ORIGINAL ARTICLE

Tumour markers and hepatitis C virus Infection in Nigerian Patients with Liver Diseases

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ABSTRACTS

Background: *Early diagnosis of hepatocellular carcinoma (HCC) is very important and determination of serum levels of tumour markers in patients with chronic liver diseases could be of immense contribution to their management.*

Method: *Forty- two adult Nigerian subjects consisting of 14 healthy subjects (Controls) and 28 patients with primary liver mass (es) and histological diagnoses of liver cirrhosis (LC) ± chronic active hepatitis (CAH) and hepatocellular carcinoma ± LC were studied. Their blood samples were assayed for Hepatitis B Surface Antigen (HBsAg), antibodies to HCV (anti-HCV), alkaline phosphatase (AP), Aphafeotoprotein (AFP) and Ferritin.*

Results: *The patients had HCC (10) HCC+LC(4), LC+CAH (2) and LC (12). Serum ferritin>700ng/ml, AP> 375IU/ml and AFP>200IU/ml were detected in 32%, 11% and 32% of the patients respectively with corresponding specificities of 100%, 86% and 100%. Elevated serum levels of AFP and ferritin were found in patients with HCC ± LC while raised serum AP occurred in those having PHCC without LC. Only combination of either AFP or ferritin to AP gave significant increase in the diagnostic yield of HCC among the patients than the use of only AP. Elevated levels of serum AFP correlated with both HBV and HCV while raised serum levels of ferritin were associated with only with HBV.*

Conclusion: *Although combination of the tumour markers gave a higher diagnostic yield for HCC among Nigerian patients, serum AFP >200IU/ml seems the best tumour marker in the diagnosis of PHCC among the patients.*

KEY WORDS: AFP; AP; Feritin; HCV; HCC; Nigeria.

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INTRODUCTION

Of all the liver diseases, hepatocellular carcinoma (HCC) and liver cirrhosis (LC) (apart from metastatic liver diseases) are two common diseases associated with solid mass (es) or nodule(s) in the liver and both are of variable incidences world wide[1,2]. Both may be consequent to hepatitis B and C viral infections among other aetiological factors[2,3]. Chronic infections of either or both viruses may progress directly or via LC to HCC. Up to about 95% of the patients with HCC had associated LC[1]. Clinical features of the both diseases are protean. Early diagnosis of HCC is important especially in Nigeria where the majority of the patients with the disease present late in hospitals when only palliative therapy could be offered despite the different armamentaria of therapies[4]. To ensure early detection of the tumour, screening with tumour markers have been found useful[2] in patient's with chronic liver diseases. Serum ferritin and alpha-feotoprotein (AFP) have been reported to be of value in diagnosing the tumour among Nigerians[5,6]. However, patients with HCC have been found to have low serum levels of either serum ferritin or AFP. Combination of tumours markers such as serum ferritin and AFP in addition to serum alkaline phosphatase (AP) have been found useful in the diagnosis of HCC in some European populations[7,8]. Similarly, the sero-detection of these markers have been positively influenced by hepatitis B and C viral infections[6,7,9,10]. However, there has been paucity of data on these findings among Nigerians with HCC. Our study on tumour markers among Nigerian patients with primary liver mass(es) - PLM who reported at the University College Hospital (UCH), Ibadan, Nigeria is hereby presented.

MATERIALS AND METHODS

Forty- two adult Nigerian subjects who consented to involvement in the study were recruited and they comprised 14 healthy volunteers as controls and 28 patients each with PLM. The exclusion criteria for inclusion of the subjects in the study were presence of iron medication, fever, blood transfusion or donation within 6 months and evidence of metastatic liver disease at histology.

The controls were matched with the age and sex of the patients. Diagnoses of their diseases were made from clinical features, abdominal ultrasonography and the histological findings of liver biopsy specimens. Ten

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millilitres of blood was collected from each subject, distributed out into capillary tube (Packed Cell Volume estimation - PCV), Citrate bottle (Prothrombin time), plain and EDTA tubes for serology/tumour markers estimation and liver cell function tests respectively. The serum was tested for serum bilirubin, alanine (ALT) and aspartate (AST) aminotranferases, alkaline phosphatase (AP), total protein and albumin following standard routine laboratory methods using the Hitachi Auto-analyser. The serum was tested for the presence of HBV and HCV infections by assay of Hepatitis B Surface Antigen (HBsAg) and antibody to HCV (anti-HCV) using Enzyme Linked Immuno-abSorbent Assay method - ELISA (Murex Diagnostic Ltd, Datford, United Kingdom, Lot No K891510 for HBsAg and K886910 for Anti-HCV). Both serum AFP and Ferritin were assayed by Radio-ImmunAassay (RIA) method using Amersham AFP Kit IM97 and Ferritin Kit IM1051 respectively. The biochemical tests and serological work were carried out at the hospital Departments of Chemical Pathology and Virology. The patients also had their Pugh Child Score determined.

The study was conducted following ethical clearance given by the Joint UI/UCH Ethical Review Board. The data were statistically analyzed using appropriate instruments at a significant p-value <0.05.

RESULTS

The 2 groups of subjects studied had male / female ratio of 11:3 each, the 14 controls and 28 adult Nigerian patients with PLM aged 49 ± 14 and 52±13 respectively, Table I. The latter group consisted of 10, 4, 2 and 12 patients with histological diagnoses of HCC, HCC+LC, chronic active hepatitis (CAH) +LC and LC respectively. These patients had a lower PCV than the controls, p<0.001 and had Pugh Child Score of A (8 patients) and B (20). Serum ferritin>700ng/ml, AP> 375IU/ml and AFP>200IU/ml were detected only among the patients with PLM in 32%, 11% and 32% respectively. The respective specificities for diagnosing HCC among the patients were 100%, 86% and 100% and the corresponding sensitivities were 64%, 50% and 21% (Table II). Although, each of AFP and ferritin had greater sensitivity than AP for PHCC, p<0.05, AFP had the best diagnosing value. Elevated serum levels of AFP and ferritin were found in patients with histologically diagnosed HCC ± LC while elevated serum AP occurred only in those having HCC without LC.

The sero-prevalences of HBsAg and anti-HCV were similar among the patients and healthy subjects and HBV infection was commoner than HCV in both groups, p<0.05.

Serum AP was unrelated to the serum level of total bilirubin and AP's highest value of 703IU/ml was found in a patient with HCC who also had normal serum bilirubin level. The serum values of all the three tumour markers were unrelated to any of the liver function tests as well as the Pugh Child Score. Elevated serum AP was also unrelated to either HBV or HCV infection while elevated levels of serum AFP and ferritin correlated with both infections and only with HBV respectively, Table III. Elevated serum levels of AP, AFP and ferritin were unrelated with each other in the patients. Combination of either AFP or ferritin to AP gave significant increase in the diagnostic yield of HCC among the patients than the use of only AP (p<0.05 each). Furthermore, combination of AFP and ferritin resulted in an insignificant higher number of patients diagnosed of HCC. In addition, there was a rise in the number of patients diagnosed with HCC by combination of the three tumour markers over AP+AFP or AP + ferritin although it was not statistically significant.

Table I. Biodata and Investigations in Nigerian adult subjects

Parameters	PLM N=28	Normal N=14
Male/Female	22/6	11/3
Age (M ± SD)	52±13	49 ± 14
Duration of disease (M ± SD)weeks	19±11	Nil
Pugh Child score A	8	-
B	20	-
C	-	-
PCV (M ± SD)	35±8	43 ± 4
$HBsAg^+$	17(61)	6(43)
$Anti\text{-}HCV^+$	6(21)	2(14)
$HBsAg^+$ & $Anti\text{-}HCV^+$	4(14)	-
AFP >200ng/ml	9(32)	-
Ferritin >700ug/l	9(32)	-
ALP >375IU/l	3(11)	-
AFP or Ferritin	13(46)	-
AFP or Ferritin or ALP	13(46)	-
AFP or ALP	10(36)	-
Ferritin or ALP	9(32)	-

$HBsAg^+$- Hepatitis B surface antigen positive, Parenthesis-percentage, $Anti\text{-}HCV^+$ - Antibody to Hepatitis C Virus positive, AFP-Alpha-feotoprotein, AP -Alkaline Phosphatase, Primary Liver Mass (es) PLM, PCV -Packed Cell Volume.

Table II. Diagnostic roles of tumour marker for HCC in adult Nigerians with Primary Liver Mass (es)

Tumour markers	Statistical Parameters - %			
	Sensitivity	Specificity	P PV	NPV
AFP >200ng/ml	64	100	100	74
Ferritin >700ug/l	50	86	78	63
AP>375IU/l	21	100	100	56

NPV - Negative Predictive value PPV - Positive Predictive value, AFP Alpha-feotoprotein, ALP. Alkaline Phosphatase.

Table III. Tumour markers for HCC, HBV and HCV infections in adult Nigerians with Primary Liver Mass(es)

Patients	HBsAg		Anti-HCV	
	+ve	-ve	+ve	-ve
Primary liver mass(es)				
AFP >200ng/ml	8(28.6)	1(3.6)	2(7.1)	7(25)
Ferritin >700ug/l	7(25)	2(7.1)	2(7.1)	7(25)
ALP >375IU/l	3(10.7)@	-	-	3(21.4)@
Hepatocellular Carcinoma				
AFP >200ng/ml	8(57)##	1(7.1)	2(14.3)#	7(50)
Ferritin >700ug/l	6(42.9)**	1(7.1)	1(7.1)*	6(42.9)
ALP >375IU/l	3(21.4)@	-	-	3(21.4)@

@All the patients had Ferritin >700ug/l while only two had AFP >200ng/m **X^2 with Yates correction = 5.04, p=0.025 for Ferritin & HBsAginPLCC *P=0.64 for ferritin & HCV in PLCC
##P<0.00007 for AFP & HBsAg in PHCC, P<0.035 for AFP & HCV in PHCC. AFP- Alpha-feotoprotein, AP-Alkaline Phosphatase, Parenthesis -percentage, Anti-HCV+ - Antibody to Hepatitis C Virus positive, HBsAg - Hepatitis B surface antigen positive

DISCUSSION

Patients with PLM such as PHCC and LC are common in Nigeria particularly because of the endemicity of both HBV and HCV infections among other aetiological factors[3]. These patients account for a significant proportion in the morbidity and mortality of Nigerians[1]. Early diagnosis of HCC is important especially in poor economic resource countries such as Nigeria as this would ameliorate the gloomy picture of the patients when left undiagnosed until advanced stage.

Use of tumour markers such as AP, AFP and ferritin are of major contributions in the diagnosis of PHCC[8] hence their determination among Nigerian patients with PLM. Our study shows that elevated serum AP >375IU/ml was diagnostic of HCC in Nigerian patients compared to 300IU/l among European patients[7]. It is significant that elevated level of AP was present even in one patient without raised serum bilirubin level, an observation previously described a feature of HCC rather than benign liver disease[12]. Similarly, this study shows that elevated serum level of Ferritin > 700IU/ml was a better diagnostic value for HCC among Nigerian patients than the value of 400IU/ml previously described among similar group of patients[6] and is comparable to a value of 800ng/ml reported among Europeans patients with HCC[7]. However, serum AFP >200IU/ml compared to serum ferritin and AP has been shown as the best tumour marker diagnostic of PHCC among Nigerian patients[7,8]. It is not surprising that serum ferritin and AFP have better sensitivities than serum AP among the Nigerian patients because elevated serum AP was found only among the patients having HCC without LC, a type of tumour less common among Nigerian patients[13]. The differences in the sensitivities of these tumour markers are related to the uniqueness of the metabolic characteristics of each marker[7]. The non-correlation of any of the tumour marker with any of the parameters of liver function, PCV and the Pugh Child Score is not unexpected as these show their roles as tumour markers for HCC[7].

The observation of high rates of HBV and HCV infections among our subjects is similar to respective prevalence of 59% and 10.9% in previous report among similar group of Nigerian patients[11]. Also from this study, the observation of the correlation of both infections with serum AFP and only HBV infection with serum ferritin in patients with HCC is a pointer to the usefulness the two tumour markers in the diagnosis of HCC. Hence, AFP might be important in HCC patients having either / both HBV or / and HCV, serum ferritin in those with HBV[9,10] while AP may be useful in patients with HCC unrelated to HCV, HBV but due other aetiologies such as alcohol or inheritable metabolic disorders[7].

Combination of the tumour markers has been suggested to be useful in the diagnosis of HCC in patients[7,8,14]. This study shows that serum AP is diagnostic of HCC in only a small proportion of Nigerian patients and addition of either AFP or ferritin to AP gives a significant increase in the diagnostic yield of HCC among the patients. Furthermore, each of AFP and ferritin gives threefolds of the diagnostic value of AP for HCC and combination of AFP and ferritin may give a better but non-statistically significant diagnostic yield of HCC among Nigerian patients than the use of either marker singly. In addition, each marker seems to be unique in the diagnosis of HCC with different peculiarities because of the relationship of each one with HCV and HBV infections.

Overall, in view of the high specificity and sensitivity of AFP compared to those of either ferritin or AP in the

diagnosis of HCC among Nigerian patients, AFP seems the best tumour marker for the diagnosis of HCC among the patients. This observation is corroborated by previous reports especially among other populations of patients[2,7,8,14]. In view of the relatively few number of patients involved in this study, another one involving a greater number of Nigerian patients will be required to consolidate our findings.

In summary, serum ferritin>700ng/ml, AP> 375IU/ml and AFP>200IU/ml are diagnostic of HCC among Nigerian patients with liver diseases. Although combination of the three tumour markers offers some improvement in the diagnostic yield of HCC among Nigerian patients, serum AFP seems the best tumour marker in view of its comparatively higher specificity and sensitivity.

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