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Research Article

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Clinical Significance and Association Between Any Two Liver Function Tests

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ABSTRACT

Liver function tests are routinely investigated for suspected liver disorders due to infection by blood borne viruses such as HCV, HbsAg which leads to a host of liver disorders. A total of 7 tests are collectively done to evaluate the role and normal functioning of synthetic, metabolic and excretory functions of liver. Each test has its specific diagnostic use to assess the above three standard functions of liver. While individual tests are specific to one particular function and as the exact malfunction of the liver is not known, all the tests are done together, and a statistically significant association should exist between any two liver function tests whether the patients liver functions are normal or abnormal.The outcome of this study has established that there is always statistically significant association between any two liver function tests suggesting the need to order all the liver function tests collectively or specific tests depending upon clinical diagnosis as pointed out in many studies mentioned under literature review.

Keywords: *LFT, GVHD, ALT, GGTP, ALP.*

INTRODUCTION

The liver is the most important internal organ which carryout many diverse functions. It detoxifies toxins entering the body, synthesize proteins that are used for blood clotting factors and other proteins that help draw fluid into our blood vessels (e.g., albumin). Liver Function tests are used to guide the physician along with the history and physical examination, in the diagnosis and management of a number of liver diseases.Liver function tests measure the concentrations of various different proteins and enzymes in the blood that are either produced by liver cells or released when liver cells are damaged. Liver function tests are very common investigations carried out in patients with suspected liver disease. Liver Function tests include tests for bilirubin, a breakdown product of hemoglobin, and ammonia, a protein byproduct that is normally converted into urea by the liver before being excreted by the kidneys. The other tests includesseveral enzymes synthesised in the liver which are special proteins which aid in the metabolies of ingested food materials. Enzymes that are often measured include gamma-glutamyltransferase; alanine aminotransferaseaspartate aminotransferase and alkaline phosphatase. Liver function tests help to detect defects in synthetic, metabolic and excretion functions. The term “liver function tests” is actually a misnomer as several of the tests do not measure total liver function at all.

Review of literature

Liver Function tests (LFT) are usedin the diagnosis and management of acute and chronic liver disease. Their routine use has led to the increased detection of liver enzyme abnormalities in otherwise asymptomatic patients. These tests consist of markers of hepatocellular injury, tests of liver metabolism, and tests of liver synthetic function. Liver injury can be characterized as primarily hepatocellular versus

cholestatic based on the degree of elevation of aminotransferases compared with Alakline phosphatase (ALP)[1].

Serum bilirubin exerts antioxidant and cytoprotective effects. In addition, elevated serum bilirubin levels are associated with the decreased metabolic and excretory function of the liver as well as cardiovascular diseases[2]. Graft-versus-host disease (GVHD) is the most frequent complication after allogeneic hematopoietic cell transplantation. Statistically significant associations between asymptomatic continuous increase ofbilirubin,Alaninetransferase (ALT), andGamma glutamyltranspeptidase (GGTP) and later liver GVHD manifestation were found. A continuous increase of bilirubin and/or ALT, GGTP before the standard liver GVHD criteria are met can be a sign of coming liver GVHD[3].

GGTP level was elevated more consistently along with the ALT level in all types of anicteric nonalcoholic chronic liver diseases. The ALTtest has already been proved to be a marker in the diagnosis of chronic anicteric nonalcoholic liver diseases. The elevated GGTP level can also be used as a noninvasive bio marker of chronic anicteric nonalcoholic liver diseases for both diagnostic and therapeutic purposes[4].

Serum albumin is associated with improved prediction of waiting list mortality. If validated and shown to be associated with reduced mortality, adoption of a Model for End-Stage Liver Disease (MELD) as the basis for liver allograft allocation may improve outcomes on the liver transplant waiting list[5]. Serum albumin level has a prognostic value in head and neck cancer patient with liver cirrhosis. The perioperative albumin levels can be utilized for risk stratification to potentially improve surgical and postoperative management of these challenging patients[6]. As compared with standard antibiotic therapy alone, treatment with albumin together with antibiotics has beneficial effects on the renal and circulatory function and shows a potential survival benefit. Further studies with large sample sizes should be performed to confirm these findings[7]. A plasma albumin level below 20 g/L was not found to be an independent marker for severe preeclampsia because all women with a low plasma albumin level had other adverse conditions[8].

The latest indication for albumin use in cirrhotic patients is extracorporeal albumin dialysis, which has shown promise for the treatment of hepatic encephalopathy; its role in hepatorenal syndrome or acute or chronic liver failure has not been established. Efforts should be made to define the indications for albumin use, dose of albumin required and predictors of response, so that patients gain the maximum benefit from its administration[9].

Albumin infusion in combination with the administration of a vasopressin analogue may be able to reverse established hepatorenal syndrome; however, no controlled studies have been published. Whereas the use of albumin infusion with large-volume paracentesis is strongly supported by the available evidence, additional conclusive studies of the use of albumin for spontaneous bacterial peritonitis are awaited[10].

Patients with metabolic syndrome (MetS) have higher serum GGTP and C-reactive protein (CRP) levels compared with controls. This increased GGTP level might be a marker of increased oxidative stress and premature atherosclerosis[11]. Serum albumin level is an independent risk factor for mortality and improvement of hypoalbuminaemiaand it should be considered as improvement of prognosis[12].

The Aspartate transferase AST/ALT ratio is significantly elevated in patients with alcoholic hepatitis and cirrhosisas well as in patients with alcoholic liver cirrhosis[13]. The increased AST/ALT ratio is due primarily to the low activity of ALT in liver. The less than expected elevation of ALT in serum of patients with alcoholic hepatic reflects the diminished hepatic ALT activity and less amount of this enzyme available to leak into serum from damaged hepatocytes[14].

The relatively greater decrease in ALT compared with AST in advanced liver diseases was not mainly due to leakage of the enzyme from the liver, but to a specific mechanism associated with hepatic injury or its progression. Other pathological conditions of the liver such as those in obstructive jaundice and alcoholic liver injury also appeared to result in reduced liver ALT activity, which was reflected in the serum as an increased AST/ALT ratio[15]. The incidences of abnormal ALT, AST, and GGTP in obese

subjects were frequently significantly higher than in the controls and it was most clearly shown in ALT. The incidences of abnormal ALT in the obese females were significantly lower than those in the obese males, but were significantly higher than the controls. Higher incidences of abnormality in the school children were ascribed to the higher degree of obesity in the children. The extent of increase in ALT was considerable, andsometimes higher than 6 times the normal upper limit[16].

The dynamics of Alkaline phosphatase (ALP) activity in serum is a reflection of the delicate biochemical and histochemical changes in the liver wound. The follow-up of serum ALP together with the clinical course of healing could be an useful index for evaluation of diet and working capacity of the patient[17]. In patients with elevated serum Alpha Feto Protein (AFP) levels, theGGTP trends were less informative, although patients with the highest AFP levels had a worse prognosis if their GGTP levels were simultaneously elevated. The results suggest that following trends in serum GGTP levels, in the context of specific ranges of AFP and bilirubin levels, might be useful in diagnosis of small Hepatocellular Carcinoma (HCC)[18].

The increased hepatic GGTP activity is neither specific for alcoholic liver disease nor essential for serum GGTP to be elevated[19]. The enhanced hepatic GGTP activity was higher in alcoholic and nonalcoholic patients than in the normal controls. No statistical significance of difference could be found in hepatic GGTP activity between alcoholic and nonalcoholic patients, while serum GGTP activity was markedly elevated in alcoholic patients compared to nonalcoholic patients. There was better correlation between hepatic and serum GGTP activity in alcoholic patients. Furthermore the enhanced hepatic GGTP activity could contribute to the pathogenesis of liver fibrosis and histological liver fibrosis[20]. Serum GGTPhas been widely used as an index of liver dysfunction and marker of alcohol intake[21].

Strong associations of serum GGTP activity with many cardiovascular risk factors and/or events might be explained by a mechanism related to oxidative stress. Even though studies on serum and/or cellular GGTP is at a beginning stage, epidemiological findings suggest that serum GGTP might be useful in studying oxidative stress-related issues in both epidemiological and clinical settings[22]. Based on current experimental and epidemiological studies, GGTP present in the serum, even within its laboratory reference intervals regarded as physiologically normal, is a promising biomarker for cardiovascular risk[23]. Lack of clear-cut benefits for survival, and fear of transmitting unknown viruses add to the controversy.

Several folds of variation in the analyzed enzymes were found between healthy control and case groups. Comparative elevation of Liver associated enzymes was observed to indicate the degree of Hepatic Damage in Viral Hepatitis, Alcoholic liver diseases and cirrhosis[24].

In clinical practice, “liver function” is assessed by either measuring the activities of enzymes /analytes produced by the hepatocyte, measuring the serum activities that are changed by hepatocyte damage, evaluating the serum level released from the cells as a result of injury, assessing the ability of the liver to perform a metabolic task such as conjugation or detoxification, or by measuring enzyme activity and substrate content of the cell and its organelles[25].

Delta-bilirubin is a bilirubin covalently bound with albumin, which is nontoxic and excreted neither in urine nor in bile. The increase in the percentage of serum delta-bilirubin indicates an effectiveness of biliary drainage in man. An analysis of serum delta-bilirubin for 7 days can distinguish the good drainage patients from the poor drainage patients[26].

Absence of one or both organic anion transporting polypeptides proteins thus may have serious impact on toxicity of commonly used drugs cleared by this system such as statins, sartans, methotrexate or rifampicin. The liver-blood cycling of conjugated bilirubinis impaired in cholestatic and parenchymal liver diseases and this impairment most likely contributes to jaundice accompanying these disorders[27].

A statistically significant increase, above normal, in serum AST andGGTP was observed in the favic subjects during the crisis. All the values reverted to normal in the asymptomatic period[28]. Tissue-specific patterns have not been described, and disease-specific patterns cannot be reproduced with confidence. Whereas exciting advances are being made in understanding the molecular structure, mechanism, and functions of the enzyme it has yet to find a genuinely useful diagnostic role substantiated by a convincing body of scientific data[29].

MATERIAL METHODS

A total of 600 non hospitalised patients consisting of males and females in the age group of 5 to 74 years attending the liver clinic and who were investigated for liver function tests were enrolled for this study. As the sole aim of this study was to find out the association between any two liver function tests, inclusion or exclusion criteria were not followed. Based on the mean results obtained for LFT they were sub divided into 300 as normal and 300 abnormal for statistical evaluation of the associations.

Olympus AU400 analyser and Roche diagnostic reagents were used to measure all the analytes. The accuracy of all LFT were validated by the use of Bio-Rad accuracy controls at two levels. For statistical analysis of data, a software downloaded from the website **http://www.vassarstats.net** was used to calculate correlation coefficient (r) , students 't' distribution (t) and probability (p) between two tests for both normal and abnormal liver function test groups.

RESULTS

Table I: **NORMAL GROUPn=300 (Mean for all patients, males & females)**

Analytes	TB	TP	ALB	GLB	A/G	AST	ALT	ALP	GGTP
All Patients n=300	0.65	6.65	3.85	2.89	1.43	24.84	20.82	87.82	24.93
Males n=159	0.72	6.58	3.78	2.81	1.42	26.02	22.30	89.15	27.53
Females n=141	0.72	6.46	3.71	2.76	1.41	26.25	22.12	85.59	27.36
Normal Range	0.5-1.0	6.0-8.0	3.5-5.0	2.5-3.0	1.0-1.8	8-40	5-35	40-125	10-50

Table II: ABNORMAL GROUPn=300 (Mean for all patients, males & females)

Analytes	TB	TP	ALB	GLB	A/G	AST	ALT	ALP	GGTP
All Patients n=300	3.63	6.52	3.47	3.05	1.24	203.56	173.31	160.04	152.99
Males n=216	4.13	6.62	3.53	3.09	1.25	203.78	179.46	159.03	165.59
Females n=84	2.80	6.55	3.56	3.00	1.29	165.34	151.60	145.61	180.90
Normal Range	0.5-1.0	6.0-8.0	3.5-5.0	2.5-3.0	1.0-1.8	8-40	5-35	40-125	10-50

Table III: Statistical Parameters r, t,p (normal group n=300)

ANALYTESCOMPARED	R	p	p
TB VsAST	0.1092	1.896	<0.05
TB VsALT	0.1998	3.52	<0.001
TB VsGGTP	0.2229	3.947	<0.0001
TP Vs ALB	0.7003	16.935	<0.0001
TP Vs GLB	0.1900	3.341	<0.0001
TP VsAST	-0.1522	-2.658	<0.001
ALB VsAST	-0.1087	-1.888	<0.05
ALB VsGGTP	-0.1141	-1.983	<0.05
ASTVsALT	0.6379	14.299	<0.0001
ALTVsGGTP	0.4684	9.153	<0.0001

Table IV: Statistical Parameters r, t,p (abnormal group n=300)

ANALYTESCOMPARED	r	t	p
TB Vs TP	0.2061	-3.636	<0.001
TB Vs ALB	0.3727	-6.933	<0.0001
TB Vs GLB	0.1494	2.617	<0.01
TB Vs ALP	0.1604	2.805	<0.01
TB VsGGTP	0.0726	1.257	<0.05
TP Vs ALB	0.5796	12.778	<0.0001
TP Vs GLB	0.5475	11.295	<0.0001
TP Vs ALP	0.1531	-2.674	<0.01
ALB Vs GLB	0.3634	-6.734	<0.0001
ALB Vs ALP	0.2559	-4.57	<0.0001
ASTVsALT	0.7877	22.072	<0.0001
AST VsGGTP	0.1121	1.947	<0.05
ALP VsGGTP	0.4318	8.264	<0.0001

Table V: Statistical Parameters r, t, p (normal Males n=159)

ANALYTESCOMPARED	r	t	p
TB Vs ALB	0.1310	1.656	<0.05
TP Vs ALB	0.6622	11.073	<0.0001
TP Vs GLB	0.5808	8.94	<0.0001
TP VS AST	-0.1786	-2.274	<0.05
ALB Vs GLB	-0.2254	-2.899	<0.01
ALB VsALT	0.1496	1.896	<0.05
GLB VS AST	-0.1717	-2.184	<0.05
GLB VsALT	-0.2009	-2.57	<0.01
ASTVsALT	0.6043	9.503	<0.0001
ASTVs ALP	-0.0161	-0.202	<0.05
ASTVs GGTP	0.2389	3.083	<0.01
ALTVs GGTP	0.2940	3.854	<0.0001

Table VI: Statistical Parameters r, t, p (normal Females n=141)

ANALYTES COMPARED	r	t	p
TB Vs ALB	0.1310	1.656	<0.05
TP Vs ALB	0.6622	11.073	<0.0001
TP Vs GLB	0.5808	8.94	<0.0001
TP VS AST	-0.1786	-2.274	<0.05
ALB Vs GLB	-0.2254	-2.899	<0.05
ALB VsALT	0.1496	1.896	<0.05
GLB VS AST	-0.1717	-2.184	<0.05
GLB VsALT	-0.2009	-2.57	<0.01
ASTVsALT	0.6043	9.503	<0.0001
ASTVs ALP	-0.0161	-0.202	<0.05
ASTVs GGTP	0.2389	3.083	<0.01
ALTVs GGTP	0.2940	3.854	<0.0001

Table VII: Statistical Parameters r, t, p (Abnormal Males n=216)

ANALYTESCOMPARED	r	t	p
TB Vs TP	-0.2287	-3.437	<0.01
TB Vs ALB	-0.4124	-6.622	<0.0001
TB Vs GLB	0.1878	-2.797	<0.01
TB Vs ALP	0.1416	2.093	<0.05
TP Vs ALB	0.5307	9.16	<0.0001
TP Vs GLB	0.4888	8.16	<0.0001
TP VS AST	-0.1050	-2.219	<0.05
TP Vs ALP	-0.1411	-2.085	<0.05
ALB Vs GLB	-0.4800	-8.004	<0.0001
ALB Vs ALP	-0.2473	-3.734	<0.0001
GLB VS AST	-0.1180	1.738	<0.05
GLB VsALT	-0.1322	-1.951	<0.05
ASTVsALT	0.9593	49.7	<0.0001
ALTVs GGTP	0.0612	0.897	0.18557
ALP Vs GGTP	0.3608	5.66	<0.0001

Table VIII: Statistical Parameters r, t, p (Abnormal Females n=84)

ANALYTES COMPARED	r	t	p
TB Vs TP	-0.4049	-4.01	<0.0001
TB Vs ALB	-0.3977	-3.925	<0.0001
TB Vs ALP	0.3460	3.339	<0.0001
TB Vs GGTP	0.2768	2.608	<0.01
TP Vs ALB	0.6855	8.526	<0.0001
TP Vs GLB	0.6964	8.787	<0.0001
TP Vs ALP	-0.1928	1.779	<0.05
ALB Vs ALP	-0.2915	-2.759	<0.01
GLB VS AST	0.2599	2.437	<0.01
ASTVsALT	0.4726	4.856	<0.0001
ASTVs GGTP	0.1996	1.845	<0.05
ALP Vs GGTP	0.7064	9.037	<0.0001

Tables I and II shows the mean results for normal and abnormal groups respectively for all the patients along with the normal ranges followed in the author's laboratory. While the mean values obtained for the normal group are within the normal range, for the abnormal group, all analytes except TP, Alb andA/G are higher compared to the upper limit of normal, suggesting that these group of patients indeed have disturbed liver functions and all the main markers are elevated.

Tables III to VIII gives the statistical parameters category wise as mentioned in the title for each Table. It is interesting to observe that almost all comparisons, irrespective of patients classification, gives highly significant correlation between any two analytes baring few in which the level of significance range from 0.05 to 0.01. These data suggests that when liver function is affected irrespective of the etiological factors, there is always elevation of key enzymes like AST, ALT, ALP and GGTP, and significant association between any two analytes will be observed, whether liver is functioning normally or get altered due to disease status. ALP, an enzyme that helps to diagnose cholestatic jaundice was found to be associated with bilirubin, TP, albumin and AST, suggesting this enzyme is also important for the differential diagnosis of jaundice.

All the statistical data shown in Tables III to VIII suggests that a total of 7 tests as mentioned in this study should be investigated for any type of suspected liver disorder.

DISCUSSION

This study shows that if one liver enzyme gets elevated, there is always found to be an increase in other enzymes and our observation in the study is found to be in accordance with previous observations[4] and continuous increase in Bilirubin along with ALT & GGTP is also observed[3]. The AST/ALT ratio is also found to be high in abnormal group as shown by previous studies[6]. In abnormal group, GGTP increase was always found to be associated with an increase in AST and ALT, notably the later one, confirming our findings with earlier one[21]. We have observed several fold increase in liver enzymes compared to normal groups, a fact that altered liver function induces rapid increase in those enzyme. [(24)] It has been predicted in earlier studies that the three key enzymes altered are AST, ALT and GGTP among which alterations in ALT and GGTP are prominent[14,16,20].

Our study has proved beyond reasonable doubt that all the seven tests investigated in this study should be collectively investigated to evaluate total liver function and individual elevation in analyte level should be compared for corresponding increase in other and the factors leading to such elevations should be explored before treatment options are initiated.

CONCLUSION

Standard liver function tests consists of 7 tests and usually many clinicians investigate all tests as LFT. There is some practice prevailing among hepatologists in some hospitals to order only few tests such as bilirubin for suspected jaundice, AST & ALT for liver cirrhosis and ALT and GGTP for GVHD. Individual test may help to diagnose specific cause, but not other factors inducing alterations in liver function. Whenever total bilirubin increases, it is important to investigate liver enzymes also as they are found to increase as observed in this study, particularly ALP for differential diagnosis of jaundice. In conclusion, this study recommends that to assess liver function it is important that clinicians order all the seven tests as outlined in this study and to interpret the results collectively as well as in comparison with other analyteto assess exact clinical diagnosis to proceed correct treatment protocol.

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