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THE DIAGNOSTIC VALUE OF SERUM ALPHAFETOPROTEIN FOR HEPATOCELLULAR CARCINOMA IN PATIENTS WITH CHRONIC LIVER DISEASE

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Abstract

Background: This study aims to determine the value of using Alpha Fetoprotein for early detection of Hepatocellular Carcinoma among patients with chronic liver disease in African population.

Methods: 50 patients with clinical and radiological evidence of Chronic Liver disease attending the gastroenterology clinic of Aminu Kano Teaching Hospital and 50 healthy volunteers were recruited by systematic sampling over 3 months. The patients were grouped according to their definitive diagnosis into: Hepatocellular carcinoma, Hepatitis B and C co-infection, Hepatitis B infection, Hepatitis C infection, Liver cirrhosis and unclassified/Miscellaneous. Alpha fetoprotein was assayed using an Electrochemiluminescence Immunoassay.

Results: Hepatocellular carcinoma accounted for 22% of the study group, while Hepatitis B and C co-infection 16%, Hepatitis B infection 12%, Hepatitis C infection 6%, Liver cirrhosis 34% and Miscellaneous group 10%. The validity of Alpha Fetoprotein for Hepatocellular Carcinoma at a cut-off value of >200IU/L were: Sensitivity- 73%, specificity-100% and predictive value positive-100% respectively.

Conclusion: AFP is useful for early detection of HCC in CLD patients' of African descent.

Introduction

Alpha fetoprotein (AFP) is a large serum glycoprotein that was discovered more than 50yrs ago from human fetuses obtained by hysterotomy.1 It is a protein that appears in both fetal and maternal serum. Levels normally fall as pregnancy advances until it becomes undetectable. Changes in the serum AFP during pregnancy is useful in detecting congenital abnormalities like Down's syndrome and spina bifida.2 Several other Studies have established the usefulness of serum AFP in detecting Hepatocellular Carcinoma (HCC). 3,4,5 Because the level of AFP correlates well with the clinical stage of HCC it is used in monitoring the disease. AFP has variable sensitivity of between 24.7% to 76% for HCC with a more variable specificity and PPV.6,7,8

Hepatocellular carcinoma (HCC) ranks as the fourth most common cancer worldwide with Sub- Saharan Africa accounting for one of the areas with a significantly high incidence. 9 The burden of such disease is quite evident in our environment as our gastroenterology units are loaded with such cases. In contrast to western countries, most of our patients present with advanced disease increasing the burden of such disease.10 Hepatocellular carcinoma is the third most frequent cause of death from cancer and the eighth most commonly occurring cancer in the world.11 HCC is the most common abdominal malignancy, representing 80-90% of primary liver malignancies around the world. It accounts for upto 31% of liver biopsies in patients with chronic liver disease and affect adults between ages 21 and 56yrs in Nigeria.12 In Africa, Southeast Asia and China, HCC accounts for up to 75% of all cancer cases mainly due to the high percentage of hepatitis B virus carriers.11

The disease is characterized by very few non-specific symptoms its early phase with most of the clinical features occurring when the disease is already advanced. The prognosis of HCC in Sub-Saharan Africa is generally poor with

patients usually presenting late with advanced disease.10 This contrasts to what is obtainable in developed countries where it is detected early through regular screening of at risk groups. . This improves the prognosis as the disease is detected when it is amenable to treatment. This screening methods utilize clinical suspicion, screening protocols, radio diagnosis, serology and histological techniques.

Alpha fetoprotein has not found much usefulness in our patients due to lack of access to reliable assay methods. With the recent availability of sensitive immunoassay based methods in most of our tertiary healthcare institutions it is likely that alpha fetoprotein will be of importance in detection and monitoring treatment of patients with HCC . Since early detection of HCC is the cornerstone of diagnosis and treatment, detection of raised or rising levels of serum AFP has been found useful for its early diagnosis. Various levels of AFP have been described as diagnostic of HCC in different populations. With the advent of highly sensitive immunoassays for the detection of AFP in this environment it is likely that there will be increasing demand for AFP test utilization in HCC patients. Hence this study aims to determine the usefulness of AFP in screening and monitoring of HCC in our population. To the best of the authors' knowledge no similar study has been conducted in our locality.

Materials and methods

Study Design

The study design was Cross Sectional, conducted at the Gastroenterology Clinic (Specialty Clinic) of AKTH, on patients with a clinical diagnosis of chronic liver disease. The control group comprised of healthy volunteers matched for age and sex with the study group. This group of participants were recruited at the blood donor and Vaccination center and comprise of 'healthy' blood donors and mothers who are six weeks post-delivery.
Before commencing the study, approval was obtained from the ethical research board of AKTH. Furthermore, informed written consent was obtained from all participants in the study after verbal explanation has been made to each participant.

The inclusion criteria include:

1. Clinical evidence of shrunken liver (span <8cm) or Enlarged Liver (span > 12cm) of >6 months duration ± Low plasma Albumin and prolonged Prothrombin Time.
2. Ultrasonographic/Computed Tomographic evidence of cirrhosis and/or tumour.
3. A Hepatitis B core antigen antibodies (IgM anti-HBc) negative and a positive result on one of the following tests:

A-Hepatitis B surface antigen (HBsAg), hepatitis B e antigen (HBeAg), or hepatitis B virus (HBV) DNA or
B- HBsAg positive or HBV DNA positive or HBeAg positive two times at least 6 months apart.

Study participants with testicular or Extragonadal Germ cell Malignancies were excluded.
Systematic random sampling was used to select patients for the study from the record unit of the clinic. Information regarding their bio-data, definitive diagnosis, and hepatitis B and C status was obtained from their case notes.
This was then followed by sample collection and analysis. The data was collected over a period of 3 months from July to September 2011 with an average recruitment of 5 cases per week.

Materials

Materials used for this research were sourced from the department of Clinical Biochemistry, AKTH, Kano. This include Elecsys 2010 auto analyzer (MODULAR ANALYTICS E170) which measures the AFP using ECL Technoogy and a Bench top centrifuge for sample processing.
Alpha-Fetoprotein- AFP reagent kit manufactured by Roche diagnostics. These consist of the following:
M- Streptavidin-coated microparticles and preservative (0.72mg/mL)
R1- Anti-AFP-Ab-BIOTIN, Biotinylated monoclonal anti-AFP antibodies (4.5mg/mL)
R2- Anti-AFP-Ab-Ru(bpy) Monoclonal anti-AFP anti-AFP antibodies labeled with ruthenium complex 12 mg/L.

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Collection and Preparation of Sample

5mls of blood was collected in a lithium heparin bottle with the aid of a vacutainer needle from the antecubital vein while adhering to standard protocols. The samples were immediately transported to the laboratory and the plasma was separated from the cells using a bench centrifuge at the speed of 1200xg for 5 mins. The Samples were immediately frozen and stored after separation.

Methods

Samples were analyzed by an Electrochemiluminescence based immunoassay using Elecsys 2010 automated analyzer (Roche Diagnostics). Normal precautions for handling all laboratory reagents, samples, laboratory wares and machines was observed. Quality control samples were assayed along with the test samples and analytical runs with control values that fall outside ± 2 standard deviation from the mean repeated.

Principle

Sandwich principle. Total duration of assay: 18 minutes.

1. Patient sample combine with biotinylated antibody and ruthenium-labelled AFP

First step, patient sample is combined with a reagent containing biotinylated AFP antibody and a ruthenium-labeled AFP-specific antibody in an assay cup. During a nine-minute incubation step, antibodies capture the TSH present in the sample.

In the second step (9 minutes), streptavidin-coated paramagnetic microparticles are added. During a second nine-minute incubation, the biotinylated antibody attaches to the streptavidin-coated surface of the microparticles.
After the second incubation, the reaction mixture containing the immune complexes is transported into the measuring cell; the immune complexes are magnetically entrapped on the working electrode, but unbound reagent and sample are washed away by ProCell.

The ECL reaction uses a ruthenium based conjugate and the chemiluminescent reaction is electrically stimulated to produce light. The amount of light produced is directly proportional to the amount of AFP in the sample.
Evaluation and calculation of concentration of the AFP are carried out by means of a calibration curve that was established using standards of known antigen concentration.

Ethical Clearance

Permission to carry out this study was obtained from the Ethical Committee of Aminu Kano Teaching Hospital, Kano, Nigeria: (Reference Number: AKTH/MAC/SUB/12A/P-3/VI/909).

Statistics

Descriptive statistics was utilized with the aid of Excel Spreadsheet in the analysis of data.

Results and Discussion

Sex Distribution of the Study Subjects

Of the 50 Study subjects 36 were males while 14 were females. This data shows a significant Male preponderance of CLD. Male to Female ratio of 2.6:1. Their age ranges from 45 years to 75 years, with a mean age of 53.

Aetiology of CLD

The patients were grouped according to the primary cause of chronic liver disease and the result is shown in table 1 below. The group includes 11 patients with a Clinical and radiological diagnosis of Hepatocellular carcinoma, 17 with an Ultrasound diagnosis of Liver Cirrhosis, 8 with chronic hepatitis B and C infection, 6 with chronic hepatitis B and 3 had chronic hepatitis C infection. 5 Patients with Chronic liver disease for which no definite cause is identified constitute the “undiagnosed cause” group while Hepatocellular carcinoma accounts for 22% of all Chronic liver disease cases.

Table 1. Grouping of the study participants according to Aetiology of CLD

S/N	CLD Etiology	Number of cases	Percentage of the disease occurence
1	Hepatocellular Carcinoma	11	22
2	Liver Cirrhosis	17	34
3	Hepatitis B and C	8	16
4	Hepatitis B only	6	12
5	Hepatitis C only	3	6
6	Undiagnosed cause	5	10
	Total	50	100

Validity of AFP for HCC Screening

For the diagnosis of HCC the commonly utilized high cut off value of >200 IU/L was used.[13] The Sensitivity, Specificity and Positive Predictive Value of AFP for HCC at a cutoff of 200 IU/L were 73%, 100% and 100% respectively.

Table 2. Relationship of Hepatocellular Carcinoma with elevated Serum Alpha Fetoprotein concentration (> 200IU/L) presented in a 2 by 2 table

	Hepatocellular	Carcinoma	
α-fetoprotein	Yes	NO	Total
Positive	8(A)	0(B)	8
Negative	3(C)	39(D)	42
Total	11	39	50

Cells A – D are shown in parenthesis
Sensitivity was calculated as (A / [A+C]) x100
Specificity was calculated as (D / [B+D]) x 100
Positive predictive value was calculated as (A / [A+B]) x100

AFP Levels in Various Forms of CLD

The table below show the mean AFP values in the various study groups

Table 3. Table Showing Mean±SEM AFP levels in the Study Groups

	STUDY GROUP	AFP(IU/L)
I	Hepatocellular carcinoma (n= 11)	606 ± 235
2	Hepatitis B and C Co-infection (n= 8)	221 ± 73.8
3	Hepatitis B infection (n=6)	196 ± 64.7
4	Hepatitis C infection (n= 3)	121 ± 23.9

5	**Liver cirrhosis (n= 17)**	**67 ± 31.9**
6	**Undiagnosed Causes (n= 5)**	**277 ± 65.4**
7	**Controls (n= 50)**	**6.3 ± 3.34**

NB: The values in the table above represent Mean of study group AFP levels

Discussion

Alpha-fetoprotein is a useful tumor marker for detecting HCC. As with many malignancies the cornerstone of achieving any hope for cure is to detect it early enough for complete surgical resection. Screening for HCC is advocated in high-risk populations to optimize early detection and treatment. The most common screening tools are AFP concentration and ultrasonography (USS), which are recommended at regular intervals for initial surveillance and follow-up of the patients with CLD.

This small scale study showed that there was a high prevalence of HCC accounting for 22% of all patients with Chronic Liver disease. The mean age of presentation of 53yrs in this study is comparable to studies done in Jos, Nigeria.[12]

There is a significant Male predominance 2.6:1 ($P<0.05$). This finding agrees with previous studies which hypothesize that this is due to androgen receptors on HCC or an increased prevalence of viral hepatitis, iron loading, and/or alcoholic cirrhosis in men.[14,15]

Table 2 shows the cause of Chronic Liver disease among the study subjects. The commonest was Liver Cirrhosis in 34% of the subjects. This observation is not unusual as liver cirrhosis is the final end result of most disorders that induce hepatocellular damage. HCC is the second commonest cause of CLD in this study underscoring the magnitude of the problem in our environment.

The validity of AFP for HCC in this study group was evaluated and is presented in a 2 by 2 contingency table (table 3). The sensitivity of 73% means that at the cut off value of > 200IU/L the test will detect 73% of cases of HCC. However the remaining 27% cases have AFP values less than the cut off. The test specificity of 100% is the most ideal for any screening test and means that at the cut off value of 200 IU/L there is no likelihood of any false positive and no case will be wrongly diagnosed with HCC. Out of all the measures of validity the predictive positive value (PPV) is the most useful in clinical setting. This is because PPV informs the physician of the probability that the patient actually has the disease given that the patient had a positive test result. However, it is influenced by the prevalence of the disease in the practice setting. The higher the prevalence, the greater is the PPV of the test and vice versa. This phenomenon has led to a wide range of results on the diagnostic value of AFP necessitating the need to evaluate it in our practice setting. The results showed that the PPV was 100% and thus support AFP as a powerful aid in the diagnostic workup of HCC. This result is different from that reported by Tong and colleagues[16] who conducted a seven year surveillance study of over 600 patients using AxSYM and reported sensitivity, specificity and PPV of 65%, 90% and 12%, respectively. The low PPV coincided with a 5% prevalence of HCC in their study sample compared to 22% in this study. The values in this study however agrees with studies by Ola[13] in the southern part of the country who reported a PPV of 100%, specificity of 100% and sensitivity of 64% at a high cut off value of 200 IU/L.
The mean serum level of AFP in patients with various forms of CLD is shown in table 4. Comparison of the mean values of various groups showed HCC (**606** IU/L)> HBsAg and HCV (**221** IU/L)> HBsAg (**196** IU/L)> HCV (**121** IU/L)>LC (**67** IU/L)> control (**6.3** IU/L) ($P<0.05$). It can be deduced from the values that the results for HCC are all above the high cut off value of 200IU/L with a mean of 606 IU/L. The high values recorded in this study may be due to the fact that most of our patients presented late.

This data also showed that the levels of AFP in patients with Hepatitis B and C co-infection is much higher than that in either alone suggesting a dual role of both viruses in inducing production of AFP. It can also be deduced that hepatitis B infection is more efficient in inducing AFP production than hepatitis C Virus as the mean level is significantly higher than in the former ($p<0.05$). The results confirm further that the secretion of AFP is augmented by Hepatitis B virus as the virus is incorporated into the hepatocyte DNA. Therefore AFP is useful in screening for HCC in patients with HBV infection. This result is similar to that reported by Olubiyide et. al[17] among Nigerian patients. The result of AFP in patients with HCV infection also demonstrates the role of HCV in inducing AFP production. Other workers have described the limitations of AFP in patients with HCV infection.[18]

Patients with liver cirrhosis display only mild elevations in AFP as the mean value in the group is low (67IU/L). This is because in cirrhosis there is marked destruction of the hepatocytes with replacement fibrosis hence only a few parenchymal cells are available to produce AFP.

The level of serum alpha fetoprotein in the control group was significantly lower than that in the study subjects. All the healthy subjects in this group have levels lower than 20 IU/L. The mean level in the group is 6.3IU/L. This agrees with several studies within and outside Nigeria.[13,19,20]

Conclusion

The results of this study demonstrated that serum Alphafetoprotein is a very useful tool for detecting Hepatocellular carcinoma in patients with chronic liver disease in black African setting.

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