

SIGNIFICANCE OF GAMMAGLUTAMYL TRANSFERASE ESTIMATION IN HEPATOBILIARY DISEASES

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ABSTRACT: The present study was conducted to evaluate the significance of gamma glutamyl transferase (GGT) in hepatobiliary diseases and made to prove the GGT as a significant differential factor in diagnosis of high serum alkaline phosphatase conditions. In 166 patients of hepatobiliary diseases. The results were compared with 32 healthy age matched volunteers, patients were subjected to detailed clinical examination and laboratory investigations. Blood samples were collected for estimation of serum Bilirubin, AST, ALT, ALP and GGT. Ratio of serum AST/ALT and serum ALP levels were significantly ($P < 0.001$) increased in alcoholic liver disease and hepatic carcinoma. The results give enough evidence of increased GGT in microsomal induction by alcohol and other factors.

Key words: Gamma Glutamyl Transferase, Alcoholic liver disease, Amino transferases, Extra and Intra hepatic cholestasis.

INTRODUCTION:

Gamma glutamyl transferase(E.C.2.3.2.2) is a membrane bound glycoprotein enzyme, which catalyses the transfer of the gamma glutamyl moiety of glutathione to various peptide acceptors(Zein and Discombie,1970;Reyes and Miller,1980;Anton et al, 2002; Niemela, 2002; Conigrave et al;2003).it's present in the plasma membrane of renal tubular cells and endoplasmic reticulum of hepatocytes and bile ductule cells. Gamma glutamyl transferase is also participates in peptide nitrogen storage and in protein bio synthesis.

GGT present in serum appears to originate primarily from the hepatobiliary system (J.K. Limdi & GM Hyde, 2003). Alcohol causes the microsomal induction there by increasing the levels of GGT, over the past decades it is considered as a significant enzymatic parameter in alcoholic liver disease, even though its increasing in all hepatobiliary diseases (Rosalki SB, 1975). In this work we explored the significance of GGT in other hepatobiliary disorders along with alcoholic liver disease.

In this work we have taken six different types of hapatobiliary diseases as Viral Hepatitis, Alcoholic liver Disease, Cirrhosis of liver, Liver abscess, Cholestasis and Hepatocellular Carcinoma. The alcoholic liver disease patients are again classified in to Moderate and Heavy drinkers based on their consumption of alcohol per day according to Paton (Paton A, 1994) and the cholestasis group is also subdivided in to intra hepatic and extra hepatic cholestasis patients. This work was initiated to gain further insight on GGT as a significant differential diagnosis marker in hepatobiliary diseases.

MATERIALS AND METHODS:

The study protocol was in keeping with the ethical guidelines of the 1975 declaration of Helsinki and all the patients gave written informed consent to the study. Patients were selected from those who had visited general medicine department of Govt. Medical College, Jagdalpur and the major clinical presentations of patients

- 1) Jaundice with portal hyper tension
- 2) Right upper quadrant pain

Institutional ethics committee approved the procedures and study was conducted on 166 patients of six different types of hepatobiliary diseases within the age group of 25 to 65 years. 32 Healthy adult subjects more than 25 years of age both sexes ,taking good diet, non smoker, non alcoholic and free of medication for at least one month prior to study were selected as controls.

5ml of venous blood was collected individually from both control and study group after overnight fasting under aseptic precautions.

The blood sample was obtained in a sterile bulb and allowed to clot at room temperature for 30 minutes and the serum was separated after centrifugation at 3000rpm for 20 min. it was then used for assessment of various biochemical parameters.

The parameters taken to evaluate for this study are

- 1) Serum Bilirubin (Total & Direct) by Jendrassik method. (Jendrassik, L., Grof, P, 1938)
- 2) Aspartate amino Transferase by Optimized U.V test according to I.F.C.C (Bergemeyer HU, Bernt E, 1963)
- 3) Alanine amino Transferase by optimized U.V.test according to I.F.C.C
- 4) Alkaline Phosphatase by P-Nitro Phenyl Phosphate (pNPP) Kinetic method (Linhardt K, Walter K, 1963)
- 5) Gamma Glutamyl Transferase by Modified Kinetic colorimetric method of Szasz (Gowelock, A.H, 1988)
- 6) Serum Total Protein and Albumin levels were measured by Biuret and Bromocresol green reagents respectively (Doumas BT, Peter T, 1997)

Were measured on all patients using Hitachi 704 fully automated analyzer with reagents supplied by Boehringer Mannheim diagnostics. Setting up of the instrument was checked by measuring calibrators for automated system. Accuracy and precision was evaluated by measuring quality control precipnorm U and precipath U from boehringer Mannheim.

In the present study we have investigated changes in above clinical parameters in patients with viral hepatitis, 46 patients (28 male and 18 female) alcoholic liver disease, liver abscess 16 patients (10 male and 6 female), cirrhosis of liver 23 patients (16 male and 7 female), cholestasis (intra and extra hepatic) 13 patients (5 male and 8 female) and hepato cellular carcinoma, 8 patients (5 male and 3 female). Alcoholic liver disease patients are further classified as described by Paton in to, A high alcohol intake group (ALD-H; those had been drinking more than 80g alcohol per day for at least one year) and b) Moderate alcohol intake group (ALD-M; those who had been drinking less than 80g alcohol per day) 22 male and 13 female in high intake group and 20 male and 5 female in moderate taking group, and the patients of Liver abscess are abstained from alcohol drinking. These parameters were compared with normal values obtained from normal healthy people. The individuals who were diabetic or had myocardial infarction, pancreatic disease, chronic obstructive pulmonary disease, pregnancy, renal failure, dystrophia myotonica and who consume drugs-carbamazepine, phenytoin and barbiturates were excluded from this study.

Their mean age, height and weight were noted. Body Mass Index (BMI) was measured by the formula: $BMI = \text{weight in kg} / (\text{height in meters})^2$

Results have been expressed as MEAN±SEM. Statistical significance was determined by students't' test for unpaired data. The values of significance were evaluated with 'p' values. The difference were considered significant at p<0.001.

RESULTS AND DISCUSSION:

In this present study patients with above mentioned six different Hepatobiliary diseases were compared with normal healthy persons and there is also an intra comparison between the persons with Alcoholic liver disease and in Cholestasis.

The normal physical parameters of mean age, height, weight and BMI are determined (Table-1) in all the normal healthy persons and in patients.

Table -1. Demographic profile: Age, Height, Weight and Body Mass Index (BMI) of normal healthy and different Hepato biliary diseases

Values are mean±SEM of number of observations (n). * indicates p<0.05 when compared with normal healthy controls, @ indicates p<0.05 when compared with moderate alcoholic

Group	Age(yrs)	Height (cm)	Weight (kg)	BMI (Kg/m ²)
Normal healthy persons(n=32)	43.47±1.32	161.43±0.13	64.47±0.34	23.75±0.23
Viral hepatitis (n=46)	37.87±1.24	163.32±0.21	63.60±0.65	23.01±0.19
Alcoholic liver disease(moderate) (n=25)	42.34±2.56	161.85±0.25	61.23±0.32	22.56±0.12
Alcoholic liver disease(heavy) (n=35)	45.25±1.35	162.23±0.51	57.23±0.15*@	21.25±0.43*@
Cirrhosis of liver (n=23)	43.25±1.72	163.12±0.85	54.72±0.15*¥	19.85±0.61*¥
Liver abscess (n=16)	41.19±1.06	162.13±0.63	61.29±0.32	22.31±0.06
Intra hepatic cholestasis (n=6)	42.21±1.53	161.23±0.35	63.21±0.21	23.25±0.45
Extra hepatic cholestasis (n=7)	42.31±1.23	162.31±0.41	62.85±0.35*#	22.85±0.56*#
Hepatic carcinoma (n=8)	43.21±2.31	161.35±0.32	56.23±0.25*¥#	20.13±0.58*¥#

liver disease, ¥ indicates p<0.05 when compared with alcoholic liver diseases,# indicates p<0.05 when compared with intra hepatic cholestasis

The persons with alcoholic liver disease (heavy), chronic cirrhosis of liver and hepatocellular carcinoma are showing significantly lower body weight ($p < 0.05$). The patients with extra hepatic cholestasis also have significant low body weight ($p < 0.05$) when compared with intra hepatic cholestasis patients. Either due to reduced adipose tissue or due essentially fat mass reduction there is a weight loss in alcoholics. Loss of protein as a source of energy in the hepatocellular carcinoma causes significant reduction in total body weight. Hepatic carcinoma patients had low weight, low BMI compared to other groups.

In viral hepatitis Hyper Bilirubinemia (Figure-1) is a common symptom as like in various other liver diseases (Szmunes W, et al, 1974). Serum amino transferase concentrations are moderately raised in chronic and milder cases of acute viral hepatitis. (Davison, and J.Mohammad, 2000). Alanine amino Transferase is statistically elevated ($p < 0.001$) than Aspartate amino Transferase and compared to the GGT the Alkaline Phosphatase is somewhat statistically significant ($p < 0.01$). The total protein and serum albumin values are almost normal and the ratio between the AST/ALT is lower than the normal ratio one. (David Mc Cathy et al, 2000). In acute hepatic disorders the both Transaminases are elevated compared to the biliary canaliculi enzymes, it is observed that infection due to hepatitis B and hepatitis C virus elevated the GGT activity in serum.

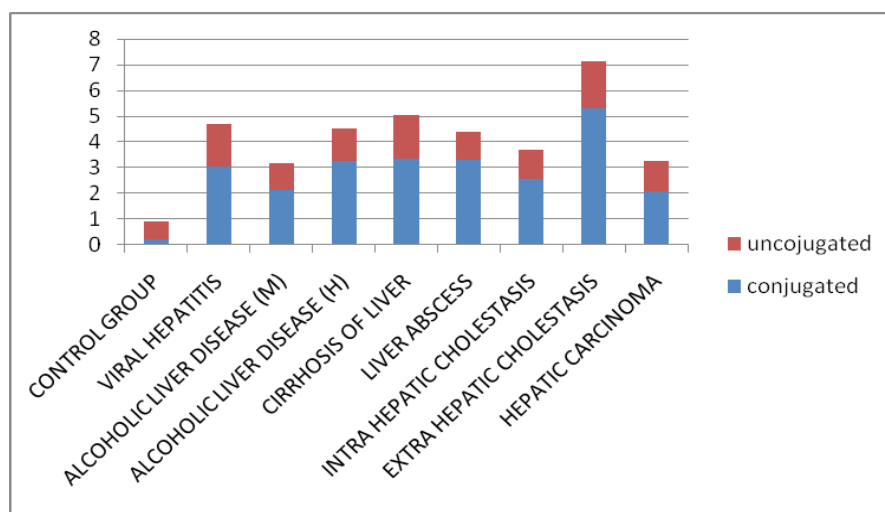


Figure-1. Unjugated and conjugated bilirubin levels of normal healthy persons and different types of hepatobiliary diseases.

Ethanol is toxic to the liver. Alcoholic beverages and the problems they endanger have been familiar in human societies since the beginning of recorded history, overall 3.5% of the global burden of disease is attributable to alcohol (Racial and Ethnic, 2002). Its excessive consumption is the most common cause of liver disease in many hepatobiliary diseases. Ethanol associated endotoxemia and subsequent release of inflammatory mediators such as Acetaldehyde, which is a oxidation product of ethanol may cause hepatocyte injury via oxy radical dependent and independent mechanisms (Flicker P., Zatloukal, K, 2002). In alcoholic liver disease bilirubin (Table-2) concentrations are elevated and causes a common symptom jaundice in both types, but the conjugated bilirubin levels are significantly elevated in Alcoholic liver disease (H) (Das SK, et al, 2003). Deficiency of pyridoxal-5-phosphate, a necessary coenzyme for both Amino transferases, is common in alcoholic liver disease. This deficiency decreases hepatic ALT to a greater extent than AST, with corresponding changes in serum concentration (Krastev Z , et al, 1992).

Table -2. Serum enzyme activities for Normal and different types of Hepato biliary disease patients.

Group	Total Bilirubin(mg/dl)	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	GGT (IU/L)	Total Protein(g/dl)	Albumin (g/dl)
Healthy persons(n=3)	0.85±0.03	24.42±1.10	26.63±0.89	143.10±11.27	27.88±1.46	7.34±0.02	1.34±0.01
Viral hepatitis(n=46)	4.71±0.78*	68.66±7.23	106.46±17.85*	357.4±35.43	90.80±10.8	7.12±0.14	1.24±0.32
Alcoholic liver disease(M)(n=25)	3.12±0.56	98.35±3.62*	85.32±23.51	296.23±21.69*	209.74±53.66*	6.92±0.13	0.93±0.18
Alcoholic liver disease(H)(n=35)	4.44±0.49*	128.75±46.29@*	114.05±23.90*	335.86±42.62*@	352.25±34.63* @	6.72±0.09*	0.72±0.21*
Cirrhosis of liver(n=23)	5.05±1.96*	78.9±16.12*	54.22±5.80	371.95±88.68*	89.82±38.20*	6.53±0.23*	0.85±0.05*
Liver abscess(n=16)	4.37±0.87*	100.28±4.05*	81.42±26.07	360.02±67.92*¥@	100.42±43.52*	7.23±0.35	1.31±0.02
Intra hepatic cholestasis(n=6)	3.52±2.31*	86.32±26.15	75.36±23.58	515.26±29.78*¥	138.76±8.57*	6.83±0.15*	0.97±0.13
Extra hepatic cholestasis(n=7)	6.95±2.95*©	101.33±19.64* @	102.33±17.35*¥	614.0±116.86*¥@ ©	278.05±16.76* @©	7.13±0.03	1.21±0.12
Hepatic carcinoma(n=8)	3.25±0.84	44.5±18.49	57.5±16.49	706.5±172.49*¥@	198.5±9.49*¥	6.81±0.19*	0.82±0.23*

Values are mean±SEM of number of observations (n). * indicates p<0.001 when compared with normal healthy control, @ indicates p <0.001 when compared with alcoholic liver disease (moderate), ¥ indicates p<0.001 when compared with alcoholic liver disease (heavy), © indicates p<0.001 when compared with intra hepatic cholestasis,

Alcoholic liver disease group with heavy alcohol (ALD-H) intake showed significant elevation in the AST/ALT ratio in comparison to alcoholic liver disease with moderate alcohol intake (ALD-M) group. Hence, the AST/ALT ratio is also a good marker of alcoholic liver disease. Alcohol causes the microsomal induction of both biliary canaliculi enzymes which are under study. GGT levels are useful to differentiate the drinking patterns of a person and the rate of elevation of GGT is directly proportional to the amount of alcohol consumed (Rosalki S, 1984). GGT levels are statistically significant ($p < 0.001$) along with the ALP in alcoholic liver disease of both types. The pattern of total protein and albumin are slightly near to normal range in alcoholic liver disease (M), but the levels are significantly decreased in alcoholic liver disease (H).

Cirrhosis of liver due to other than alcoholic liver disease, there is a significant raise in conjugated bilirubin ($p < 0.001$) than unconjugated bilirubin and in Amino Transferases ALT is not much deviated from their normal range and it is statistically less significant ($p < 0.05$) when compared with AST, ALP and GGT, reliable biochemical parameters for chronic liver disease are raised more than double to their reference levels.

Although some of the workers proved that ALP is statistically significant than GGT in cirrhosis liver disease (17), but in this study we found that there is marked elevation of GGT ($p < 0.001$) along with ALP in cirrhosis of liver. Liver is the main site of synthesis for proteins, so its concentration in plasma reflects the functional capacity of liver, there is a marked decrease in total protein values along with serum albumin levels ($p < 0.001$) when compared with normal subjects. Because of low serum albumin values there is a reduction in effective blood volume with arterial hypotension, recognized principle of hemodynamic disturbance in cirrhosis is observed.

Liver abscess affects 10% of world population, patients taken all are abstained from alcohol, most of the them have amoebic liver abscess, conjugated bilirubin is increased than unconjugated bilirubin, in both transaminases AST levels ($p < 0.001$) are statistically elevated than ALT, AST/ALT ratio is more than 1 (Figure-2). G. Rajagopal and Mohammed Rafi (18) proved that GGT is 334% in alcoholic liver abscess when compared to normal control subjects, in this study also similar results found and its once again proved as a sensitive index in the diagnosis of liver abscess, compared to the alcoholics ALP levels are markedly raised than GGT ($p < 0.001$), there is no marked difference in total protein and serum albumin levels when compared to the normal control group.

In cholestasis groups, real time Ultrasonography allowed distinction between extra hepatic cholestasis with dilated bile ducts within the liver and intra hepatic cholestasis, where intra hepatic biliary radical dilation was not seen

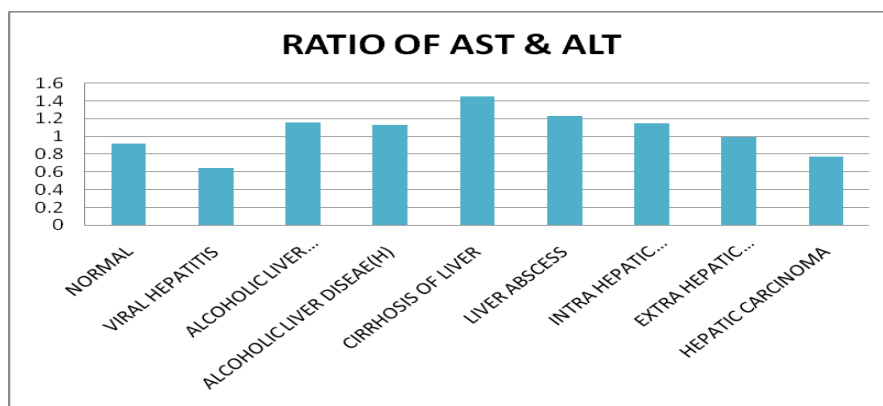


Figure-2. Ratios of AST and ALT in different groups of study, each bar represents mean±SEM of number of observations (n).

In intra hepatic cholestasis there is slight increase in the conjugated bilirubin, both transaminases increased in two folds but statistically insignificant.

ALP is increased significantly when compared to GGT ($P < 0.001$ & $P < 0.05$) respectively during cholestasis bile acids accumulate in hepatocytes and solubilize the plasma membrane, there by resulting the release of ALP(19). No marked change in the total protein and albumin in serum. But results of this study show a significant difference in GGT values in the intra and extra hepatic cholestasis, GGT is statistically more significant in extra hepatic cholestasis (Waern AU, Helling K, 1980) and it can be considered as a factor to differentiate the both types of cholestasis.

Hepatic carcinoma constitutes the sixth most frequent form of cancer worldwide and it holds third place concerning malignancy related mortality (Parkin 2005) chronic hepatitis B is the major risk factor for developing hepatic carcinoma in India. More than 80% of hepatic carcinoma cases are arising in the background of cirrhosis, the process of formation of new vasculature from pre existing capillaries called angiogenesis is observed and it is characterized by sinusoidal capillarization, increased expression of enzymes in endothelial cells along with deposition of extra cellular matrix and micro vessel formation (Arensen, k. F et al, 1970).

The liver is the most common site of distant metastasis, due to unique vascular architecture of the liver enables a tumor to acquire adequate nutrients and oxygen through various mechanisms such as vessel co option and traditional angiogenesis. The 8 cases observed in hepatic carcinoma are 'C' grade of hepatic carcinomas according to Barcelona clinical liver classification, tumor stage is advanced, general stage of health is reduced and tumors are characterized by large multiple nodules with vascular invasion and extra hepatic secondaries.

The total bilirubin, aminotransferase levels are not significantly increased ($P < 0.05$) when compared with normal control group and remaining enzymes ALP & GGT levels are elevated statistically significant ($P < 0.001$) due to angiogenesis, total protein and serum albumin levels are significantly decreased ($P < 0.001$).

CONCLUSION:

Current guidelines recommend liver biopsy as a part of the management of chronic liver disease. This procedure provides important information regarding the degree of liver damage and severity of fibrosis (Bravo A.A, et al, 2001) unfortunately liver biopsy has a potential sampling error, is invasive, costly and prone to complications, up to 30% of patients experience pain following the procedure, 0.3% have severe complications and mortality approaches 0.01%.

As a result of those problems as well as patient reluctance to undergo liver biopsy, the estimate of liver injury using non invasive biomarkers has gained a growing importance.

According to some workers there is a limitation of normal routine liver biochemical tests to precisely predict the severity of liver disease (Carl.A.Lehmann, 2005), warrants a cautious approach in interpreting the results of these tests. But taken together the present data supports the concept that the diagnostic potential of GGT measurements is so wide not confined only as a marker of the ethanol consumption, it's very much useful to decide the origin of increased ALP in serum either from bone or liver, very much useful in the differentiation between mechanical biliary obstruction and intra hepatic cholestasis represents one of the classical diagnostic challenge in clinical medicine. Most of the physicians considered 5' nucleotidase as a significant indicator instead of GGT in obstructive colestasis conditions; However the rise in serum 5'NT levels is noticeable several days after obstruction of the biliary ductual system and may lag behind the elevations in serum ALP and GGT.

So ultimately based on our study we conclude GGT is a significant, Non invasive biochemical marker useful in the differential diagnosis of all hepato biliary diseases.

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