Is liver function test of any diagnostic relevance in patients presenting with hepatocellular carcinoma?

Original Article
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Received: March 30th, 2011
Accepted for Publication: October 31st, 2011

ABSTRACT

Background: Hepatocellular carcinoma (HCC) is a tumor with very poor prognosis in Nigeria because of late diagnosis. This underscores the need for cheap and available investigations to aid early diagnosis. Liver function test (LFT) is affordable, available and minimally invasive. The recognition of specific fluctuations in liver function test in HCC will facilitate pragmatic clinical, radiological and histopathological evaluation in individuals at risk.
Aim / Objective: To determine if specific fluctuations in LFT is suggestive of HCC in patients presenting with this tumor at a tertiary health center in South-East Nigeria.
Methods: This was a case-control study. Sera from 64 patients with HCC and 120 patients without HCC were analyzed for bilirubin, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP) and albumin.
Results: A total of 184 patients were studied. Among the patients with HCC, AST and ALT were elevated in 30(46.9%) and 31(48.4%) patients, respectively, while ALP was elevated in 33(52%). Hyperbilirubinaemia was present in 34(53%) and hypoalbuminaemia in 54(84.3%) of the patients. Except for bilirubin, LFT was more frequently abnormal in HCC than in non-HCC cases. However, the difference was not statistically significant between HCC and liver cirrhosis ($p > 0.05$).
Conclusion: No specific pattern of LFT is diagnostic of HCC. Abnormal LFT in a high risk patient should prompt urgent imaging and histopathological evaluation.

Keywords: *Hepatocellular carcinoma, Liver function test*

INTRODUCTION

Hepatocellular carcinoma is the fifth most common cancer globally and one of the foremost causes of cancer-related deaths.[1] In Nigeria, HCC is diagnosed after the development of clinical deterioration, by which time the patient survival is measured in months, due to paucity of diagnostic equipment and late presentation.[2] This is unlike what is obtained in developed countries where health facilities are adequate and HCC can be diagnosed early enough, often in asymptomatic individuals when it will still be amenable to curative therapeutic options.[3]

The term 'liver function test' (LFT) is a constellation of biochemical tests that assess both hepatic secretory and synthetic functions. The serum bilirubin level, aspartate aminotransferase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) assess hepatic secretory/excretory functions, while serum albumin evaluates hepatic synthetic function.

Over the years, highly sophisticated techniques have evolved in the diagnoses of liver diseases particularly ultrasonography, computed tomography, magnetic resonance imaging, digital subtraction angiography, and percutaneous/endoscopic liver biopsies. In spite of this, many clinicians continue to routinely request for LFT for a variety of liver diseases including hepatocellular carcinoma.

In a resource poor country like Nigeria, LFT is destined to retain its place in the clinical biochemical milieu because it is affordable, available and minimally invasive and also, the modern equipment needed for the newer investigative modalities are virtually non-existent in our primary and secondary health centers. With the exception of ultrasonography which can be found in most tertiary health institutions, the other major imaging modalities can only be found in a small number of Teaching Hospitals. Even then, the cost of the tests is prohibitive. Though the private health sector attempts to make up for the deficiencies in government owned hospitals, the skill and qualifications of some of these operators are highly questionable.Diverse opinions as to the value of LFT in the diagnosis of HCC have been documented by various authors.[4,5]

This study was undertaken to ascertain if LFT serves a worthwhile purpose in the evaluation of patients with HCC, and if fluctuations in its parameters can be used to facilitate further evaluation of high risk individuals.

METHODOLOGY

This was a hospital based study on the diagnostic value of liver function test in hepatocellular carcinoma. The study was carried out at the Nnamdi Azikiwe University Teaching Hospital (NAUTH) Nnewi. The Medical Out-patient Department and the Medical Wards of this hospital were the main sites of the study. Ethical clearance was obtained from the Nnamdi Azikiwe University Teaching Hospital Ethical Committee.

Sample size was calculated using a prevalence of 7%, a precision of 5% and a formula proposed by Lwanga *et al*.[6,7] Blood from 64 HCC patients, 60 patients with benign chronic liver diseases (20 chronic hepatitis and 40 liver cirrhosis) and 60 patients with diverse medical problems not related to the liver were used in the study. Each subject provided written, informed consent to conduct an interview, collect blood specimen, undergo a liver biopsy, and perform serological assays. All the cases were interviewed in person with the use of a structured questionnaire.

The diagnosis of HCC in all the patients was based on typical clinical features such as hard, nodular and tender hepatomegaly with or without arterial bruit, weight loss, elevated alpha-fetoprotein level and ultrasound finding of various sizes of masses in the liver which maybe hypoechoiec or hyperechoiec. The diagnosis of benign chronic liver disease in all of the patients was based on a combination of stigmata of chronic liver disease, reduced liver span <8cm, evidence of complications from cirrhosis such as ascites, hepatic encephalopathy, upper gastro-intestinal bleeding, laboratory evidence of chronic HBV and/or HCV infection and characteristic abdominal ultrasound finding of coarse irregular hepatic parenchyma which maybe small or enlarged, with increased echogenicity. All those who were eligible had liver biopsy and histological confirmation of the diagnosis.

Five milliliters(mls) of venous blood was drawn from the ante-cubital fossa under observance of universal precautionary measures for liver function test (bilirubin, AST, ALT, ALP and serum albumin). The samples were left to clot for a period of 30minutes at 37^0 C and then, spun at a rate of 4000rpm for 5minutes. The supernatant sera

were analyzed at the Nnamdi Azikiwe University Teaching Hospital Chemical Laboratory. The methods for bilirubin and albumin assay were that of Evelyn and Mallory and the brom-cresol green dye binding of Doumas *et al*, respectively.[8,9] The Raithel-Franknell[10] method was used for AST and ALT assays, while ALP was assayed using the King Armstrong colorimetric method.[10,11] The Pearson's *chi squared* test was used to test for association between categorical variables and the one-way ANOVA for association between continuous variables; *p-value* <0.05 was considered significant.

RESULTS

A total of 184 patients were studied. They were 64 patients with HCC, 40 patients with liver cirrhosis, 20 with chronic hepatitis and 60 patients with non-liver related conditions, with an age range of 19 - 86years, and a mean age of 50years. The results showed that for those with HCC, the range of serum bilirubin was 0.6-27mg/dl with a mean of 4.82±6.9; the range of serum ALT was 4-78IU/L with a mean of 20.8±14.7; serum AST was 4-181 IU/L with a mean of 25.04±25.2; serum ALP had a range of 5-672 IU/L with a mean of 130±123.7, and the range of serum albumin was 15- 52g/l with a mean of 29.3±6.4 (Table 1).

Table 1. Mean, median and range of LFT parameters in patients with HCC

LFT	Cut-off	Mean	Media	Range
Total S Bil (mg/dl)	1.2	4.82	1.35	0.6-27
ALP (IU/L)	92	130	95	5-672
ALT (IU/L)	15	20.8	14	4-78
AST (IU/L)	18	25	16	4-181
S Alb. (g/1)	35	29	29	15-52

Amongst those with liver cirrhosis, the range of serum bilirubin was 0.6-16mg/dl with a mean of 3.38±4.01; serum ALP was 14-476 IU/L and a mean of 84±82.4; serum ALT 4- 90 IU/L and AST from 8 - 100 IU/L, and their mean values were 21.9±20.7 and 24.1±24.3, respectively. The range of serum albumin was 14-38g/L with a mean of 28.2±6.92.

In patients with chronic hepatitis, the range of serum bilirubin was 0.3-1.3mg/dl with a mean of 0.66±0.2, the range of serum ALT was 3-43 IU/L and the mean was 12.7±8.47, serum AST was 4-41 IU/L with a mean of 12.5±7.66, and serum ALP ranged from 21-79 IU/L with a mean of 38.5±17.4.

In patients without liver disease, the range of serum bilirubin was 0.4-2.8mg/dl with a mean value of 0.82±0.35mg/dl, serum ALT was 4-21 IU/L with a mean of 12.6±2.94, AST was 5-23 IU/L and the mean was 14.2±3.36, ALP was 18-76 IU/L and the mean was 39.9±15.2, and serum albumin had a range of 22-55g/l and the mean was 38.0±17.03.

By comparing the mean value of LFTs in patients with HCC and in those without, there were statistically significant higher values in HCC patients than in patients with chronic hepatitis and non-liver diseases (p<0.01) respectively while for liver cirrhosis, LFTs were not statistically significantly different (Tables 2 and 3).

Table 2. Mean values of LFT in HCC, BCLD and NLD patients

Test	HCC	Chronic Hepatitis	Liver Cirrhosis	Non-Liver Disease
Total S Bil (mg/dl)	Mean value±SD	Mean value±SD	Mean value±SD	Mean value±SD
	4.82±6.9	0.66 ±0.18	3.38±4.01	0.82±0.35
ALP (IU/L)	130±123.66	38.45±17.36	84±82.4	39.85±15.19
ALT (IU/L)	20.75±14.68	12.7±8.47	21.9±20.7	12.61±2.94
AST (IU/L)	25.04±25.16	12.5±7.66	24.12±24.3	14.18±3.36
S Alb. (g/l)	29.25±6.36	35.4±5.23	28.15±6.92	38.0±7.03

Key: HCC = Hepatocellular carcinoma, BCLD = Benign chronic liver diseases, NLD = Non-liver disease

Table 3. Pairwise comparison of mean difference in LFT between HCC, CH, LC and NLD Patients

LFT	Mean Difference	Significance
Bilirubin		
HCC vs. NLD	3.99	0.000
HCC vs. CH	4.15	0.000
HCC vs. LC	1.44	0.69
ALP		
HCC vs. NLD	90.24	0.000
HCC vs. CH	91.64	0.000
HCC vs. LC	45.96	0.14
ALT		
HCC vs. NLD	8.14	0.000
HCC vs. CH	8.05	0.21
HCC vs. LC	-1.16	1.00
AST		
HCC vs. NLD	10.86	0.006
HCC vs. CH	12.49	0.005
HCC vs. LC	0.92	1.00
Albumin		
HCC vs. NLD	-8.75	0.000
HCC vs. CH	-6.15	0.002
HCC vs. LC	1.10	0.84

LFT = Liver function test, HCC = Hepatocellular carcinoma, CH = Chronic hepatitis
LC = Liver cirrhosis, NLD = Non-liver disease

This study showed that out of the 64 patients with HCC, 34 (53%) had hyperbilirubinaemia, and ALP was elevated in 33 (52%). Of those with elevated ALP, 25 (75.7%) had hyperbilirubinaemia while 8 (24.3%) had bilirubin levels within the normal values. The serum amino transferases (ALT and AST) were elevated in 30 (46.9%) and 31 (48.4%) patients, respectively, whereas hypoalbuminaemia was seen in 54 (84.4%) patients. Among those with liver cirrhosis, 23 (57.5%) had hyperbilirubinaemia, 12 (30%) had elevated serum ALP out of which 11(91.6%) had hyperbilirubinaemia and only 1(8.4%) patient had normal bilirubin level. Raised ALT and AST levels were documented in 17 (42.5%) and 15 (37.5%) patients, respectively; while 29 (72.5%) patients had hypoalbuminaemia.

In chronic hepatitis, 1 patient each (5%) had hyperbilirubinaemia and elevated AST, 5 (25%) each had elevated ALT and hypoalbuminaemia and none had elevated serum ALP. In those without liver disease, 3 (5%) had hyperbilirubinaemia, 6(10%) and 4(6.7%) had elevated serum ALT and AST levels, respectively, while 16 (26.7%) had hypoalbuminaemia, and all of the patients had ALP values within the normal range (Table 4). The relationship between bilirubin, HCC and liver cirrhosis in patients with raised serum ALP is shown in Table 4.

Table 4. Percentage of abnormal LFT results among those with and without HCC

TEST	HCC (%)	LC (%)	CH (%)	NLD (%)
TotalSBil (mg/dl)				
Normal	30(47)	17(42.5)	19(95)	57(95)
Elevated	34(53)	23(57.5)	1(5)	3(5)
ALP (IU/L)				
Normal	31(48)	28(70)	20(100)	60(100)
Elevated	33(52)	12(30)	0(0)	0(0)
ALT (IU/L)				
Normal	34(53)	23(57.5)	15(75)	54(90)
Elevated	30(47)	17(42.5)	5(25)	6(10)
AST (IU/L)				
Normal	33(52)	25(62.5)	19(95)	56(93.3)
Elevated	31(48)	15(37.5)	1(5)	4(6.7)
S Alb. (g/l)				
Normal	10(15.6)	11(27.5)	15(75)	44(73.3)
Reduced	54(84.4)	29(72.5)	5(25)	16(26.7)

LFT = Liver function tests, HCC = Hepatocellular carcinoma, CH = Chronic hepatitis
LC = Liver cirrhosis, NLD = Non-liver disease

Table 5. Relationship of bilirubin with HCC and Liver Cirrhosis in patients having elevated ALP

Bilirubin	HCC (%)	Liver Cirrhosis (%)	Chi-sq	*p*-value
Elevated	25(75.7)	11(91.6)		
Normal	8(24.3)	1(8.4)	1.392	0.23
Total	33(100)	12(100)		

DISCUSSION

The clinical significance of Liver function test (LFT) in the diagnosis of liver diseases is that it may show a predominantly hepatocellular necrosis or cholestatic pattern. However, a substantial number of patients show abnormalities in their LFT that characterize a mixed pattern of hepatic injury. With the exception of bilirubin, the other LFT parameterswere more frequently abnormal in HCC than in chronic hepatitis and liver cirrhosis - conditions which precede it - and this observation is in keeping with reports from other studies.[12] Although the mean values of all the LFTs except for ALT were higher in HCC than in the other chronic liver diseases, these differences were statistically significant for HCC and chronic hepatitis but not for liver cirrhosis.

The average bilirubin value was 4 times the upper limit of normal and 53% of the HCC patients had hyperbilirubinaemia. In liver cirrhosis, average bilirubin was 2.8times the upper limit of normal and 57.5% of patients had elevated bilirubin levels. The average bilirubin level was normal in patients with chronic hepatitis. This shows that hyperbilirubinaemia is not a distinguishing feature of HCC as was reported by Lopez *et al* in their series.[12] However, the serum bilirubin level is an indicator of prognosis (the higher the level, the worse the prognosis) and has been incorporated in the Child-Pugh's and Model for end-stage liver disease scores which are prognostic models for chronic liver diseases including HCC.[13]

Increased serum activity of ALP may result from cholestasis or compression of small intra-hepatic bile ductsby a tumor nodule. The average ALP in HCC was 1.4 times the cut-off value for this study, while the average value in other chronic liver diseases were within normal limits. Nevertheless, such mild to moderate elevations as observed in this study are seen in a wide range of liver diseases and are not specific to HCC.[14] On the other hand, it has been recognized that isolated rise in ALP without a corresponding rise in bilirubin is often a feature of focal intra-hepatic ductal obstruction by a tumour nodule and is a presumptive evidence for the presence of HCC.[15] Of the 33 patients with HCC that had elevated ALP, 8(24%) had normal serum bilirubin levels as against 1(8%) of the 12 patients with liver cirrhosis and elevated serum ALP, although this relationship was not statistically significant (p=0.23). Nevertheless, serum ALP may remain normal in extensive liver disease, and is not a reliable marker of the severity or prognosis of underlying liver disease.[16]

Elevated plasma activity of AST and ALT are regarded as indicators of hepatocellular necrosis. Marked elevations are seen in acute viral and toxic liver damage while mild to moderate elevations are characteristic of chronic liver diseases.[17] Our result showed only mild elevation which was slightly higher for AST than ALT (1.39 vs. 1.38 times the upper limit of normal) in HCC, while in liver cirrhosis, the elevation was more for ALT than AST (1.46 times and 1.34 times), respectively. In chronic hepatitis, the values were within normal. The percentage of patients with elevated aminotransferases was higher for HCC than liver cirrhosis and chronic hepatitis and it bears out previous observations that although aminotransferases are only mild to moderately elevated in chronic liver diseases, patients with HCC are more likely to have abnormal results and AST

values are somewhat higher than those of ALT.[4,12]

Serum albumin concentration is regarded as an index of hepatic synthetic function and in patients with liver disease, a value below the lower limit of the reference range is taken to imply chronicity. The mean value of 29g/l among HCC patients is significantly lower than the 38g/l and 35g/dl observed in patients without hepatic disease and chronic hepatitis, respectively but is not significantly different from the 28g/l seen in those with liver cirrhosis. The result of serum albumin in HCC in this study is comparable to the 28.5g/l reported by Lai *et al*, however, it is much higher than the 21g/dl reported by some other studies.[18,19] Hypoalbuminaemia was also more frequently a feature of HCC than other benign chronic liver diseases and is in keeping with reports from other studies.[12,19]

Some researchers had earlier reported that LFT may provide evidence for HCC when its parameters are considered collectively rather than individually, and proposed the following pattern of LFT as indicative of HCC: moderately elevated AST and ALT levels, rising ALP and normal bilirubin levels that only begins to rise as liver failure develops and serum albumin that is generally low.[20] In spite of these observations, our study showed that excluding albumin, LFT were deranged in approximately half of the patients with HCC and even then, it was not specific for HCC as all its parameters were not significantly different from what was observed in liver cirrhosis, its immediate precursor. However, LFT was significantly different between HCC and chronic hepatitis and non-liver disease patients.

In conclusion, LFT alone is inadequate in differentiating HCC from other benign chronic liver diseases especially liver cirrhosis. For the diagnosis of HCC, clinicians should direct scarce resources towards other modalities of investigation such as radiology and histopathology.

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