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Estimation of Serum ALP, GGT and LAP Levels in Liver Carcinoma

Dr.Sujata Sahay[1], Dr.Deependu Kumar Sinha[2*]
[1] *Associate Professor, Department of Biochemistry, M.G.M Medical College, Jamshedpur*
[2] *Professors, Department of Surgery, M.G.M Medical College, Jamshedpur*
Corresponding Author: Dr.Sujata Sahay

Abstract:
Introduction: *Hepatobiliary carcinoma includes carcinoma of liver, pancreas, bile ducts, ampulla of Vater and gall bladder. Hepatocellular carcinoma (HCC) is the most common primary malignant tumor of the liver. It is the fourth most common cancer in the world[1]. Metastases are the most common malignant tumors of the liver[2]. Metastasis of liver is commonest ranking second only to cirrhosis as a cause of liver damage. The incidence of carcinoma of the pancreas has markedly increased over the past several decades and ranks as the fourth leading cause of cancer death in the United States[3]. In pathological conditions there is usually some derangement in the metabolic processes which is reflected by a change in enzymatic patterns. The detection of these biochemical agents is simple and noninvasive.*
Materials and methods: *50 patients diagnosed with heptobiliary carcinoma were enrolled for the study and 50 healthy individuals of matched age and sex served as controls. Serum Leucine Aminopeptidase (LAP), Alkaline Phosphatase (ALP) and Gamma-glutamyl Transpeptidase (GGT) were determined and compared in study and control groups.*
Results: *The mean levels of serum ALP, GGT and LAP in the study group were 785.3±465.14 IU/L, 190.7±106.60 IU/L and 97.65±33.86 IU/L respectively. These values were significantly higher than in the control groups. Serum LAP showed abnormal levels in maximum number of cases (93.3%) followed by serum GGT in 83.3% and serum ALP in 76.6% cases. ALP, GGT and LAP levels were significantly higher in icteric and nonicteric groups as compared to control group.*
Conclusion: *Serum ALP, GGT and LAP were significantly elevated in the patients with hepatobiliary carcinoma and the elevations were significantly higher in icteric as compared to nonicteric groups. Serum LAP is a better indicator of hepatobiliary carcinoma.*
Key words: *hepatobiliary carcinoma, ALP, GGT, LAP*

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I. Introduction

Worldwide, liver cancer is the second leading cause of cancer-related death in men and the sixth in women[1]. Hepatocellular carcinoma (HCC) accounts for >80% of liver cancer cases. Approximately 78% of HCC was attributable to hepatitis B virus (HBV) or hepatitis C virus (HCV) infection. Also, presence of cirrhosis from any cause markedly increases HCC risk [2]. The overall age-adjusted HCC incidence rate in the United States tripled between 1975 and 2005, partially accounted for by the increase of HCV infection and the influx of immigrants from HBV endemic regions. According to the World Health Organization, HCC has the second highest increase in overall death rate of all malignancies and its burden is expected to continue to increase in the next a few decades [3]. The five-year survival rate for HCC is <5% in all patients whereas >30% in patients diagnosed in early stages and receive surgery or liver transplantation [4]. These facts highlight the importance of clinical surveillance, risk prediction, targeted prevention, and early diagnosis in HCC management.

Serum liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), and γ-glutamyltransferase (GGT), are tested routinely and automatically in current clinical settings. These enzymes are commonly elevated in patients with liver diseases and thus may reflect the status of liver injury[5]. Physicians generally use significant elevations of liver enzyme levels as complementary markers to aid the diagnosis of various diseases. For example, elevations of ALT and AST may indicate the presence of hepatocellular predominant disorders while elevations of ALP and GGT may implicate cholestatic predominant diseases [6]. Recent epidemiological studies have shown the associations between abnormally high liver enzyme levels and risks and mortalities of many diseases [7]. However, as yet, no population-based study has been reported on the associations of these enzymes and the risk of HCC in HBV patients, especially for baseline

enzyme levels measured at the initial clinic visit of patients. In the current study, we sought to use a prospective approach to evaluate the effects of these four commonly tested serum liver enzymes on the long-term risk of developing HCC in a clinic-based Korean American HBV patient cohort. To the best of our knowledge, this is one of the first prospective studies that comprehensively evaluated these enzymes in HCC risk.

II. Materials and Methods

The study was done on 50 patients diagnosed with heptobiliary carcinoma visiting surgery and Biochemistry department of M.G.M Medical College, Jamshedpur. 50 healthy aged and gender matched adults served as control. Clinically proven cases of hepatobiliary carcinoma, confirmed on radiological investigations, laparotomy, and ultrasound, CT scan or MRI were included in the study group. Detailed history was taken and a thorough clinical examination was done. The study group was further divided into icteric and non- icteric groups. All cases which had received chemotherapy, gave history of bone fracture or bone disease in last six months, children, pregnant women and chronic alcoholics were excluded from the study. Serum Leucine Aminopeptidase, Alkaline Phosphatase and Gamma-glutamyl transpeptidase were determined using optimized kinetic method.

Investigations done were Hemoglobin (Hb), Total leucocyte count (TLC), Bleeding time(BT), Clotting time (CT), Erythrocyte sedimentation rate (ESR), Fasting blood sugar (FBS), Blood urea, Serum creatinine, TSP, DSP (serum albumin and glopulin), serum amylase, serum bilirubin, Aspartate Transaminase(AST) and Alanine Transaminase(ALT). Serum leucine aminopeptidase (LAP) was determined by optimized kinetic method according to the recommendations of the German society of Clinical Chemistry[7]. When L-leucyl-p-nitroanilide is acted upon by the enzyme leucine aminopeptidase, p-nitroaniline is liberated.

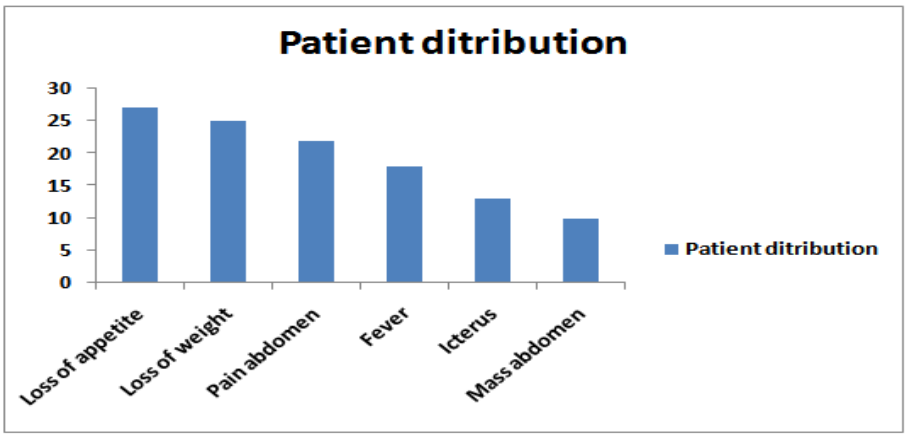
The absorption of p-nitroaniline is very high at 405 nm, whereas the substrate hardly absorbs at all at this wavelength. The absorbance is read at 405 nm and is directly proportional to the enzyme activity. Reagents: Buffered substrate solution (1.6 mM in 0.05M tris Buffer, pH- 7.2): 40.2 mg of L-leucyl-p-nitroanilide was dissolved in 2 ml of 96% ethanol and made upto 100 ml with tris buffer. The freshly prepared solution had an absorbance between 0.090 and 0.095 against distilled water at 405 nm. Reagent was freshly prepared each time and was stored in dark colored bottle. Serum ALP was measured by kinetic method and the kit was supplied by Accurex Biomedical Pvt. Ltd., Mumbai. For GGT, the methodology used was of Ssaz[8], using single reagent chemistry by kinetic (IFCC) method and the kit used was supplied by Erba/transasia Biomedical Pvt. Ltd., Mumbai. Serum levels were again estimated and compared after one month of treatment in the form of surgery, radiotherapy or chemotherapy.

III. Results

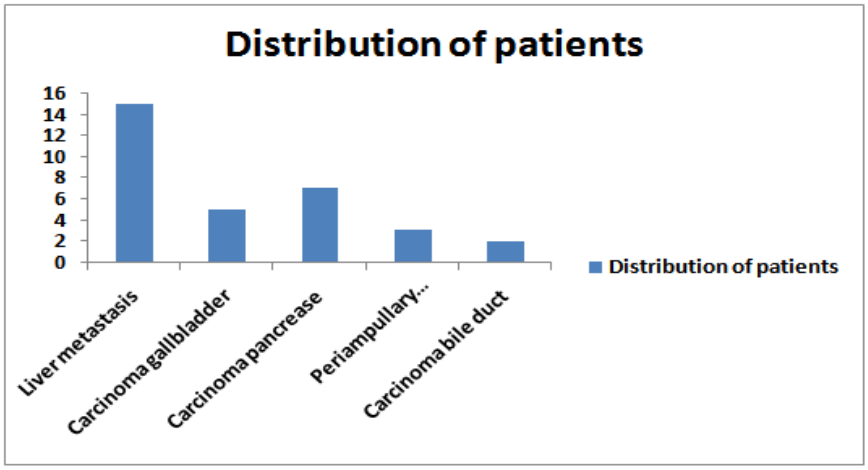
There was no statistical significant difference in the mean age and sex distribution of study and control groups. Loss of appetite and weight were the main presenting symptoms. (Figure I).Distribution of patients according to diagnosis is depicted in Figure 2. The study group was further divided into icteric and non-icteric groups depending upon the presence or absence of icterus (Figure 3). Serum bilirubin, AST and ALT were found to be significantly higher in study group as compared to control group (Table 1). The mean levels of serum ALP, GGT and LAP in the study group before initiation of treatment were 786.3±465.05IU/L, 193.3±116.73IU/L and 96.86±34.74 IU/L respectively. These values were significantly higher than in the control group (Table 2). Out of 30 patients of hepatobiliary carcinoma, serum LAP showed abnormal levels in maximum number of cases (93.3%) followed by serum GGT in 83.3% and serum ALP in 76.6% cases (Table 3).

Thus, it is observed that out of the three enzymes, serum LAP is the most sensitive index of hepatobiliary carcinoma. Levels of all the three enzymes i.e., ALP, GGT and LAP were significantly higher in icteric and non-icteric groups as compared to control group. Also, icteric group showed higher values than the non-icteric group, the difference being highly statistically significant. LAP was significantly raised ($p<0.001$) in the icteric as compared to the non-icteric group. 15 out of 16(93.75%) nonicteric cases had elevated LAP.

This was followed by GGT, raised in 12/16(75%) nonicteric cases and ALP raised in just 9/16(56.25%) nonicteric cases. Therefore, it is observed that LAP is the most frequently elevated enzyme in non-icteric patients. The serum levels of ALP, GGT and LAP before and after treatment are compared in table 7. Out of 30 patients in the study group, only 26 could be followed up because three patients had expired and one refused to follow-up. Though the mean levels of all three enzymes decreased on the follow-up, the difference was not significant statistically



Graph 1: Distribution of patients according to general presenting symptoms



Graph 2: Distribution of patients according to diagnosis

Parameter	Group	Range	Mean±SD	P value
Bilirubin Mg%	Study	0.37-13.5	3.15±3.65	<0.001
	Control	0.1-0.9	0.48±0.19	
AST(IU/L)	Study	35-220	106.5±70.1	<0.001
	Control	10-35	21.35±7.5	
ALT(IU/L)	Study	18-160	65.34±43.57	<0.001
	Control	12-32	22.16±6.32	

Table1: showing serum Bilirubin, AST and ALT levels in study and control groups

Parameter	Group	No of patients	Range (IU/L)	Mean±SD (IU/L)	P value
ALP	Study	50	220-1650	7.85±465	<0.001
	Control	50	112-240	159.6±38.19	
GGT	Study	50	30-441	193.5±116.73	<0.001
	Control	50	8-42	16.02±7.55	
LAP	Study	50	36-176	96.65±34.57	<0.001
	50	27-40	33.16±4.32		

Table 2: Comparison of serum ALP, GGT and LAP levels in study and control groups.

Level of enzyme	ALP		GGT		LAP	
	No	% Age	No	% Age	No	% Age
Normal	17	23.33%	15	30%	22	44
Above normal	33	76.77%	35	70%	28	56
Total	50	100	50	100	50	100

Table 3: Showing frequency of elevation of serum ALP, GGT and LAP in study group

IV. Discussion

It has been suggested that in the hepatobiliary diseases, there is increased synthesis of ALP by the hepatocytes which results in increased enzyme levels in circulation[14]. Hepatic ALP is normally present on the apical domain (i.e., canalicular) of the hepatocyte plasma membrane and in the luminal domain of bile duct epithelium. In cholestasis, retained bile acids solubilize the hepatocyte plasma membrane and facilitate release of ALP[16-18]. The increase levels of serum GGT result from cholestasis in which the bile acids solubilize the hepatic membrane bound enzyme. It is also suggested that the tumor itself may contribute to raised GGT levels because of the pronounced GGT activity of malignant liver cells[16].

Studies from several authors have shown raised levels of serum GGT in liver metastasis[10, 11]. GGT was increased in most of the patients (70%) of liver metastases[23]. Serum GGT is an important marker for Hepatitis B virus-related combined hepatocellular-cholangiocarcinoma. It is shown that serum GGT is not elevated in bone disorders. Thus measurement of serum GGT helps us to distinguish whether bone or liver is the source of increased levels of serum ALP. Elevation of serum GGT levels is an indicator of aggressive tumor behaviors and a predictor of poor clinical outcomes. It may prove to be a useful biomarker for identifying intrahepatic cholangiocarcinoma (ICC) patients at high risk of early recurrence and unfavorableprognosis. Out of the three enzymes, serum LAP was found to be raised in maximum number of cases (93.3%) followed by GGT and ALP in 83.3% and 73.3% cases respectively. LAP is found to be the most sensitive enzyme in hepatobiliary carcinoma.

Abnormal levels of serum LAP were reported in100% of cases of carcinoma pancreas and 93% cases of liver metastasis. LAP is raised in diseases of liver and hepatobiliary duct system and the diseases not involving liver and bile duct system are seldom associated with increased LAP. Increased LAP was due to obstruction of common bile duct by the tumor or liver metastasis or both. Significantly elevated LAP levels were observed in liver metastasis. Arise in serum LAP is detected in patients of hepatobiliary pancreatic carcinoma. LAP was found to be elevated in papillary adenocarcinoma of bile duct and in cholestatic liver disease.Non-significant elevations of LAP was observed in cases of carcinoma gallbladder without liver metastasis. Serum LAP is not significantly elevated in malignant liver disease as compared to benign liver disease. Patients with liver metastasis of non-pancreatic origin and without jaundice had increased LAP levels suggesting that hepatic infiltration is the cause of rise in liver metastasis[12].

Cholestatic liver diseases are characterized by impaired hepatocellular secretion of bile, resulting in intracellular accumulation of bile acids which result in a shift in the oxidant/prooxidant balance in favor of increased free radical activity and injury of different tissues.It is concluded that rise in LAP seen in both icteric and non-icteric groups was due to hepatocellular dysfunction. Whereas ALP and GGT showed greater rise in icteric group as compared to non-icteric group indicating that hepatic dysfunction with jaundice was the cause of elevated levels, LAP rises with hepatic dysfunction irrespective of jaundice, it is a better indicator of hepatobiliary malignancy. Lowering of LAP levels was either due to removal of primary tumor or suppression of primary tumor with subsequent decrease in size of secondaries by various modes of treatment (surgery, radiotherapy or chemotherapy).

Fall in levels were not statistically significant because the residual tumor still remained in the body. Moreover these estimations were done when patients were still taking the treatment in the form of radiotherapy or chemotherapy and high levels may have not disappeared from the circulation. These patients may have shown fall in the levels after completion of treatment.

V. Conclusion

All the three enzymes i.e. ALP, GGT and LAP are significantly elevated in the patients with hepatobiliary carcinoma and the elevations are significantly higher in icteric patients as compared to nonicteric patients. Out of these enzymes, LAP is the most sensitive in diagnosis of hepatobiliary carcinoma. It is more useful in the screening of non-icteric cases of hepatobiliary carcinoma as it rises more frequently in non-icteric cases. Thus serum LAP is a better indicator of hepatobiliary carcinoma. Monitoring LAP is a simple, low cost, and relatively sensitive screening tool for detecting hepatobiliary carcinoma.

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