective indicators of alcoholic liver disease.

Increased in AST and ALT activity was considered as an index of liver cell injury of various etiological factors. Increase activity of these enzymes in serum serve to identify and confirm liver diseases. AST and ALT are nonspecific indicators of liver diseases and they are not of much importance in the diagnosis of severity of liver disease, as they are non specific indicators of hepatocellular damage. Also the AST, ALT assay is of limited value and/or differential diagnosis[12-13].

5. CONCLUSION

Estimation of GGT will be of immense important in annual / periodic medical examinations particularly in suspected case of alcohol dependence.

AST and ALT are less sensitive than GGT. Mitochondrial isoenzyme (m-AST) is more specific in the diagnosis of liver disease and in the detection of alcohol abuse (m-AST) accounts for about 80% of total AST activity with in the liver cells[14].

To conclude increase activity of these enzymes in serum serves to identify or to confirm liver disorders. The increased activity of these enzymes is found in many liver diseases hence an abnormal result is of limited value for differential diagnosis[14].

Selection of appropriate liver function tests depends on particular clinical problem and on understanding the use and limitation of application of each test in various liver diseases. The liver function test can be of value prognostically in screening for liver diseases are vital in monitoring of the liver disease in response to treatment.

Further this study requires isoenzyme activity of GGT, m-AST and m-AST / t-AST ratio which are helpful in differential diagnosis of liver diseases.

Table – 1 showing levels of Mean ± SD of GGT, AST, ALT ratio in 50 control Subjects and Acute Hepatitis, Chronic Hepatitis and Cirrhosis

	No.	GGT IU/L	AST IU/L	ALT IU/L
Controls	50	23.7±8.1	13.6±6.4	10.1±5.2
Acute viral hepatitis	21	150.6±78.2	109.9±77.2	95.8±71.0
Chronic hepatitis	23	136.0 ±79.1	87.1±40.7	57.7±49.2
Cirrhosis of liver (non alcoholic)	10	49.7±10.8	52.7±14.4	32.0±26.3
Alcoholic cirrhosis (combined)	20	161.5±75.6	70.0±22.1	42.7±28.3
Alcoholic cirrhosis (> 10 Years)	10	201.3±76.4	74.8±15.2	49.9±35.6
Alcoholic cirrhosis (< 10 years)	10	121.8±52.8	65.3±27.4	35.6±17.6

Table – 2 showing Comparison of GGT, AST, ALT between Non-Alcoholic Cirrhosis and Alcoholic Cirrhosis

Group	GGT IU/L	AST IU/L	ALT IU/L
Non-alcoholic	49.7±10.8	52.7±14.4	32.0±26.3
Alcoholic	161.5±75.6	70.0±22.1	42.7±28.3
p-value	< 0.001	< 0.05	NS
Significance	HS	SS	NS

The value are expressed as their Mean *± SD*

P>0.05 NS *Not significant*
P>0.05 SS *Statistically significant*
P>0.001 HS *High significant*
P>0.01 VS *Very significant*

Table – 3 showing Comparison of GGT, AST, ALT between Alcoholic Cirrhosis of > 10 and < 10 years

Group	GGT IU/L	AST IU/L	ALT IU/L
> 10 years	201.3±76.4	74.8±15.2	49.9±35.6
< 10 years	121.8±52.8	65.3±27.4	35.6±17.6
p-value	< 0.001	> 0.05	> 0.05
Significance	HS	NS	NS

The values are expressed as their Mean ± SD
P > 0.05 NS = Not Significant
P < 0.01 HS = Highly Significant

Table – 4 showing Comparison of GGT, AST, ALT between Acute Viral Hepatitis, Chronic Hepatitis and Cirrhosis

Group	GGT IU/L	AST IU/L	ALT IU/L
Acute Viral Hepatitis	15.0±78.2	109.9±77.2	95.8±71.0
Chronic Hepatitis	136.0±79.1	87.1±40.7	57.7±49.2
Cirrhosis	124.3±81.6	64.2±21.3	39.2±27.7
Acute Viral Hepatitis V/s Chronic Hepatitis	> 0.05, NS	> 0.05, NS	< 0.05, SS
Acute Viral Hepatitis V/s Cirrhosis	> 0.05, NS	< 0.001, HS	< 0.001, HS
Chronic Hepatitis V/s Cirrhosis	> 0.05, NS	< 0.05, SS	> 0.05, NS

The values are expressed as their Mean ± SD
P < 0.05, SS = Statistically Significant
P > 0.05, NS = Not Significant
P < 0.001, HS = Highly Significant

Table – 5 showing Comparison of GGT, AST, ALT between Chronic Hepatitis, Non Alcoholic Cirrhosis and Alcoholic Cirrhosis

Group	GGT IU/L	AST IU/L	ALT IU/L
Chronic Hepatitis	136.0±79.1	87.1±40.7	57.7±5.2
Non Alcoholic Cirrhosis	49.7±10.8	52.7±14.4	32.0±26.3
Alcoholic Cirrhosis	161.5±75.6	70.0±28.1	42.7±28.3
Chronic Hepatitis V/s Cirrhosis (NA)	< 0.01, SS	< 0.001 HS	< 0.001, HS
Chronic Hepatitis V/s Cirrhosis (AL)	> 0.05, NS	> 0.05, NS	< 0.05, SS

The values are expressed as their Mean ± SD
P < 0.05, SS = Statistically Significant
P > 0.05, NS = Not Significant
P < 0.001, HS = Highly Significant

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