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The Combined Diagnostic Accuracy of Serum Alpha Fetoprotein and Des-Gamma Carboxyprothrombin in Hepatocellular Carcinoma among Chronic Liver Disease Patients in Ilorin

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Abstract

Introduction: Chronic liver disease (CLD) is a disease of public health importance. CLD is defined as a clinical syndrome of liver disease lasting for at least six months with histology showing varying degree of hepatocellular necro-inflammation and fibrosis with or without neoplastic transformation. The disease is a spectrum that manifests initially as chronic hepatitis which may progress to liver cirrhosis and ultimately hepatocellular carcinoma (HCC). The current practice in the field of Gastroenterology has shifted from invasive methods of diagnosing HCC to non-invasive methods using tumor biomarkers. Various biomarkers of HCC have been proposed, but the largest body of evidence exists with alpha-fetoprotein (AFP). Most of the studies on the combined diagnostic accuracy of AFP and des-gamma-carboxyprothrombin (DCP) were done in other populations outside Nigeria. It is necessary to determine the combined diagnostic accuracy of the two tumor markers for early detection of HCC in North-central Nigeria. **Materials and Methods:** This study was a cross-sectional study and ethical clearance was obtained from the ethical and research committee of UITH, Ilorin. A total of 190 participants consisting of 125 cases and 65 healthy controls that were age and sex-matched were studied. Patients with extra-hepatic malignancies were excluded. The serum levels of AFP and DCP were determined using the enzyme-linked immunoassay (ELISA) technique. A detailed questionnaire was used to document the socio-demographic characteristics, clinical features as well as results of laboratory/radiologic parameters. Percutaneous liver biopsy was carried out on patients that were fit. Test of association between categorical va-

riables was carried out using the Chi-Square Test. The sensitivity, specificity, positive and negative predictive values of the two tumor markers were determined by the area under curve (AUC) at various cut-off levels using the receiver operating characteristic (ROC) curve analysis. Statistical significance was set at p value < 0.05. AFP Quantitative test kit (alfabeto-RiakiDainabot Radioisotope laboratory, Japan) and DCP Qualitative test kit (EiTest MONO P-II kit) were used to assay AFP and DCP respectively. Liver biopsy needle (Menghini needle) was used to carry out liver biopsy. **Results:** Using a cut-off of 400 ng/ml, the sensitivity of serum AFP for diagnosing HCC was 51.3%. The specificity of AFP at the same cut-off was 87.8%. The positive and negative predictive values were 92.8% and 49.3% respectively. Using a cut-off of 7.5 ng/ml, the sensitivity of serum DCP for diagnosing HCC was 57.1%. The specificity of DCP at the same cut-off was 63.4%. The positive and negative predictive values were 76.2% and 41.9% respectively while the accuracy was 59.2%. The diagnostic accuracy of combined serum AFP and DCP for diagnosis of HCC in University of Ilorin Teaching Hospital, Ilorin was 64.9%. The sensitivity of combined serum AFP and DCP for diagnosing HCC was 55.6%. The specificity of combined serum AFP and DCP was 95.6%. The positive and negative predictive values were 96.2% and 52.3% respectively. **Conclusion:** Combining these two tumour markers does not significantly improve the diagnostic accuracy of HCC and chronic HBV remains a strong aetiological agent of HCC in UITH, Ilorin.

Keywords

Necro-Inflammation, Liver Cirrhosis, Hepatocellular Carcinoma, Alpha Fetoprotein, Des-Gamma-Carboxyprothrombin, Enzyme-Linked Immunoassay, Percutaneous, Tumor Markers

1. Introduction

Chronic liver disease (CLD) is a disease of public health importance. CLD is defined as clinical features of liver disease lasting for at least six months with histology showing varying degree of hepatocellular necro-inflammation and fibrosis with or without neoplastic transformation [1]. The disease is a spectrum that manifests initially as chronic hepatitis which may progress to liver cirrhosis and ultimately hepatocellular carcinoma (HCC). Liver disease is an important cause of morbidity and mortality globally with a varying prevalence in different geographical locations. Liver disease accounted for 7.9% of medical admissions at the University of Nigeria, Enugu [2].

Hepatocellular carcinoma is a primary neoplasm of the liver. Although its incidence is increasing worldwide, striking geographical differences are observed for both risk factors and occurrence [3]. The incidence in developing countries is two to three times higher than in developed countries and it represents more than 5% of all cancers [3]. HCC is the fifth most common cancer worldwide, and the third most common cause of cancer mortality [4]. The highest age-adjusted

incidence rates were reported from countries in south-east Asia and sub-Saharan Africa (SSA) where hepatitis B virus (HBV) endemicity is high [5]. Liver cirrhosis is present in about 80% - 90% of HCC patients and is thereby the largest single risk factor. The other risk factors include hepatitis C virus (HCV), aflatoxins, hepatitis D virus (HDV) in the background of HBV infection, and possibly obesity and diabetes mellitus [5] [6]. The presence of several well-documented environmental potentially preventable risk factors such as aflatoxins, non-alcoholic fatty liver disease (NAFLD) and alcoholic liver disease (ALD) are also important [4]. This study aims to assess the combined diagnostic accuracy of serum AFP and DCP in HCC by studying a group of patients with CLD at UITH. Findings generated from this study may allow for early detection of HCC using AFP and DCP which may avail patients the opportunity of treatment with curative intent.

2. Material and Methods

2.1. Study Setting/Location

This study was a hospital-based cross-sectional study and was carried out on patients of Department of Internal Medicine, University of Ilorin Teaching Hospital, Ilorin from April 2018 to February 2019. A total of 125 adult subjects (both male and females) and 64 age- and sex-matched controls greater than 18 years, were used in this study.

2.2. Study Design

This study was a hospital-based cross-sectional study.

2.3. Study Location

This study was carried out in a tertiary care teaching hospital at the Gastroenterology and Hepatology Clinic, Medical wards and Medical emergency unit of the University of Ilorin Teaching Hospital.

2.4. Sample Size

125 patients.

2.5. Sample Size Calculation

The required sample size was determined using Fisher's statistical formula for estimating minimum sample size for cross-sectional studies [7]. The target population from which we selected our sample using convenient sampling technique was less than 20,000. The sample size actually obtained using Fischer's exact formula was 112. However, we used 125 patients and 65 controls putting an attrition rate of 10%.

2.6. Subjects and Selection Method

The study population was drawn from chronic liver disease patients who presented to the University of Ilorin Teaching Hospital (UITH) Ilorin between

April 2018 and February 2019.

2.7. Inclusion Criteria

1) All consenting patients aged 18 years or older.

2) Patients with histopathologic evidence of CLD or cytologic evidence of HCC (with clinical features of CLD such as jaundice, parotid fullness, loss of axillary hair, spider naevi, gynaecomastia in males, finger clubbing, leuconychia, loss of thenar and hypothenar eminences, testicular atrophy in males and bilateral pedal oedema).

2.8. Exclusion Criteria

1) Patients below 18 yrs of age.

2) Patients with known extra-hepatic malignancies presenting with or without hepatomegaly.

3) Patients with extra-hepatic malignancy (such as gastric cancer, pancreatic cancer, biliary cancer, teratoma) which are known to release serum alpha-fetoprotein.

4) Patients with acute liver disease.

3. Literature Review

3.1. Epidemiology of Hepatocellular Carcinoma

Hepatocellular carcinoma ranks as the fifth most common cancer in the world, and the third most common cause of cancer mortality [8]. An estimated total of one million new cases occur each year [9]. The East and Southeast Asia, and sub-Saharan Africa (SSA) are regarded as the high-risk regions [10]. The incidence is about two to three times more in men than in women, this sex ratio is more pronounced in the high-risk regions [10]. The prevalence of HCC in Nigeria is between 0.4% - 17.0% [11]. The collection and analysis of epidemiologic data of HCC will play a critical role in guiding future disease prevention strategies and optimizing patient management [8]. Chronic HBV infection is by far the most important risk factor for primary HCC in humans, and it is estimated that 80.0% of HCC worldwide is aetiologically associated with HBV [10]. Other risk factors associated with HCC are HCV, aflatoxins, alcohol, metabolic disease such as haemochromatosis, Wilson's disease, hereditary conditions such as alpha1 anti-trypsin (AAT) deficiency and autoimmune hepatitis [12]. Obesity and diabetes mellitus can lead to non-alcoholic steatohepatitis (NASH), which is also an established risk factor for HCC, most likely via progression of the steatohepatitic disease to cirrhosis and HCC [13]. Estimates of the true prevalence of the tumor in most areas of the SSA is difficult as majority of the people live in the rural areas and do not present to the hospital hence, do not have record in a cancer registry. HCC does not have a uniform world-wide distribution, majority occur in resource constrained areas. In high incidence areas, HCC accounts for 90.0% to 95.0% of all primary malignant tumors of the liver, whereas in regions

with a low or intermediate incidence it accounts for 70.0% - 85.0% of these tumors [14]. Another region of concern is SSA, particularly the western region of Africa, including Gambia, Guinea, and Mali, and also the Republic of Mozambique in south-east Africa [15].

3.2. Pathogenesis of Hepatocellular Carcinoma

Malignant transformation of hepatocytes may occur as a consequence of various aetiologies, such as chronic viral hepatitis, alcohol, and metabolic disorders, in the context of increased cellular turnover induced by chronic liver injury, regeneration and cirrhosis [16]. Activation of oncogenes, inactivation of tumour suppressor genes, genomic instability, including defects in DNA mismatch repair and impaired chromosomal segregation, over expression of growth and angiogenic factors and telomerase activation all contribute to the development of HCC [16].

Hepatocarcinogenesis is a slow and progressive process during which genomic changes alter the hepatocellular phenotype to produce cellular intermediates that evolve into HCC [17]. During the long pre-neoplastic stage, in which the liver is often the site of chronic hepatitis, cirrhosis, or both, hepatocyte cycling is accelerated by up-regulation of mitogenic pathways, in part through epigenetic mechanisms [17]. Continuous hepatic insult leads to development of dysplastic hepatocytes that have telomerase re-expression, sometimes microsatellite instability, and occasionally structural aberrations in genes and chromosomes [17]. In chronic HBV infection, both viral and host factors have been implicated in hepatic carcinogenesis. Most HCC contain clonally integrated HBV DNA and microdeletions in the cellular DNA, which could deregulate cellular growth control mechanisms (**Figure 1**).

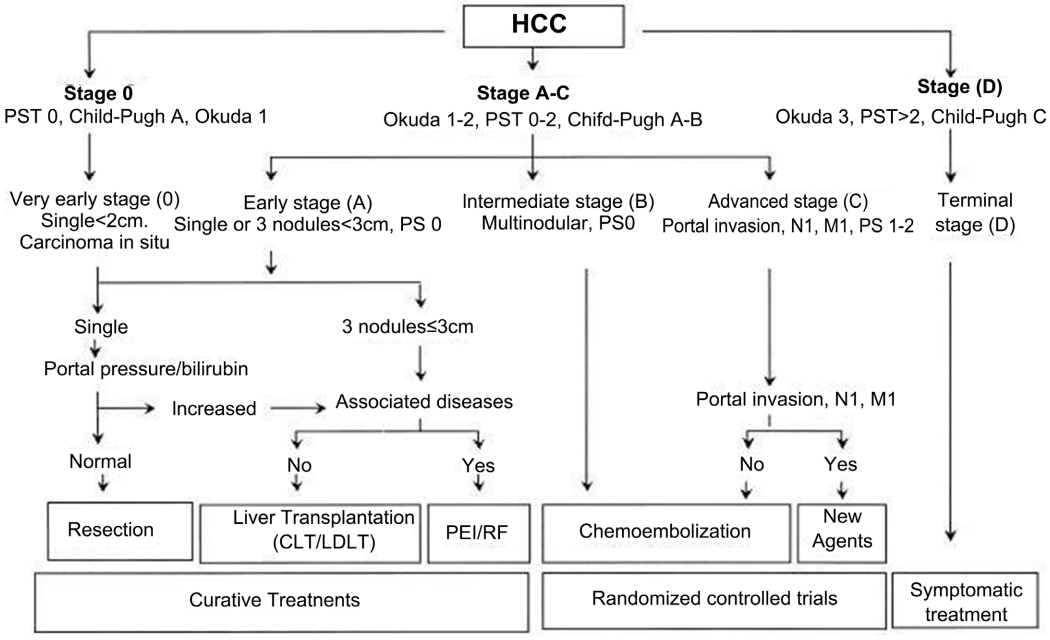

Figure 1. Adapted from journal of clinical and experimental hepatology, 2014 Courtesy of Elseiver.

3.3. Clinical Features of Hepatocellular Carcinoma

The commonest symptoms are anorexia and malaise [18]. Other features are weight loss, abdominal pain, jaundice, fever, recurrent vomiting, ascites, hepatomegaly and bilateral pedal edema [19]. Patients could also have hepatic bruit as a result of angiogenesis by the tumor cells. The paraneoplastic syndromes associated with HCC are hypoglycemia, hypercholesterolaemia, hypercalcaemia and erythrocytosis [20]. HCC is characterized by extrahepatic metastasis. In a study carried out by Uka *et al.* [21], the incidence of extrahepatic metastasis was found to be 15.2%. In this study, the most frequent metastatic sites were the lungs, lymph nodes, bones, and adrenal glands. HCC is thought to spread mainly *via* the haematogenous route, thus causing intra/extra hepatic metastases.

3.4. Diagnosis of Hepatocellular Carcinoma

A diagnosis of HCC is frequently made in the presence of supporting clinical features. From the time of diagnosis, patient's survival is usually measured in months. Serum AFP used alone can be helpful if levels are markedly elevated, but this occurs in fewer than half of cases at the time of diagnosis [22]. There are other serum markers currently in use in clinical practice as a method for early detection of HCC.

3.5. Alpha-1 Fetoprotein

Under physiological conditions, AFP is a foetal specific glycoprotein with a molecular weight of around 70 kDa [15]. AFP is more useful in HCC of non-viral aetiology than viral induced liver cancer [23] [24]. Serum AFP of more than 500 ng/ml is strongly suggestive of HCC [25]. High serum AFP levels have been found in 60% - 70% of patients with HCC, nevertheless, there are other causes of increased levels, such as liver cirrhosis, lung cancer, biliary cancer, gastric cancer, pancreatic cancer, teratocarcinoma of the testis, and tyrosinaemia [26]. AFP levels < 20 ng/ml are considered normal [25]. In tumors that secrete AFP, the concentration is related to the size of the tumor and less sensitive for small cancer less than 3 cm [27]. Monitoring of HCC with six monthly AFP assay with USS has been advocated. The serum AFP of HCC patients is characterized by fraction that reacts with lens culinaris (L3) [28]. Some clinical researchers have indicated that the simultaneous determination of supplementary markers along with AFP could significantly increase the sensitivity in the diagnosis of HCC [29]. Serum AFP levels can be determined using a) Electroimmunodiffusion, and b) Counterimmunoelectrophoresis [30]. Electroimmunodiffusion method which was used in this study is simpler, quicker and more sensitive than counterimmunoelectrophoresis. HCC patients with a high AFP concentration (≥400 ng/ml) tend to have greater tumor size, bilobar involvement, portal vein thrombosis, and a lower median survival rate [31]. Total AFP can be divided into three different glycoforms, namely AFP-L1, AFP-L2 and AFP-L3 according to their binding capability to lectin lens culinaris agglutin (LCA). AFP-L1, as the non-LCA-

bound fraction, is the major glycoform of AFP in the serum of non-malignant hepatopathy patients [31]. On the contrary, AFP-L3, as the LCA-bound fraction, is the major glycoform of AFP in the serum of HCC patients [31]. The AFP-L3 can be detected in approximately 35.0% of patients with small HCC (<3 cm), especially when the tumor mass is supplied by the hepatic artery. HCC patients with percentage of serum AFP-L3 over 15.0% also showed a higher incidence of infiltrative type HCC with sensitivities ranging from 75% - 96.9% and specificities of 90% - 92.0% [31]. It also identifies a tumor with irregular margin ($p < 0.05$) and a higher frequency of poorly differentiated HCC ($p < 0.05$). Therefore, it could be used as a valuable indicator of poor prognosis.

3.6. Des-Gamma-Carboxyprothrombin

Des-gamma-carboxyprothrombin (DCP), is also called protein induced by vitamin K absence or antagonist II (PIVKA-II); it is an abnormal form of prothrombin [32]. DCP was discovered by Liebman *et al.* in 1984 and has since been used as an important tumor marker of HCC [32]. DCP is more accurate, albeit complementary to AFP as a marker of HCC [33]. DCP was found to have direct correlation with tumor size and was not elevated in any patients without HCC [34].

Marrero *et al.* reported that the sensitivity and specificity of serum DCP (at the cut-off value of 125 mAU/ml) in discriminating HCC from non-malignant hepatic disease were 89% and 86.7% respectively [31]. The cut-off of DCP used in this study was 100 mAU/ml (7.5 mg/dl). Serum DCP could also be used as a prognostic indicator for HCC patients, and may be more useful than AFP in reflecting the invasive characteristics of HCC [31]. It has been reported that patients with DCP sero-positive and AFP sero-negative have a higher frequency of primary HCC with a distinct margin, large nodule more than 3 cm, and poorly differentiated [31]. Moreover, the simultaneous determination of serum DCP levels and tissue DCP expression is more valuable than either fraction alone in predicting the prognosis of HCC patients. It has been shown that in HCC and increase in the prothrombin precursor concentration does not equate to vitamin-K-dependent carboxylase activity, this leads to an overproduction of prothrombin precursor with reduced γ-carboxylation [35].

3.7. Alpha-1-Fucosidase

Alpha-1-fucosidase (AFU) is an enzyme that hydrolyzes fucose glycosidic linkages of glycoprotein and glycolipids. Its activity increases obviously in the serum of HCC persons. It has been reported that the sensitivity and specificity of AFU at the cut-off 870 nmol/ml are 81.7% and 70.7% respectively [31]. It has been reported that HCC will develop within a few years in 82.0% of patients with liver cirrhosis, if their serum AFU activity exceeds 700 nmol/ml, and the activity of AFU has been found to be elevated in 85% of patients at least 6 months before the detection of HCC by USS [31]. Thus, serum AFU could be a good tumor

marker in detecting HCC at an earlier period.

3.8. Heat Shock Protein

Heat shock protein (HSP) is a highly conserved stress response protein. It can protect cells and promote them to repair the damage caused by a variety of stimuli. HSP is expressed under physiological and stress conditions, including carcinogenesis [36]. By immunohistochemical staining, Zhao *et al.* identified the positive rate of HSP 70 and HSP 27 to be 56.3% and 61.9% respectively in HCC tissues [35]. The stained intensity of HSP 70 was positively correlated with tumor size, portal vein invasion and tumour stage. HSP 70 may be used as an indicator of prognosis for HCC. Furthermore, the expression of HSP 70 is correlated with differentiation and apoptosis of tumour cells. It promotes tumour cell growth by stabilizing cyclin D1 and suppresses the apoptosis of tumour cells by inhibiting the p53 pathway [36].

3.9. Glypican-3

Glypican-3 (GPC3) is a family of heparin sulfate proteoglycans that is linked to the cell membrane by a glycosylphosphatidylinositol (GPI) anchor. GPC3 is involved in the process of regulating cell growth, development, differentiation and migration. The expression of GPC3 was up-regulated in HCC tumour tissues compared with normal and benign liver diseases and contributed to promoting the growth of HCC by stimulating wnt—a signaling transduction pathway which is a pro-tumorigenic growth factor [37]. The sensitivity and specificity in the diagnosis of HCC were found to be 77.0% and 96.0% respectively. On this strength, GPC3 is a potential marker for HCC [36].

4. Squamous Cell Carcinoma Antigen

It has been reported that squamous cell carcinoma antigen (SCCA), a member of the high molecular weight family of serine protease inhibitors and is strongly expressed in a number of different epithelial cancers such as cervix, head and neck tumors [36]. Recently, Stefaniuk *et al.* first reported a high SCCA expression in HCC tissues which seems very interesting, as the liver does not possess squamous epithelial cells [37]. The sensitivity and specificity for SCCA in HCC diagnosis are 84% and 46% respectively [37].

5. Result

All the controls recruited in this study were normal individuals. However, among the study subjects, 75 (60%) of them had jaundice while 92 (72.8%) had palmar erythema. Furthermore, leuconychia, finger clubbing and weight loss were seen in 78 (62.4%), 67 (54.4%) and 100 (80%) of the study subjects respectively. Abdominal pain was present in 98 (78.4%) of the patients' melaena in 30 (24.0%) of the patients and hematemesis 38 (34.4%) of the patients. Other clinical features are as listed in **Tables 1(a)-(c)**.

Table 1. (a) Comparison of socio-demographic characteristics of participants; (b) clinical features of the study subjects; (c) anthropometric measurements of study subjects and controls.

(a)

Variables	Patient (N = 125)	Control (N = 65)	X^2	p value
	n (%)	n (%)		
Age group (yrs)				
≤30	14 (11.2)	5 (7.7)	1.782	0.776
31 - 40	50 (40)	25 (38.5)		
41 - 50	42 (33.6)	21 (32.3)		
51 - 60	8 (6.4)	7 (10.8)		
≥61	11 (8.8)	7 (10.8)		
Mean ± SD	42.81 ± 10.77	44.05 ± 11.77	0.729**	0.463
Gender				
Male	88 (70.4)	44 (67.7)	0.148	0.701
Female	37 (29.6)	21 (32.3)		
Marital status				
Single	40 (32)	45 (69.2)	36.67[y]	<0.001
Married	50 (40)	10 (15.4)		
Divorced	10 (8)	10 (15.4)		
Widowed/Separated	25 (20)	0 (0.0)		

(b)

Clinical features	Frequency (n)	Percentage (%)
Pallor	20	16.0
Jaundice	75	60.0
Pedal edema	80	64.0
Hepatic bruit	55	44.0
Ascites	73	58.4
Gynaecomastia	30	30.0
Sparse axillary hair	80	64.0
Spider naevi	5	4.0
Leuconychia	78	62.4
Hepatomegaly	76	60.8
Palmar erythema	91	72.8
Finger clubbing	67	54.4
Parotid swelling	53	42.4
Hyperpigmentation	73	57.6
Weight loss	100	80.0
Anorexia	96	76.8

Continued

Abdominal pain	98	78.4
Melaena	30	24.0
Haematemesis	43	34.4

The controls are normal subjects

(c)

Variable	Study subjects	Control	T	p value
	Mean ± SD	Mean ± SD		
Weight	63.94 ± 8.38	64.0 ± 8.73	0.043	0.966
Height (m)	1.63 ± 0.07	1.64 ± 0.07	0.533	0.595
BMI (kg/m^2)	24.18 ± 2.81	24.19 ± 2.81	0.027	0.978

The mean height for the patients was 1.63 m while the mean height for the control was 1.64 m. There was no significant difference in mean of the height between the patients and the control at a p value of 0.595. The mean weight for the patients was 63.94 kg while the mean weight for the control was 64.0 kg. There was no significant difference in mean of the weight between the patients and control at p value of 0.966. The mean BMI of the patients was 24.18 ± 2.81 kg/m^2 and 24.19 ± 2.81 kg/m^2 for the control participants with a p value of 0.978. There was no statistically significant difference in mean BMI between the patients and control.

Table 2 shows the investigative modalities used in the diagnosis of HCC among the study subjects. Histology was done in 35 (41.7%) patients, 29 (34.5%) patients had FNAC performed which was subjected to cytology and 20 (23.8%) had abdominal CT scan.

Table 3 shows the comparison of the serum AFP and DCP between HCC patients and controls. The mean serum DCP for the subjects was 7.90 ng/ml while for the controls the serum DCP was 4.66 ng/ml. There was a significant difference in the serum DCP between the study subjects and controls at a p value of 0.001.

The mean serum AFP for the study subjects was 504.62 ng/ml while for the mean serum AFP for controls was 4.60 ng/ml. There was a significant difference in serum AFP between the study subjects and control at p value of 0.001.

Figure 2 depicts the spectrum of diagnoses among the patients. Majority of the patients had HCC 84 (67.2%), followed by liver cirrhosis 25 (20%) then, CHB 16 (12.8%).

Out of the 84 patients who were diagnosed as having HCC, 61 (72.6%) patients had serum DCP levels in the range of 0 - 10 ng/ml while 23 (27.4%) patients had DCP in the range of 11 - 20 ng/ml.

Out of the 16 patients who were diagnosed as having CHB, 14 (87.5%) patients had serum DCP levels in the range of 0 - 10 ng/ml while 2 (12.5%) patients had serum in the range of 11 - 20 ng/ml.

Table 2. Investigative modalities used in the diagnosis of HCC among the study subjects.

Variables	n (%)
Histology	35 (41.7)
FNAC	29 (34.5)
CT Scan	20 (23.8)

Table 3. Comparison of the serum AFP and DCP between HCC patients and controls.

Variable	Groups	n	Mean (ng/ml)	Std Deviation	p value
DCP	Study	84	7.90	5.08	0.001
	Control	65	4.66	6.74	
AFP	Study	84	504.62	674.39	<0.001
	Control	65	4.60	6.75	

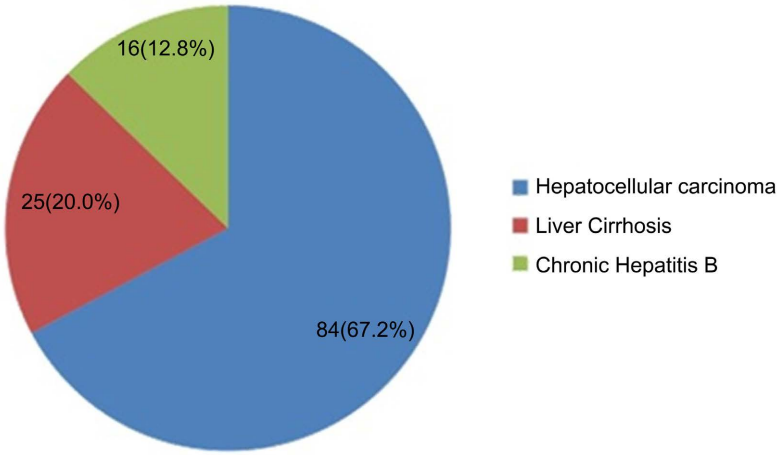

Figure 2. The Spectrum of pathological diagnoses of the study subjects using combination of the clinical history, histology, FNAC and CT Scan.

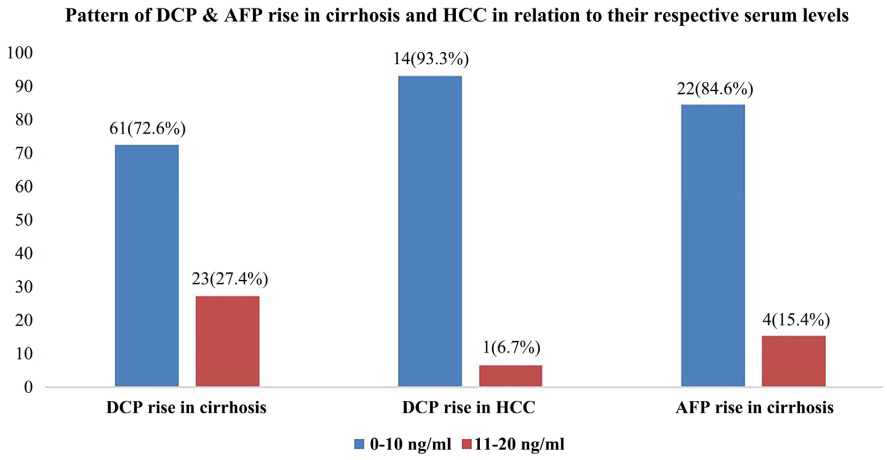

Figure 3. Pattern of serum DCP levels in the subgroups of HCC, Chronic hepatitis and cirrhosis subjects enrolled in the study.

Out of the 25 patients who were diagnosed as having cirrhosis, 22 (88%) patients had serum DCP levels in the range 0 - 120 ng/ml while 3 (12%) patients had serum DCP levels in the range of 11 - 20 ng/ml (Figure 3).

Twenty-nine (34.5%) HCC subjects had serum AFP level in the range of 1 - 100 ng/ml. Six (7.1%) HCC subjects had serum AFP level in the range of 101 - 200 ng/ml while 2 (2.4%) HCC subjects had serum AFP levels in the range of 201 - 300 ng/ml. Eight (9.5%) HCC subjects had serum AFP levels in the range of 301 - 400 ng/ml while 39 (46.4%) HCC subjects had serum AFP levels greater 400 ng/ml.

Five (33.3%) CHB subjects had serum AFP level in the range of 1 - 100 ng/ml. Another 5 (33.3%) CHB subjects had serum AFP level in the range of 101 - 200 ng/ml. Four (26.7%) CHB subjects had AFP levels in the range of 201 - 300 ng/ml while 1 (6.7%) CHB subject had serum AFP levels between 301 - 400 ng/ml. One (6.7%) CHB subject had serum AFP level greater than 400 ng/ml (Figure 4).

Using a cut-off of 400 ng/ml, the sensitivity of serum AFP for diagnosing HCC was 51.3%. The specificity of AFP at the same cut-off was 87.8%. The positive and negative predictive values were 92.8% and 49.3% respectively.

Using a cut-off of 7.5 ng/ml, the sensitivity of serum DCP for diagnosing HCC was 57.1%. The specificity of DCP at the same cut-off was 63.4%. The positive and negative predictive values were 76.2% and 41.9% respectively while the accuracy was 59.2% (Table 4).

The sensitivity of combined serum AFP and DCP for diagnosing HCC was 55.6%. The specificity of combined serum AFP and DCP for diagnosing HCC was 95.6%. The positive and negative predictive values were 96.2% and 52.3% respectively.

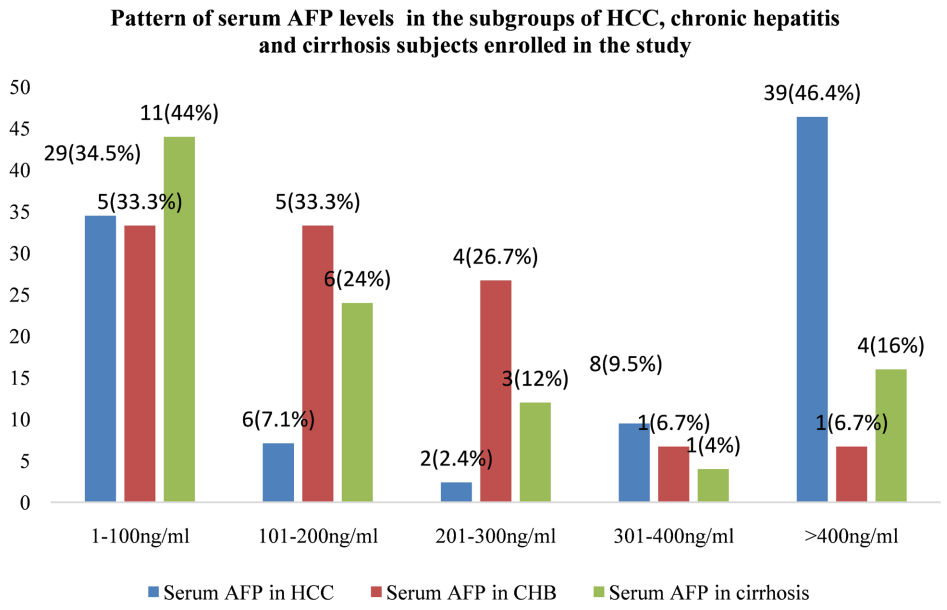

Figure 4. Pattern of serum AFP levels in the subgroups of HCC, chronic hepatitis and cirrhosis subjects enrolled in the study.

Table 4. Sensitivity, specificity, positive and negative predictive values of serum AFP in HCC among CLD Patients.

Evaluation	Serum AFP (400 ng/ml)	DCP (7.5 ng/ml)	AFP + DCP
Sensitivity (%)	51.3%	57.1%	55.6%
Specificity (%)	87.8%	63.4%	95.6%
Positive predictive value (%)	92.8%	76.2%	96.2%
Negative predictive value (%)	49.3%	41.9%	52.3%
False positive rate (%)	12.2%	36.6%	43.5%
False negative rate (%)	48.7%	42.9%	44.4%
Accuracy	64.1%	59.2%	64.9%

6. Discussion

The mean age of the CLD patients in this study was 42.81 ± 10.77 years with a slight male preponderance of 2:1. In an epidemiologic survey by Sherman *et al.* the peak age of onset of HCC was about 40 years in Mali, SSA where HbsAg seroprevalence was found to be high [38]. This peak age of onset of HCC is similar to the mean age of CLD in this study. In Ethiopia, the peak age incidence of HCC was between 41 and 60 years [14]. In an epidemiologic review by El-Serag *et al.*, a shift in the age incidence HCC from typically elderly patients to relatively younger ages of 40 to 60 years was reported [39]. Mittal *et al.* showed a recent increase in the age incidence of HCC among Hispanics and blacks in the United states at the ages of 45 - 65 years [40]. The younger age of HCC in this part of the world may be attributed to the predominant route of HBV transmission which is either vertical or horizontal transmission from child to child. In a case control study by Samuel *et al.*, a male: female preponderance of 2.2:1.0 was noted in the HbsAg positive patients in South Africa [41].

The mean BMI for the patients was 24.18 ± 2.81 kg/m^2 and 24.19 ± 2.81 kg/m^2 for the control participants. From this study there was no significant difference in the BMI of the patients and control. From previous studies carried out, obesity has been shown to be associated with increased morbidity and mortality in CLD [41]. Stepanova *et al.* have shown that patients who have increased BMI or other components of metabolic syndrome have high degree of hepatic fibrosis [42]. This is different from this study because most patients had an advanced disease and have lost body fat but had anasarca which gave them falsely high BMI. There is no statistically significant difference in BMI between the case cohort and control. The reason for this observation is because of fluid retention (ascites and pedal oedema) observed in majority of the patients with decompensated CLD which falsely increased their weight.

Weight loss (80.0%), Hepatomegaly (60.5%) and ascites (58.4%) were the most prominent clinical findings in patients with HCC and this similar to findings by Lesi *et al.* who found abdominal swelling as the most common symptom

because most of the study subjects in their study subjects were cirrhotic [43]. Jaundice was seen in 68.0% of the subjects similar to the findings by Iloh *et al.* [44]. Melaena and haematemesis were seen in 24.0% and 34.4% respectively. Hepatic bruit was seen in 44.0% of the patients. Iloh *et al.* found hepatic bruit in 5.8% of the subjects because majority of the subjects had liver cirrhosis [44]. Palmar erythema and sparse axillary hair were seen in 72.8% and 64.0% of the study subjects respectively. In a multi-center cohort study by Yang *et al.*, hypoalbuminaemia 31.0 g/l and hyperbilirubinaemia 82.1 μmol/l were noted among cohorts with CLD which was similar to this study [45].

Using a cut-off point of 400 ng/ml, the sensitivity and specificity were 51.3% and 87.8% respectively. This is similar to findings by Hu *et al.* who found a sensitivity and specificity pattern ranging from 40% - 65% and 76% - 96% respectively with AUC of 0.835 using a similar cut-off [46]. In a systematic review by Sherman *et al.*, AFP was found to have a sensitivity of 60.0% at a cut-off of 400 ng/ml [38]. Soyemi *et al.* showed that AFP screening accuracy has an AUC of 0.916, sensitivity of 51.7% and specificity of 100% in a study determining the combined diagnostic accuracy of AFP and SCCA in UCH, Ibadan [47]. This sensitivity compares favourably with the sensitivity obtained in this study, however the specificity in this study is lower. This might be due to the larger tumor sizes in their study. In a multicenter case-control study by Tsuchiya *et al.* involving 836 subjects (HCC, $n = 419$ and cirrhosis, $n = 417$) AFP exhibited sensitivity and specificity as high as 66% and 82% respectively, for early stage HCC (BCLC stages 0 and A) at a lower threshold of 10.9 ng/ml [48].

In a systematic review by Li *et al.*, looking at the diagnostic accuracy of serum DCP versus AFP, the sensitivity of AFP in HCC was 59% and the specificity was 86% at CI of 95% with AUC of 0.77 in diagnosing HCC [49]. In a study carried out by Michael *et al.* AFP was found to be more specific than DCP in HCC diagnosis [50]. The reason for the slight difference in the values of sensitivity and specificity may be due to the fact that not all HCC secrete AFP. Notwithstanding its low sensitivity and accuracy, AFP is still the most widely used tumour marker for HCC surveillance and diagnosis but there is the need for discovery of better tumour markers.

6.1. Sensitivity, Specificity, Positive and Negative Predictive Values of Serum DCP for HCC Diagnosis among CLD Patients

This study demonstrated that serum DCP has a high sensitivity, specificity, positive and negative predictive values in HCC diagnosis among CLD patients in Ilorin. This is similar to findings from other parts of the world.

The overall sensitivity, specificity, positive and negative predictive values of DCP for the detection of HCC in a study carried out by Zhu *et al.* were 71% (95% CI: 68% - 73%), 84% (95% CI: 83% - 86%), 6.48 (95% CI: 4.22 - 9.93), and 0.33 (95% CI: 0.25 - 0.43), respectively. The area under the ROC curve was 0.8930 [51]. The difference in the sensitivity and specificity between this and other studies may be due to differences in the size of HCC. The sensitivity of se-

rum DCP was 57.1% and specificity was 63.4% at cut-off of 7.5 ng/ml with an AUC of 0.608. This is similar to findings by Gomaa *et al.* who found a sensitivity of 48% to 62%, a specificity of 81% to 98% and a diagnostic accuracy of 59% to 84% at cut-off of 40 mAu/ml in diagnosing HCC in several large case-controlled studies [15].

In a cross-sectional study by Ette *et al.* at Ile Ife, southwest Nigeria, DCP was found to have higher sensitivity and specificity of 96.8% and 98.3% respectively at a cut-off of 140 mAU/ml of the ROC [52]. The AUC was 0.99. The higher sensitivity and specificity obtained from that study might be due to the higher cut-off value and the fact that majority of the patients had tumor size > 5 cm and few patients had tumor size of between 3 - 5 cm. Another plausible explanation for their higher sensitivity, specificity and accuracy compared with this study was because they grouped the patients into two arms viz: HCC and non-HCC group [52]. In a systematic review by Li *et al.*, the sensitivity of DCP at a cut-off of 140 mAU/ml was 63% (95% CI, 58% - 67%) and specificity 91% (95% CI, 88% - 93%) [49].

6.2. Sensitivity, Specificity, Positive and Negative Predictive Values of Combined Serum AFP and DCP

Combining two tumor markers improves the diagnostic yield in HCC as most of the markers are complimentary. Grazi *et al.* proved that AFP and DCP are not correlated, so the combination of those tumor markers improves the accuracy of HCC detection with sensitivity and specificity of 74.2% (cut-off 400 ng/ml) and 87.2% (40 mAU/ml) respectively [53]. In this study, the sensitivity of the combined tumor markers was 55.6% and the specificity was 95%. In a similar study by Stefaniuk *et al.,* the sensitivity of both tumor markers was higher at 84.8% than this study but the specificity was comparable and the accuracy of the combined tumor markers was 64.9% [37]. This difference in sensitivity may be attributed to the methods of tumor markers determination in which advanced enzymatic immunoassay method was used. In this method, multistep assay was used by chemically linking antibodies with detectable label which emit radiation or produce a colour change in a solution. Giannelli *et al.* showed that combining the tumor markers provides a simple and non-invasive way of diagnosing HCC [54]. Based on satisfactory accuracy, serum tumor markers have been used as an effective method for detecting malignant tumors for a long time, and they could be valuable in further strengthening the usefulness of ultrasonography and CT scan in the diagnosis of HCC [54]. In a single center study among the European cohort by Ertle *et al.*, the sensitivity and specificity of combined AFP and DCP was 78.0% (cut-off of 400 ng/ml) and 63.4% (cut-off of 5 ng/ml) respectively compared to either of them singly [55]. The AUC for the two tumor markers was 0.91 compared to 0.676 obtained in this study. The difference in the diagnostic accuracy between this study and my study may be due to the difference in genetic makeup between Africans and Europeans cohorts.

7. Conclusion

The diagnostic accuracy of combined serum AFP and DCP for diagnosis of HCC in University of Ilorin Teaching Hospital, Ilorin was 64.9%. The sensitivity and specificity of the combined tumor markers were 55.6% and 95.6% respectively. The positive and negative predictive values were 96.2% and 52.3% respectively. The mean serum AFP and DCP in HCC patients is 504.6 ng/ml and 7.9 ng/ml respectively. The sensitivity and specificity of AFP and DCP were (51.3%, 87.8%) and (57.1%, 63.4%) respectively. Combining these two tumour markers does not significantly improve the diagnostic accuracy of HCC and chronic HBV remains a strong aetiological agent of HCC in UITH, Ilorin.

Additional Information

Disclosures

Human Subjects: Consent was obtained or waived by all participants in this study. Health Research Ethics Committee, University of Ilorin Teaching Hospital, Ilorin issued approval **ERC PAN/2017/10/1/1729 Animal Subjects**: All authors have confirmed that this study did not involve animal subjects or tissue. **Conflicts of Interest**: In compliance with the ICMJE uniform, all authors declare the following: **Payment/Services Info**: All authors have declared that no financial support was received from any organization for the submitted work. **Financial Relationships**: All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work. **Other Relationships:** All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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