A Comparative Study of Serum Gamma-Glutamyl Transpeptidase, Serum Alkaline Phosphatase and GGT/ALP Ratio in Different Liver Disorders

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ABSTRACT

Introduction: Determination of serum enzyme activity plays important role in the diagnosis of various liver diseases. Out of the commonly used enzymes, serum γ-glutamyl transpeptidase (GGT) and serum alkaline phosphatase (ALP) are considered as the markers of cholestasis but due to wide distribution their single estimation lacks specificity. As both GGT and ALP are increased in liver disease, GGT/ALP ratio could help differentiate liver disorders. Aims and objectives: To study the serum levels of GGT, ALP and GGT/ALP ratio is various liver disorders

Material and methods: A case-control study was carried out on 28 patients each suffering from acute alcoholic hepatitis (AAH), acute viral hepatitis (AVH), obstructive jaundice (OJ) and chronic liver disease (CLD). Serum GGT and ALP levels were determined in these patients and compared with healthy controls (n=28). GGT/ALP ratio was calculated and compared. Statistical significance was determined using Microsoft Excel (Microsoft Corp, Washington, USA). P value <0.05 was considered significant.

Result: Significant increase ($p<0.001$) in GGT and ALP levels were seen in all types of liver disease. Maximum elevation of GGT and ALP was observed in cholestatic etiology. Although GGT was higher in patient with OJ, this was statistically not significant when compared with patient with AAH. GGT/ALP ratio in healthy controls was 0.22±0.12. This ratio was significantly elevated ($p<0.001$) in all the liver disorder with highest being in AAH (1.43±0.36).

Conclusion: Although both serum GGT and ALP are altered in liver diseases, GGT/ALP can be useful in differentiating the liver diseases of different etiologies.

Key words: Alcohol, Viral, Cholestasis, Hepatitis

INTRODUCTION

Liver is a vital organ which performs multiple functions such as excretion, detoxification, synthesis and is considered as the principle organ of metabolism. Liver disease is a term used for any damage which alters the function of the liver. Alteration in any function of the liver can have adverse effect of health. Enzymes, predominantly those released by the hepatobiliary system, are used in evaluation of diseases having a hepatic or a cholestatic origin. Aminotransaminases; i.e. Alanine Transaminase (ALT) and Aspartate Transaminase (AST), Alkaline Phosphatase (ALP) and Gamma Glutamyl Transpeptidase (GGT) are commonly used to differentiate between hepatocellular disorder and post hepatic obstruction.[1] Although GGT is the most sensitive indicator of hepatobiliary diseases, it has a poor specificity due to its wide distribution in cells of tissues like liver, kidneys, pancreas, brain, spleen and seminal vesicles which limits in usefulness.[2,3] Ranging between 5 to 40 IU/L, elevated serum levels of GGT is observed in usually observed in alcoholics and alcohol induced hepatitis.[4]

Alkaline phosphatase belongs to zinc metalloenzymes with serine at the center. ALP originates mainly from the liver and the kidneys and are present in the mucosal lining of small intestine, proximal convoluted tubule of the kidney, bones, liver and placenta.[5] Elevated levels of serum ALP are observed in various hepatobiliary disorders such as bile duct obstruction (due to cholethiasis, tumors or strictures), primary biliary cirrhosis, primary sclerosing cholangitis, liver metastasis, infiltrative liver disease (granuloma, amyloid), viral hepatitis, alcoholic hepatitis and cirrhosis. Apart from these, elevated levels are also observed in various non-hepatic conditions such as bone disorders, chronic renal failure, congestive cardiac failure, adolescence, malignancies and drugs such as ACE inhibitors and estrogen therapy.[6]

Different liver diseases are associated with varying degree of elevation of these enzymes. Elevation in GGT is about five times its upper limit in viral hepatitis, 10 times in cholestatic hepatitis and above 20 times in alcohol induced hepatitis.[7-11] With regards to serum ALP, maximum elevation is seen in obstructive etiology and marginal to mild elevation in viral hepatitis. In case of alcoholic hepatitis, ALP may be normal or even in elevated it is disproportionately lower than GGT.[10,11] Therefore, although both ALP and GGT are useful enzymes to assess liver function, their wide distribution and wide range of elevation in the serum, limits their role as an independent marker of liver damage. More over single elevation of GGT with normal ALP levels indicates drug

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toxicity such as warfarin, NSAIDs, barbiturates, recreational drugs, anticonvulsants and alcohol toxicity.[12] Hence this study was done to study the diagnostic importance of GGT/ALP ratio in various liver disorders.

MATERIAL AND METHODS

This case-control study was conducted in the department of Biochemistry, Rajshree Medical Research Institute, Bareilly, Uttar Pradesh, India, between February 2015 to January 2016 on 140 subjects with ages ranging between 18 years to 50 years, after obtaining approval from the Institutional Ethical Committee. One hundred twelve diagnosed cases of liver diseases were registered and divided into four groups i. e. group-I (acute alcoholic hepatitis, AAH, n = 28), group-II (acute viral hepatitis, AVH, n = 28), group-III (obstructive jaundice, OJ, n = 28) and group-IV (chronic liver disease, CLD, n = 28). Twenty eight normal healthy individuals were studied as control (group-V).

Questionnaire and data collection: A questionnaire was specifically designed based on the criteria to obtain information which helped to select the cases for the study. Blood samples were collected using aseptic techniques after obtaining informed consent from each subject.

Inclusion criteria: The diagnosed cases of acute alcoholic hepatitis, acute viral hepatitis, obstructive jaundice and chronic liver disease were included.

Exclusion criteria: Individuals with other diseases associated with or without hepatitis and with H/O taking any drug altering serum GGT and/ or ALP levels, pregnant women and individuals with family history of liver disorder were excluded. Biochemical analysis included estimation of GGT and ALP from fresh separated and unhemolyzed serum. Serum GGT was estimated by Carboxy Substrate kinetic method, kit from Coral Crest Biosystems and ALP was estimated by pNPP-AMP (IFCC), kinetic method, kit from Benesphera.[13,14]

STATISTICAL ANALYSIS

Statistical analysis was done using MS Exel (2007) software (Microsoft Corporation, Redmond, Washington, USA) and results expressed as Mean±SD. Comparison of variables between two groups were performed using student 't' test. 'p' values < 0.05 were considered significant.

RESULTS

The mean serum GGT and ALP levels of patients with AAH, AVH, OJ and CLD are summarized in Table 1/Figure 1. Both the serum GGT and ALP levels were elevated in all the four types of liver diseases when compared with controls. As shown in table 2, this increase in serum GGT and ALP was statistically significant in all the liver diseases ($p < 0.0001$). On comparing the means of GGT and ALP among diseased condition it was observed that the increased GGT levels in the patients with AAH and those with AVH and obstructive jaundice were statistically significant ($p<0.001$) whereas no statistical significance was observed between AAH and obstructive jaundice patients ($p = 0.209$). Similarly, the

Groups	GGT (Mean±SD) IU/L	ALP (Mean±SD) IU/L	GGT/ALP Ratio
Acute Alcoholic Hepatitis (AAH) n=28	225.70±94.35	163.02±72.05	1.43±0.36
Acute Viral Hepatitis (AVH) n=28	87.48±40.80	194.86±58.43	0.47±0.24
Obstructive Jaundice (OJ) n=28	283.43±228.21	499.02±448.42	0.64±0.31
Chronic Liver Disease (CLD) n=28	77.05±17.86	160.94±51.51	0.51±0.15
Control (n=28)	15.83±6.39	76.57±18.13	0.22±0.12

Table-1: Serum GGT, ALP levels and GGT/ALP ratio (Mean ± SD) in different groups.

Groups	GGT		ALP		GGT/ALP Ratio	
	t-value	p-value	t-value	p-value	t-value	p-value
AAH vs Control	11.5655	<0.0001 HS	6.5278	<0.0001 HS	15.5869	<0.0001 HS
AVH vs Control	9.2316	<0.0001 HS	9.8689	<0.0001 HS	4.7718	<0.0001 HS
OJ vs Control	6.1589	<0.0001 HS	4.9422	<0.0001 HS	6.2168	<0.0001 HS
CLD vs Control	17.278	<0.0001 HS	7.8436	<0.0001 HS	6.9825	<0.0001 HS
AAH vsAVH	6.9561	<0.0001 HS	1.7383	0.0936 NS	11.7752	<0.0001 HS
AAH vs OJ	1.2871	0.2090 NS	3.9024	0.0006 NS	9.884	<0.0001 HS
AVH vs CLD	7.928	<0.0001 HS	0.1169	0.9078 NS	13.3407	<0.0001 HS
AVH vs OJ	4.6952	<0.0001 HS	3.5374	0.0015 VS	2.9184	0.007 VS
CLD	1.4268	0.1651 NS	2.7482	0.0105 S	0.9468	0.3521 NS
CLD	4.8817	<0.0001 HS	4.0458	0.0004 HS	2.3478	0.0265 S

HS=highly significant, VS= very significant, S= significant, NS= not significant.

Table-2: Comparative statistical analysis of serum GGT, ALP levels and the ratios between the groups.

Parameter	Acute Alcoholic hepatitis	Acute viral Hepatitis	Obstructive jaundice	Chronic Liver Disease
GGT	14 fold	5 fold	18 fold	5 fold
ALP	2 fold	3 fold	7 fold	2 fold

Table-3: Increase in serum GGT and ALP levels of different liver disorders when compared with health controls.

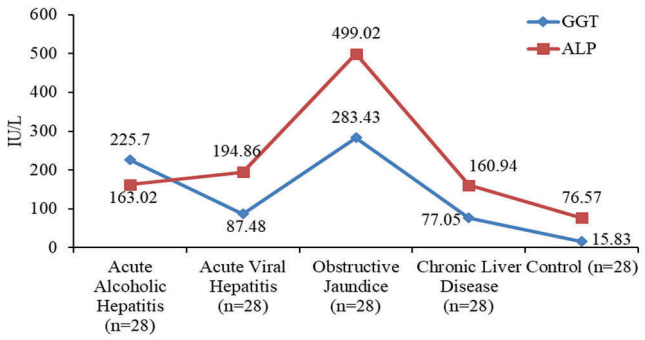

Figure-1: Graph comparing the mean GGT and ALP levels in all case groups and control

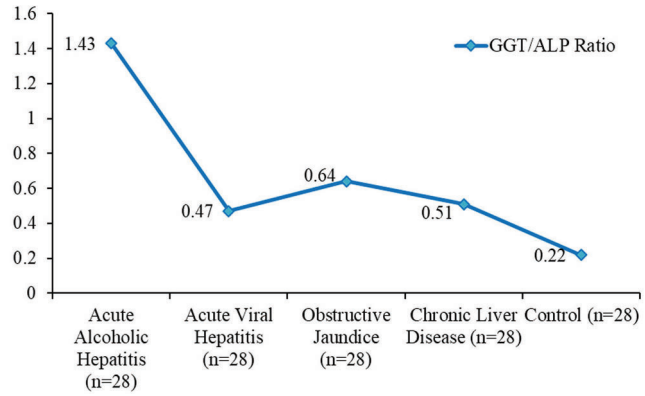

Figure-2: A graph depicting the GGT/ALP ratio in all case groups and controls

increased ALP levels in patients with obstructive jaundice when compared with increased ALP levels seen in AVH and CLD was statistically significant with p value of < 0.001 and 0.004 respectively. The GGT/ALP ratio was highest in case of AAH with 1.43 and least in case of CLD with 0.22 (Table 1/Fig 2). The variation in GGT/ALP ratio observed within all the groups were statistically significant (Table 2)

DISCUSSION

Etiology of liver diseases depends upon the different subsystem involved such as hepatocellular, mainly in the form of damage to the hepatocytes, the biliary excretory apparatus and the vascular system which is evident as portal hypertension. In order to affirm the cause of liver disease, a battery of tests is performed based of different functions of the liver. Various non functional enzymes and their disproportionate increase in the serum provides important clue to the underlying disorder. In this study the pattern of ALP and GGT were studied in four common types of liver injuries i.e acute viral hepatitis, acute alcohol hepatitis, obstructive jaundice and chronic liver disease. Gamma glutamyl transpeptidase (EC 2.3.2.2) catalyzes the transfer of gamma-glutamyl groups from peptides such as glutathione to other amino acids.[15] Although increased in most form of liver injury, Szilagyi A in their study shown it to be an early indication of alcoholic liver injury.[16,17] As expected, it was observed that both ALP and GGT was significantly elevated ($p < 0.05$) in all the four types of hepatic diseases when compared with healthy controls. As seen in table 3, highest increase in mean serum GGT levels by 18 folds were observed in obstructive jaundice followed by AAH (14 folds). Five folds increase was observed in CLD and AVH. Similar results were reported by Sunanda et al and Benerji et al.[2,18,19] With respect to ALP, a seven folds increase was observed in obstructive jaundice followed by three folds increase in AVH and two folds increase in CLD and AAH. This finding was consistent with study conducted by Hasan et al and Nandi et al who demonstrated 1.5 to 2 folds increase in ALP levels in AVH and 10 folds increase in obstructive jaundice.[20,21]

The cause of increase in ALP and GGT varies with the etiology of liver disease. In cholestatis, there is increased synthesis of ALP and GGT and their solubility and hence leaks out of the bile duct due to increased bile pressure.[22] Although there were higher levels of GGT in patients with obstructive jaundice as compared to patients with AAH, this increase was not statistically significant. This could be explained by the fact that in alcoholics, increase in GGT is more due to induction by ethanol rather than obstruction or hepatic injury. GGT probably also has a role in protection against reactive oxygen species (ROS) which is evident from extracellular metabolism of glutathione the main antioxidant of human cells.[23] Similar observations were seen with serum ALP levels too where the increase of ALP levels in patients with AAH was not statistically significant when compared with other groups.

A raised GGT is not diagnostic of alcohol abuse, with research showing it remains high in former drinkers as well as current drinkers.[23] In men, the highest levels of GGT occur in those who consume ethanol on daily basis. A GGT/ALP ratio >2.51 in association with jaundice suggests alcohol as a cause of liver disease.[24,25] Salaspuro M stated that the use of test combinations significantly improves the information received with single serum enzyme determinations.[26] If GGT to ALP ratio exceeds 1.4, the specificity of the finding in favor for alcoholic liver injury is 78% and Lai CL et al. observed the similar results in their study and stated that the ratio of GGT to ALP was significantly higher in the group with alcoholic liver disease than in any of the other four groups.[27] When the ratio was higher than 1.4, the diagnostic efficiency for distinguishing the alcohol group from the other four groups was 78% (the normal upper limit for GGT and ALP being 35 and 115, respectively). In this study it was also observed that GGT/ALP ratio of 1.43±0.36 in AAH which was significantly elevated ($p<0.05$) as compared to the results obtained in other disease groups and the control group, except AVH v/s CLD and is consistent with the studies conducted previously.[26,27] In our study, GGT/ALP ratios of 0.47±0.24, 0.64±0.31 and 0.51±0.15 were observed in AVH, OJ and CLD respectively. The values in all these case groups were found significantly elevated ($p<0.05$) when compared with the control group (mean GGT/ALP ratio of 0.22±0.12). We found significant difference ($p<0.05$) when any two of the case groups were compared except AVH v/s CLD. Hence it could be said that along with other parameters, GGT/ALP ratio could help in classifying liver diseases based on different

etiologies. However, there are few limitations in this study. Firstly, the staging of alcoholic hepatitis and chronic liver disease was not taken into consideration. Further studies correlating GGT/ALP ratio with diagnostic imaging studies could help management of hepatic disorders. Secondly, this study was done in patients who sought treatment at the hospital. Larger scale community based multi-centric studies would provide more valuable information regarding GGT and ALP as markers of liver damage.

CONCLUSION

Although both GGT and ALP is a sensitive marker of hepatic cell disorder and biliary canal cell dysfunction, they lack specificity. However, when both these markers are combined in the form of GGT/ALP ratio, it not only helps in the differential diagnosis of liver disorders but the rising value of GGT/ALP may also indicate more severe damage of liver due to alcohol consumption. However, the result of this study was based on a retrospective data. Further, prospective data in a larger population would be needed to refute or support this study.

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