LEUCINE AMINO PEPTIDASE A BETTER INDICATOR OF CARCINOMA OF LIVER, BILIARY TRACT AND PANCREAS

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ABSTRACT

Serum levels of leucine amino peptidase (LAP) was studied along with bilirubin, aspartate transaminase (AST), alanine transaminase (ALT), gamma glutamyl transpeptidase (GGT), alkaline phosphatase(ALP) and the ratio of AST/ALT and GGT/AST in 25 healthy subjects and 52 patients with hepatobiliary malignancies of which 12 were with hepatocellular carcinoma, 12 with liver metastasis, 6 with obstructive jaundice, 9 with carcinoma of gall bladder, 6 with carcinoma of pancreas and 7 with periampullary carcinoma. 24 Of the 52 patients studied had jaundice and 28 were without jaundice.

LAP as compared to the other enzymes AST, ALT, GGT, ALP and AST/ALT ratio and GGT/AST ratio showed 100% elevation in obstructive jaundice, carcinoma of gall bladder and pancreas and periampullary carcinoma, 91.7% elevation in hepatocellular carcinoma and 83.3% elevation in liver metastasis. On comparing the levels of these enzymes in non jaundiced and jaundiced groups, LAP was elevated in both jaundiced and non jaundiced groups in 95.8% and 92.9% cases respectively whereas the other enzymes AST showed increase from 67.9% to 100%, ALT from 21.4% to 83.3%, GGT from 71.4% to 95.4% and ALP from 82.1% to 100% in non jaundiced and jaundiced groups respectively indicating that LAP rises in hepatic dysfunction due to hepatobiliary malignancy whereas the other liver function enzymes showed increased hepatic dysfunction due to hepatobiliary malignancy with the onset of jaundice thereby indicating that LAP is a better indicator of hepatobiliary malignancy as compared to other enzymes.

The quantitative methods used for determination are reliable, accurate, simple, rapid and cost effective and therefore have better application in a clinical setting.

KEY WORDS : Leucine amino peptidase, carcinoma, liver, biliary tract, pancreas

INTRODUCTION

Leucine amino peptidase (LAP), a proteolytic enzyme, is capable of hydrolyzing L – leucyl peptides and is primarily involved in protein digestion in small intestinal mucosa. LAP is widely distributed in bacteria, plants and demonstrated in all human tissues with high activity in liver as well as in duodenum, small intestine, pancreas, testis and stromal cells of the uterus (1). LAP has sensitivity and diagnostic

significance. Various liver function tests like bilirubin, AST, ALT, AST/ALT ratio, ALP(2) GGT, GGT/AST ratio and LAP(3) are reported to identify patients with liver disease. In virtually all other conditions the overlap is such as not to allow the discrimination between different disease categories. For this reason AST/ALT ratio by DeRitis was advocated by Wroblewski as a better diagnostic criterion than numerical values of activities of individual tests. LAP can be used as a marker in testicular tumor (4).

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There is a general agreement amongst clinicians on the need for more specific biochemical aid in diagnosis and evaluation of the treatment of malignant

disease. Studies have demonstrated a relationship between activity of enzymes and pathologic process of tumor growth (5-7). The present study was undertaken with a view to see if it fulfils the general agreement of clinicians on the need for more specific biochemical aid in diagnosis and evaluation of malignant disease as the activity of the enzymes is very closely related to the pathologic tumor growth and also to see if jaundice was in any way related to the elevated levels.

MATERIALS AND METHODS

25 healthy ambulant subjects and 52 cases with hepatobiliary and pancreatic malignancies- 12 with hepatocellular carcinoma, 12 with liver metastasis, 6 with obstructive jaundice, 7 with periampullary carcinoma, 9 with carcinoma of gall bladder and 6 with carcinoma of pancreas - were included in this study.

All 7 patients with periampullary carcinoma had obstructive jaundice with bilirubin ranging from 3.9 – 43.0 mg% (median 14.7 mg%). Two patients with liver metastasis had bilirubin levels of 2.6 mg% and 5.2 mg%. Five patients with carcinoma of gall bladder had elevated bilirubin levels ranging from 3.5-22.6 mg% (median 11.2 mg%). Amongst 6 patients with obstructive jaundice 4 had portal nodes whereas 2 had bile duct tumor. The bilirubin levels ranged from 17.0-25.3 mg% (median 20.8 mg%).

All cases studied belonged to the age group of 25-60 years and had histological evidence of malignancy. Age and sex distribution were similar between control and study group. All had normal renal functions and none had hemolytic disorders. Blood samples were allowed to clot at room temperature, centrifuged at 10000 rpm for half an hour and separated sera analysed the same day. Haemolysed samples were excluded. Quality control sera were run during analysis to check precision and accuracy of the estimation.

LAP was determined by the optimized kinetic method using leucine p-nitroanilide as substrate

according to the recommendations of the German Society of Clinical Chemistry (8). AST and ALT were determined by the optimized standard UV method (9). GGT was determined by the quantitative method of Szasz using L-gamma-glutamyl-3carboxy-4-nitroanilide as substrate (10). ALP was estimated by optimised standard method using p-nitrophenylphosphate (11). The AST/ALT (Deritis ratio) and GGT/AST ratio were calculated. Measurement of all the enzymes was carried out on a fully automated discrete random access Hitachi 717 autoanalyser.

Students 't' test for unpaired numbers was used for statistical analysis after confirming homogeneity of variances. Cochran's correction was utilised in a group with heterogeneous variance.

RESULTS AND DISCUSSION

On comparing the mean levels of serum bilirubin, AST, ALT, AST/ALT ratio, GGT, GGT/AST ratio, LAP and ALP of normals and patients with hepatobiliary malignancies it was found that the mean levels of all the parameters were raised in malignancies in a highly significant ($p < 0.001$) (Table 1) way. The levels of bilirubin, AST, ALT, AST/ALT, GGT, GGT/AST, LAP and ALP were elevated in obstructive jaundice, carcinoma of pancreas, and periampullary carcinoma ($p < 0.001$). In hepatocellular carcinoma except GGT/AST ratio all the other parameters were elevated significantly ($p < 0.001$) whereas in carcinoma of gall bladder AST/ALT ratio did not show any significant increase. In metastatic liver disease total bilirubin and ALT did not show a significant increase.

Table 2 gives a comparison between normals and jaundiced and non jaundiced patients with hepatobiliary malignancy. The levels of all the enzymes and AST/ALT and GGT/AST ratio except ALT were elevated significantly ($p < 0.001$) in non jaundiced groups. The levels of the enzymes in jaundiced group when compared with non jaundiced group showed 2.4 times increase in AST and ALT, 2.4 times increase in GGT, 1.7 times increase in LAP and 2.0 times increase in ALP. AST/ALT and GGT/AST ratio did not show any increase on comparing the jaundiced and non

Table 1. Serum levels of bilirubin, aspartate transaminase (AST), alanine transaminase (ALT), AST/ALT, gamma glutamyl transpeptidase (GGT), GGT/AST, leucine aminopeptidase (LAP) & alkaline phosphatase (ALP) in normal subjects, patients with hepatobiliary malignancy, hepatocellular carcinoma, liver metastasis, obstructive jaundice, gall bladder carcinoma, carcinoma of pancreas and periampullary carcinoma.

Group	No of cases		Bilirubin (mg%)	AST u/l	ALT u/l	AST/ALT	GGT u/l	GGT/AST	LAP u/l	ALP u/l
Normals	25	Mean	0.292	21.72	25.32	0.87	19.4	0.949	38.16	189.36
		± SD	0.135	6.5	7.781	0.223	7.77	0.363	5.38	38.19
		Range	0.1-0.9	9-33	14-48	0.47-1.36	10-44.9	0.345-1.61	28-44	102-255
Patients with hepatobiliary malignancy	52	Mean	5.99*	106.15*	68.35*	1.836*	194.2*	2.381*	106.08*	794.62*
		± SD	9.216	92.91	55.41	1.339	187.75	2.02	59.1	635.69
		Range	0.1-43	10-438	7-257	0.631-8	20.3-736.3	0.097-8.566	42-324	223-2428
Hepatocellular carcinoma	12	Mean	1.038*	166.83*	63.42*	2.597*	137.19*	1.086	96.58*	512.08*
		± SD	0.726	135.61	33.22	1.331	114.71	0.963	48.36	278.43
		Range	0.3-2.54	49-438	20-147	1.07-5.76	30.3-446	0.4-3.912	44-191	230-1260
Liver metastasis	12	Mean	0.942	66.5*	48.58	1.44	127.19*	2.301*	73.33*	514.83*
		± SD	1.439	65	45.52	0.722	109.77	1.256	33.98	359.56
		Range	0.11-5.21	10-247	7-180	0.63-3.57	20.3-400	0.634-4.762	42-140	134-1516
Obstructive jaundice	6	Mean	17.41*	96.83*	79.17*	1.768*	388.23*	4.631*	128.17*	1271.83*
		± SD	8.27	42.53	52.82	0.952	269.67	3.086	46.52	738.8
		Range	0.18-25.28	35-167	10-168	0.631-3.5	52.4-736.3	0.528-8.566	72-206	290-2315
Gall bladder carcinoma	9	Mean	6.83*	82.78*	82.22*	1.12	185.73*	2.365*	114.67*	962.22*
		± SD	7.367	52.97	65.08	0.27	150.83	1.108	48.14	679.04
		Range	0.1-22.5	26-153	22-230	0.67-1.63	31.7-506.3	0.61-4.423	54-186	223-2352
Carcinoma of pancreas	6	Mean	0.613	78.17*	51.83*	1.73*	178.98*	2.755*	108.5*	706.67*
		± SD	0.242	58.6	44.54	1.029	152.46	1.761	50.82	676.08
		Range	0.16-0.9	29-200	24-150	0.97-4	33.9-455	0.32-5	55-198	239-2200
Periampullary carcinoma	7	Mean	16.95*	132.14*	78.14*	1.82*	264.41*	2.519*	146.43*	1176.86*
		± SD	12.23	78.23	35.15	0.86	237.44	2.489	93.63	663.85
		Range	3.85-43	70-300	34-138	0.97-3.44	29-650	0.097-7.963	62-324	409-2380

* P<0.001.

Table 2. Serum levels of bilirubin, aspartate transaminase (AST), alanine transaminase (ALT), AST/ALT, gamma glutamyl transpeptidase (GGT), GGT/AST, leucine aminopeptidase (LAP) & alkaline phosphatase (ALP) in normal subjects, jaundiced and non-jaundiced patients with hepatobiliary malignancy.

Group	No of cases		Bilirubin (mg%)	AST u/l	ALT u/l	AST/ALT	GGT u/l	GGT/AST	LAP u/l	ALP u/l
Normals	25	Mean	0.292	21.72	25.32	0.87	19.4	0.949	38.16	189.36
		± SD	0.135	6.5	7.781	0.223	7.77	0.363	5.38	38.19
		Range	0.1-0.9	9-33	14-48	0.47-1.36	10-44.9	0.345-1.61	28-44	102-255
Jaundiced	24	Mean	12.49*	154.17*	95.5*	1.858*	286.08*	2.465*	136.33*	1083.54*
		± SD	10.28	101.58	49.5	1.191	214.85	2.143	61.96	642.18
		Range	1-43	54-438	30-230	0.63-5.76	29-736.3	0.097-7.963	44-324	278-2380
Non jaundiced	28	Mean	0.436	65*	40.18*	1.705*	115.44*	2.311*	80.14*	538.82*
		± SD	0.248	59.3	29.56	0.928	111.63	1.901	41.7	485.03
		Range	0.1-0.9	10-300	7-150	0.83-4.05	20.3-455	0.313-8.566	42-198	134-2200

* P<0.001

Table 3. Frequency of elevation of bilirubin, aspartate transaminase (AST), alanine transaminase (ALT), AST/ALT, gamma glutamyl transpeptidase (GGT), GGT/AST, leucine aminopeptidase (LAP) & alkaline phosphatase (ALP).

Group	Bilirubin	AST	ALT	AST/ALT	GGT	GGT/AST	LAP	ALP
Jaundiced	24/24 (100)	24/24 (100)	20/24 (83.3)	12/24 (50)	23/24 (95.8)	14/24 (58.3)	23/24 (95.8)	24/24 (100)
non jaundiced	0/28 (0)	19/28 (67.9)	6/28 (21.4)	14/28 (50)	20/28 (71.4)	15/28 (53.6)	26/28 (92.9)	23/28 (82.1)

Figures in parentheses denote percentages. Values above 33 μ l for AST, 48 u/l for ALT, 1.36 for AST/ALT, 44.9 u/l for GGT/AST, 44 u/l for LAP and 255 u/l for ALP were considered as elevated.

jaundiced group indicating that bilirubin could be the cause of such elevated enzyme levels.

Table 3 gives frequency of elevation of all the enzymes and AST/ALT and GGT/AST ratio. In jaundiced group AST was elevated in 24 of the 24 cases (100%), ALT showed a rise in 20 of the 24 cases (83.3%), GGT was elevated in 23 of the 24 cases (95.8%), ALP in 24 of the 24 cases (100%) and LAP was elevated in 23 of the 24 cases (95.8%) whereas the AST/ALT and GGT/AST were elevated in 12 of the 24 cases (50%) and 14 of the 24 cases (58.3%) respectively. In non jaundiced group AST was elevated in 19 of the 28 cases (67.9%), ALT in 6 of the 28 cases (21.4%), GGT in 20 of the 28 cases (71.4%), ALP in 23 of the 28 cases (82.1%) and LAP in 26 of the 28 cases (92.9%) whereas AST/ALT was elevated in 14 of the 28 cases (50%) and GGT/AST was elevated in 15 of the 28 cases (53.6%).

Rise in LAP seen in both jaundiced and non jaundiced group was due to hepatic dysfunction whereas the other enzymes showed a greater rise in jaundiced group as compared to non jaundiced group

indicating that hepatic dysfunction with jaundice was the cause of elevated levels. LAP rises with hepatic dysfunction irrespective of jaundice and thus is a better indicator of hepatobiliary malignancy. Rutenburg et al (12) observed significantly elevated levels of urinary and serum LAP in all patients with cancer of pancreas, but patients with malignant lymphoma had increased urinary activity with normal or slightly elevated serum levels. An increase in serum activity of the enzyme during pregnancy and progressive fall in activity in immediate post partum period have been reported previously (13,14). Hoffman et al (15) found elevated levels of LAP in benign disease with normal levels in carcinoma. Thus, one cannot correlate the enzyme levels with neoplastic disease. Sathe et al (16) found that serum LAP can be used for detecting hepatic involvement in gastrointestinal tract cancer.

Conclusion

Our study reveals that this simple and reliable test of LAP determination is a better indicator of hepatobiliary malignancy as compared to the other conventional biochemical liver function tests.

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