Original Article

Prognostic Significance of Alpha-Fetoprotein in Staging of Chronic Hepatitis B Infection

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Abstract

Background
Ascertaining the stage of chronic hepatitis-B infection (CHBI) remains one of the major predicaments to effective therapeutic decision. There is pressing need to forestall dearth of such reliable biomarker(s). Despite the promising tendency of alpha-fetoprotein (AFP), it has not been assessed in staging CHBI.

Objective
This study was to determine the prognosis of serum AFP as a biomarker for staging CHBI.

Methods
Participants were grouped into three based on their hepatitis-B envelope antigen (HBeAg) status and alanine aminotransferase (ALT) level. By denoting HbeAg(+) as (EP), HbeAg(-) as (EN), elevated ALT as (H) and normal ALT as (I), the stages were EPH, ENH and ENI. AFP was assayed, One-way ANOVA, Multivariate linear regression and area under curve were adopted for the analysis.

Results
AFP was significantly elevated, ($P < 0.05$) in EPH, which equally has the highest prevalence of elevated AFP (64.7%). After adjusting for confounding factors, odds ratio was 1.438 (95% CI, 0.62–1.948), while area under the curve for predicting EPH was (0.828, 95% CI, 0.778 –0.895).

Conclusions
The finding of elevated AFP in CHBI is an independent prognostic marker of EPH. It is often associated with necroinflammation; thus, it is a reliable indicator for treatment initiation.
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Keywords: Alpha-fetoprotein, Alanine aminotransferase, Chronic hepatitis-B infection, Hepatitis-B virus, Hepatitis-B e-antigen

Introduction

Chronic hepatitis B infection (CHBI) is a condition of continuous presence of hepatitis B virus (HBV) in an individual beyond six months.[1] It is the foremost causes of liver cirrhosis and accountable for approximately 55% cases of hepatocellular carcinoma (HCC).[2, 3]

Currently approximately 250 to 300 million peoples are chronically infected with HBV globally.[4, 5] The population of people with CHBI in Africa was estimated to be 82 million, this constitutes almost a quarter of the global burden.[6] Unfortunately, while the mortality associated with other chronic infections such as; Human Immunodeficiency Virus, tuberculosis,

among others were on decline, death attributable to CHBI keep growing.[7] The pool prevalence and associated mortality of CHBI in Africa was 6.1% and 87,890 respectively.[6] One of the major contributory factors to these alarming figures as well as hindrances to effective treatment of CHBI is the dearth of biomarker(s) that can promptly predict/identify the patients who are prone to progression.[8] In order to avert the morbidity and death associated with liver damage, there is a need for timely detection of patients who are predispose to HBV complication and early treatment. [9] Achieving that goal practically involve utilization of suitable biomarker(s) that will better characterize the infection.

Clinically, the progression of CHBI follow multiple stages and varies from one patient to another, thus, it is somehow challenging to determine its outcome. Currently, the biomarkers frequently adopted in staging of CHBI include, HBV envelope antigen (HBeAg) status, HBV-DNA and serum alanine aminotransferase (ALT) levels. [10] The levels of those biomarkers vary as the disease progress, as such, they are commonly used to define its stages.[11] The two distinguishing features adopted in the new nomenclature of CHBI stages are HBeAg status and necroinflammation.[10]

HBeAg is an antigenic glycoprotein connected with nucleocapsids of HBV. It is circulating as soluble proteins during active HBV infection only, otherwise it is usually absent.[6,12] HBeAg(+) and HBeAg(-) status are denoted by EP (envelope positive) and EN (envelope negative) respectively. On the other hand, the presence of necroinflammation and its absence are denoted by H and I respectively.[10] Hence, the four stages of CHBI are commonly depicted as; Envelope-antigen Positive Infection (EPI), Envelope-antigen Positive Hepatitis (EPH), Envelope-antigen Negative Infection (ENI) and Envelope-antigen Negative Hepatitis (ENH). Therapeutic decision in CHBI is mainly anchored on the presence of necroinflammation which occurs during EPH and ENH.[9]

Nevertheless, there is bottleneck associated with those classical biomarkers. For HBV-DNA assay, apart from the fact that its availability is posing a great challenge in resource-limited settings,[8] its plasma levels sometimes inconsistent in the natural course of the infection.[9] Though, in the absence of HBV-DNA quantification, both HBeAg and serum ALT activity can serve as alternative biomarkers.[13] Simply because the liver has the highest ALT activity (2850 U/L) and a rise in its plasma activities is considered a sensitive indicator of hepatic necro-inflammation.[9] Nevertheless, the rate of elevation of ALT and AST are often disproportionate with that of histological finding. More so, the two enzymes level sometimes normal in liver cirrhosis.[14] Thus, apart from being multiple biomarkers and associated high cost of workup, the classic biomarkers are also not totally reflecting the intricacy of HBV induced liver damage.[15]

In view of the challenges associated with correct staging of CHBI, which is a condition for therapeutic decision.[10] The quest for an ideal biomarker(s) for staging CHBI was affirmed by the International Coalition to Eliminate Hepatitis B Virus (ICE- HBV). According to ICE-HBV, the proposed biomarker should characterize the stages of CHBI better, enhance the identification of HCC risk, reveal immune status and response to therapy.[16] In addition to the above-mentioned features, Biomarkers Definitions Working Group also added that such biomarker(s) should be sensitive, specific, reproducible, simple, rapid, non-invasive, accessible, inexpensive, correlative with disease activity and severity.[17]

Some evolving immunological biomarkers are currently under investigation, few notables' ones among them are; intrahepatic covalently closed circular DNA (cccDNA) and RNA,[17] serum HBsAg isoform,[18] HBV core-related antigen (HBcrAg) assessment,[19] soluble Program Death 1 (sPD1), its ligand (sPDL1) level,[20] and soluble cluster of derivatives 14 (sCD14). [21]

The serum and intrahepatic levels of those biomarkers as well as their dynamics under treatment varies during the course of CHBI, which makes them a promising candidate.[22] Unfortunately, they are also not flaw-free, rather they have one shortcoming or the others.[15] Among these limitations are; invasiveness, sampling bias, lack of standardization which usually resulted in inter assay conflicting results and high cost of reagents.[18] In order to achieve the WHO 2030 agenda of reducing incidences of HBV associated mortality by 60%, a panel of biomarkers are thus required for surveillance and to determine treatment outcome.

AFP is an alpha1 globulin, a glycoprotein majorly produced by fetal liver cells, yolk sac cells, and in trace amounts by the fetal gastrointestinal tract.[24] The level of AFP is usually elevated in early neonatal stage, and later subsides at about three weeks of neonatal period.[23] Reappearance of AFP in adult serum indicate pathologic conditions, such as HCC or germ-cell tumors containing yolk sac cell elements.[26] Patients with acute and chronic active hepatitis (with and without cirrhosis) often showed AFP elevations above 150 ng/ml.[27] In spite of frequently reported elevation of AFP in HBV infection,[24,28,29] its significance in staging of the infection has not been investigated. More so, the advent of Enzyme Linked Immunosorbent Assay and other technique has made AFP assay simple, rapid, accessible, reproducible and less expensive.[29, 30] In view of the foregoing, this study was embarked on to evaluate the prognostic significant of AFP in staging of CHBI. To the best of our knowledge, this study is the first the report on the involvement of AFP as a biomarker in the pathogenesis of CHB among Africa population.

Materials and methods

Study design

This study was carried out among the inhabitants of Kisi town, Irepo Local Government area of Oyo State, Southwest Nigeria between March, 2018 to March, 2019.

The participants were consecutively screened for HBsAg twice with interval of at least six months duration between the two screenings. The purpose of the first screening was to determine the HBV status (seropositive or seronegative), while the second visit was aiming at determining the chronicity of the infection. Participants were included if they were persistently remained HBsAg seropositive for at least six months. Those with other chronic infections, malignancy, and pregnancy were excluded. Age and sex matched HBsAg(-) individuals were recruited as control.

Data collection/laboratory assessment

Questionnaires were used to gather sociodemographic data such as age, gender, and marital status. Anthropometric data were taken using standard method. At first and second visits, approximately 2 mL and 5mL of blood was collected from eligible participants. The serum was screened for HBsAg, anti-Hepatitis C using rapid kits (Shanghai Eugene Biotech. Co, Shanghai) on both occasions. The concentration of ALT and AST were measured with commercial kit (Agape Diagnostic Switzerland), and that of AFP with ELISA (AccuBind, Monobind Inc. USA) from the second sample.

Prognostic evaluation

The prognostic value of serum AFP was evaluated at the cut-off point of 11.6 ng/mL (the upper limit of AFP mean value of the control group). To predict diagnosis of a patient, his/her AFP value will be compared with 11.6 ng/mL. If it is less than 11.6 ng/mL, the predicted condition (AFP prognosis ability in CHBI) is negative. But if it is greater than the cut-off value, it will be considered as positive. However, it is not expected that the prediction will be 100% accurate. Thus, there are four possible outcomes, some predictions may match the patient true status; true positive (TP) or true negative (TN), while some other predictions are discordant with diagnosis; false positive (FP) or false negative (FN) (Table 3).

Statistical analysis

Statistical analyses were carried out using IBM SPSS version 21.0 for window software (SPSS Inc. Chicago, IL USA) and MedCalc.version 15.2.2 (MedCalc. Software, Mariakerke, Belgium).

P value of ≤ 0.05 was considered statistically significant. Continuous and categorical variables were expressed as means ± SEM and percentage respectively. The Kolmogorov-Smirnov test was used to check if variables were normally distributed. One-Way ANOVA and multivariate regression model were adopted for comparing inter group differences. The receiver operating curve (ROC) and area under curve (AUC) were used to determine prognostic value of AFP in CHBI

Ethical considerations

Ethical approval for this study was obtained from the ethical review committee of Kwara State Ministry of Health, Ilorin (MOH/KS/EU/777/245). Informed consent was obtained from eligible participants prior to enrolment.

Results

Characteristic of the participants

During the period of study, a total of 198 participants with CHBI were consecutively enrolled. Among them 112 (56.6%) were male, the female-to-male ratios was similar for control and pathological groups (1.3:1). Based on their HBeAg status and ALT, participants were categorized into stages, the resulted prevalence of each stage went thus: ENI (n = 140), EPH (n = 17), and ENH (n = 40). The mean ± SEM values of AFP in ng/mL in EPH, ENH and ENI were (39.41 ± 8.15), (12.83 ± 1.10) and (15.29 ± 1.28) respectively (Table 1). The value was significantly higher in EPH than both ENH (P < 0.001) and ENI (P = 0.003). Though the AFP value was higher in ENH and ENI, but the difference was not significant. The demographic information of the participants was shown in Table 1.

Table 1. Characteristic of study subjects

Characteristics	Control	ENI	EPH	ENH	*P* value
Number (%)	110	140 (71.1)	17 (8.6)	40 (20.3)	-
Sex (M/F)	62/48	83/57	11/6	18/22	-
Age (years)	35.0±0.73	32.0±0.69	36.0±1.11	38.0±4.15	0.425
BMI (Kg/m²)	25.65±0.14	24.90±0.13	23.12±0.23	25.02±0.17	0.017
ALT (IU/L)	14.39±0.77	17.33±0.75	143.24±14.96	48.67±4.58	0.000
AST (IU/L)	15.48±0.87	20.72±0.92	76.18±12.34	39.11±3.83	0.000
AST/ALT	1.84±0.11	1.61±0.22	0.64±0.46	1.08±0.10	0.000
AFP (ng/mL)	9.85±0.35	12.83±1.10	39.41±8.15	15.29±0.35	0.000
HBsAg	Negative	Positive	Positive	Positive	NA
HBeAg	Negative	Negative	Positive	Negative	NA
Anti-HCV	Negative	Negative	Negative	Negative	NA

The values are mean ± standard error of mean; Abbreviations: ENI = Envelope-antigen Negative Infection, EPH = Envelope-antigen Positive Hepatitis, ENH = Envelope-antigen Negative Hepatitis, ALT = Alanine aminotransferase, AST = Aspartate aminotransferase, AFP = Alpha-fetoprotein, BMI = Body mass index, HBsAg = Hepatitis B surface antigen, HBeAg = Hepatitis B e antigen, Anti-HCV = Hepatitis C virus antibody. NA = not applicable.

Linear regression analysis and scatter plot

The outcome of univariate analyses, revealed a significant association between AFP and the following; BMI, male sex and HBeAg status. After adjustment for those variables, the association remain significant with ALT and AST (odds ratio, 1.438; 95% CI, 0.626 - 1.948) (Table 2). Scatter plots were used to show the relationship between AFP and ALT in different stages of CHBI. A very weak negative correlation was observed between the two parameters in ENI and ENH (Figure 1 and 3), while that of EPH was a weak positive correlation (Figure 2).

Table 2. Odd ratios of CHBI progression risk factors according to AFP as continuous variable

	Univariate analysis			Multivariable analysis		
Variables	**OR**	**95% CI**	***P* value**	**OR**	**95% CI**	***P* value**
Age	1.023	0.954–1.196	0.004	-	-	-
Sex, male	0.931	0.596–1.238	0.013	-	-	-
BMI	0.623	0.452–1.086	0.094	-	-	-
HBeAg	1.049	0.592–1.869	0.009	-	-	-
ALT	1.845	0.734–2.582	0.001	1.438	0.626–1.948	0.011
AST	1.109	0.867–1.879	0.012	0.797	0.701–1.693	0.034

Abbreviation: OR = Odd ratio, CI = confidence interval, ALT = Alanine aminotransferase, AST = Aspartate aminotransferase, AFP = Alpha-fetoprotein, BMI = Body mass index, HBeAg = Hepatitis B e antigen

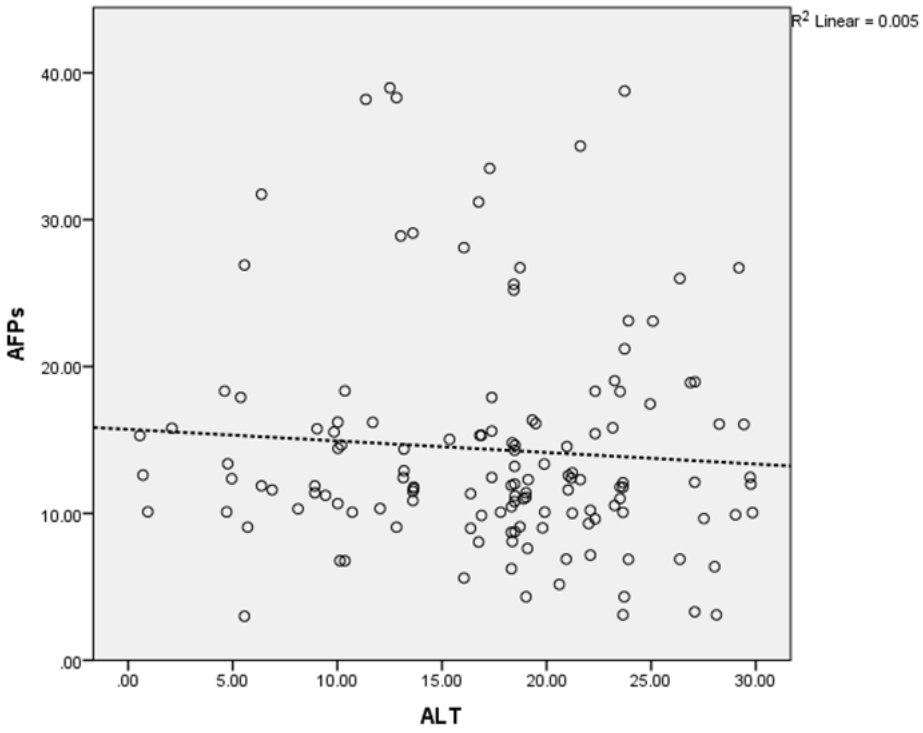

Figure 1. Scatterplot showing a very weak negative correlation between AFP and ALT in ENI.
The dotted line represents the line of best fit. Abbreviations: AFP = Alpha-fetoprotein; ALT= Alanine aminotransferase; ENI = Envelope-antigen Negative Infection.

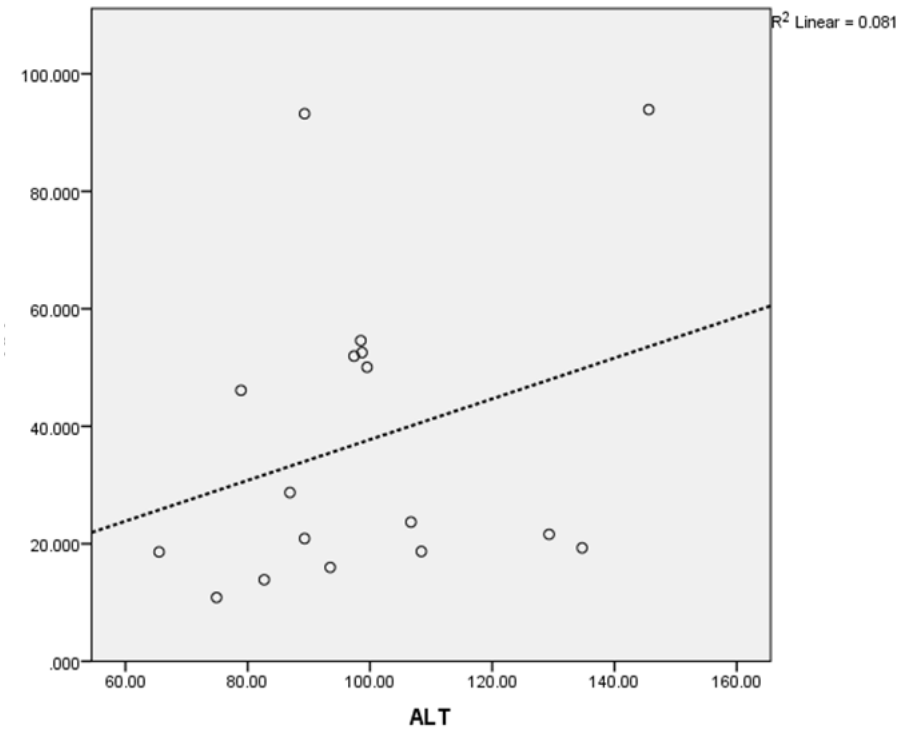

Figure 2. Scatterplot showing a weak positive correlation between AFP and ALT in EPH.
The dotted line represents the line of best fit. Abbreviations: AFP = Alpha-fetoprotein; ALT = Alanine aminotransferase ; EPH = Envelope-antigen Positive Hepatitis.

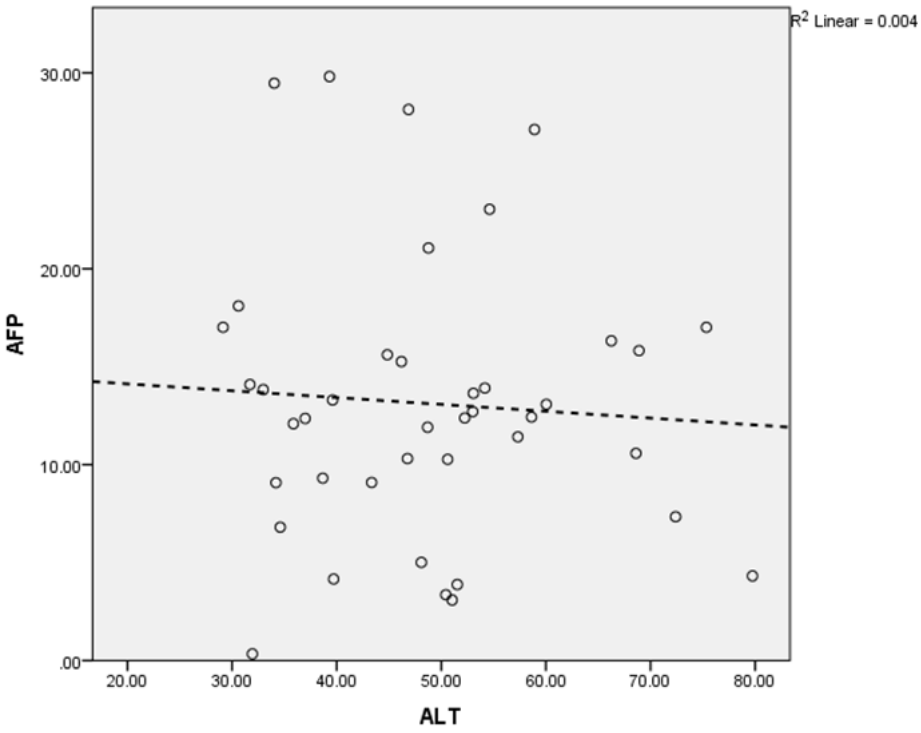

Figure 3. Scatterplot showing a very weak negative correlation between AFP and ALT in ENH.
The dotted line represents the line of best fit. Abbreviations: AFP= Alpha-fetoprotein; ALT= Alanine aminotransferase; ENH = Envelope-antigen Negative Hepatitis.

Prognostic value of AFP

Table 3. The outcome of receiver operating characteristic curve revealed a specificity of 91.82% in the three groups. The sensitivity, positive predictive value (PPV) and negative predictive value (NPV) in different stages was ENI (19.2%, 33.8% and 84.0%), ENH (25.0%, 39.8% and 85.0%) and EPH (64.7 63.13% and 85%). The area under the curve for AFP in EPH was (0.828, 95% CI, 0.778 to 0.895) and greater than that of ENI and ENH.

Table 3. Performance of serum AFP in staging of chronic hepatitis-B infection

Rates	Formula	ENI	ENH	EPH
Sensitivity (%)	TP/ (TP + FN)	19.29	25.00	64.71
Specificity (%)	TN/ (FP + TN)	98.21	98.21	98.21
Positive likelihood ratio	TP/ (TP + FN)/ (1- Specificity)	2.36	3.06	7.91
Negative likelihood ratio	FN/ (TP + FN)**/** Specificity	0.88	0.82	0.38
Positive Predictive Value (%)	TP/ (TP + FP)	33.79	39.82	63.13
Negative Predictive Value (%)	TN/ (FN + TN)	84.01	84.97	92.32
Accuracy (%)	(TP+TN)/(TP+FP+FN+TN)	78.91	79.92	86.99

Table 3 displayed the formula and performance of elevated AFP in different stages of CHBI using 11.6 ng/mL (the upper limit of mean AFP value of the control group) as cut-off value. ENI = Envelope-antigen Negative Infection, ENH = Envelope-antigen Negative Hepatitis, EPH = Envelope-antigen Positive Hepatitis, TP= true positive, TN= true negative, FP= false positive, FN= false negative.

Discussion

The natural progression of CHBI is commonly viewed as consisting of multiple stages; an infected person can progress from a less severe to a severe stage. The rate of progression also varied; it could be rapid, slow, or sporadic.[10, 31] In view of its intriguing pathophysiology complexity, making accurate diagnosis and subsequent therapeutic decision is a herculean task.[9] However, with the availability of relevant and accurate biochemical or immunological biomarkers, clinicians can be appropriately guided in deciding on the optimal timing for initiating antiviral therapy.[9, 12] Previous studies have clearly demonstrated that the combination HBeAg status, [HBeAg(+) or HBeAg(-)] and the new baseline cut-off values (30 U/L in men and 19 U/L in women) for ALT can be adopted in staging CHBI.[13, 32, 33] Based on the results of ALT and HBeAg, the participants in this study were classified thus; those with elevated ALT and HBeAg(+) were grouped as EPH, those with elevated ALT and HBeAg(-) were grouped as ENH. Those with normal ALT and HBeAg(-) were grouped as ENI. (Table 1) The prevalence of each stage as observed in this study was equally shown on the Table. ENI, which is otherwise known as inactive constituted the highest number of participants (71.1%). Other authors as well as WHO has affirmed the fact ENI usually constituted more than 80% of CHBI.[34]

An examination of male/female ratio revealed that a little above half of the participants were males (approximately 57 %). This may imply and support the notion that HBV affects men and women similarly. [35] More so, the distribution could be attributed to the pattern of exposure to risk factors among the population studied. However, higher female ratio has also been reported in Nigeria.[36]

The body mass index (BMI) was compared between different stages and controls, although the pre-morbid weights of the study subjects were not known, it was observed that ENH had significantly lower BMI compared to the control group (23.12± 0.93) vs. (24.65± 1.4) Kg/m2, (P=0.001). In the same vein, the difference between the BMI was significantly lower in EPH in relation to ENI (P = 0.419) and ENH (P = 0.992). The mechanism/environmental factors that could responsible for the changes in BMI are not understood. However, it could be attributed to the severity of the infection, as reflect in the concentration of albumin of the participants (data not presented).

Serum AFP level was assayed in CHBI and control, the outcome revealed a heterogeneity in level and distribution of AFP in different stages of CHBI. This was evident by within-group variability in AFP level (it is reflected in the scatter plots). No significant difference was observed in AFP level between ENI and ENH (P > 0.05), though the value was slightly higher in ENH. However, AFP was significantly elevated in EPH in relation to both ENI and ENH (P < 0.05) (Table 1). This outcome is in line with the finding of,[37] who reported elevated AFP among over 57% of HBeAg(+) studied. However, our finding was contrary to what was earlier reported. [38] Highest AFP was reported in ENH, follow by EPH and lowest in ENI. The reason for disparity in AFP level in ENH and EPH could be attributed to variation in dynamic interaction between the virus and the liver microenvironment (hepatic parenchymal cells, non-parenchymal cells and local immune cells).[30] The observed elevation in AFP in EPH in this study could be attributed to the presence of HBV envelope antigen (HBeAg), as it can be seen that the other two chronic stages with HBeAg(-) have a significantly lower expression of AFP. More so, as it is evident from our data, EPH has the highest percentage of participants with elevated AFP. This further support peculiarity of this stage. Of all the stages of CHBI, EPH [HBeAg(+)] has been identified to be associated with severe course and unpredictable spontaneous upsurges of hepatic inflammation that swiftly advanced to hepatic fibrosis.[32] However, the exact mechanism of induction of AFP expression by HBeAg remains unclear.

The association between AFP and ALT in different stages of CHBI has rarely been investigated in this part of the world. The outcome of correlation analysis between ALT and AFP in CHBI was represented with scatter plot (Figure 1, 2, and 3). The outcome revealed a diverse result in the three stages; there was a very weak negative correlation between the two parameters in ENI and ENH (Figure 1 and 3), and a weak positive correlation was observed in EPH (Figure 2). These observations were similar to earlier finding by [37] who reported a significant weak to moderate correlation between AFP and liver enzymes (ALT, AST and one other) in CHBI. Nevertheless, the positive correlation observed in EPH does not explain the exact mechanisms involved and could not be considered as causative.[39] It is most likely that inflammatory activities primarily or ALT released responsible for the induction of AFP expression in CHBI, because significant number of patients with elevated AFP had elevated ALT. Another interesting finding of our results is that, after adjusting for multiple confounding factors (Table 2), association between AFP and aminotransferases' in EPH remained significant. This indicates that reducing necroinflammation may lower the AFP and signify reducing risk of liver damage, which is consistent with previous study.[37] A strong relationship between pathological levels of inflammation and fibrosis with serum AFP level was reported in CHBI.

The prognostic performance of AFP in respect of the 3 stages of CHBI studied is presented on Table 3. As shown on the table, the specificity was 98.21%, i.e., the true negative rate which is the number of control (known negative), whose AFP fell within normal range. The specificity was the same for the three groups, because they were compared with the same cut-off value .[40,42] On the other hand, the sensitivity (the true positive rate) is the proportion of the CHBI participants predicted to be positive by having elevated AFP.

High sensitivity is a desirable feature of an ideal diagnostic test parameter.[41] As revealed on the table, EPH has the highest sensitivity (64.7) in respect of other two stages. Another valuable prognostic rates are the positive and negative likelihood ratios (LR+ and LR-). Both are considered to stretch evidence to affirm or to negate diagnoses respectively in most situations [43] A LR+ greater than ten is considered as a strong indication of presence of the disease, and it is favorable feature of diagnostic test.[44] For LR- the lesser the value the more favorable the new test can perform. A LR- below 0.1 is interpreted as providing strong indication to rule out disease [43]. As reflected on the Table 3, though the two values did not yield ideal values of greater than 10 and less than 0.1, despite that the performance in EPH was encouraging; it has the highest LR+, (7.91) and lowest LR-, (0.38) of the three stages studied. In addition to earlier data, the positive and negative predictive value (PPV and NPV), are the proportion of the participants who are predicted to be positive and negative (by having elevated AFP and normal AFP respectively), who are in the really sense truly having elevated ALT and normal ALT respectively.[41] 100% is considered a favorable value for both PPV and NPV, thus the closer they are to the target, the better. [44] Going by the outcome of our study, EPH has the highest PPV, (63.13 %) as well as NPV (92.32%) in relation to the remaining two stages.

Therefore, AFP is appropriate for ruling out or ruling in EPH stage. Furthermore, on the accuracy, which is otherwise known as proportion of participants who were correctly predicted or classified.[42] Our result revealed that EPH has the highest accuracy of approximately 87%, (Table 2). By considering the overall prognostic indices, it was clear that the outcome of AFP assay to a larger extent could predict EPH stage in similar manner with the combination of HBeAg and ALT. On a final note, the AUC for this regression model was 0.828, which is very high. This indicates that AFP perform outstandingly in predicting whether or not a patient is in EPH stage.[44]

It is noteworthy that about 1.79% of apparently healthy individual with neither common viral infections, nor any liver disease also have mildly elevated AFP (data not shown). Probably some other factors might also stimulate the expression of AFP.

Our study has few advantages. First, it was a prospective cohort study of at least six months. We also corrected for identified confounders, such as age, gender, and BMI. These steps made our results relatively reliable. This study equally has few limitations which include: small sample size, particularly EPH which appeared promising. Inability to carry out liver histology to correlate the extent of necroinflammation with AFP in different stages. This would have enabled us to classify CHBI better and added a further interesting dimension to the present study.

Conclusion

The impact HBV-induced immunopathology apparently interferes with the expression of AFP in the liver. The effect which is well pronounced in EPH stage than others.
More so, a mild association was reported between serum AFP and necro-inflammatory biomarkers in EPH. It's also displayed significant sensitivity, specificity, predictive value and accuracy in EPH stage.
On a final note, since EPH is one of the two CHBI stages that warrant treatment, therefore, finding of elevated AFP can be considered as an indication for initiating anti-viral treatment, and as well as an additional biochemical parameter to characterized the stage.

Further study

Study design to evaluate which fraction of AFP elevated in CHBI is recommended. Also, evaluation of AFP performance in CHBI treatment monitoring is recommended. Further study to reaffirm the potential of AFP in EPH in a larger population is recommended.

Conflict of interest

There is no conflict of interest among the authors.

Contributions of authors

OGA, conceived and designed the experiments; IES, performed the experiments, analyzed and interpreted the data; OCA, contributed to tools development and data analysis, wrote manuscript.; ALA, contributed reagents, other materials, and revised the manuscript.

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