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Identifying Possible Hepatic Fibrosis of Hepatitis B Origin Using Non-invasive Markers: A Case-control Study in the South West Region of Cameroon

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aim: HBV infection is known to cause liver fibrosis as well as some extrahepatic manifestations. We aimed at assessing hematological changes and identifying possible hepatic fibrosis of Hepatitis B origin using non-invasive markers (NIMs).
Study Design: A hospital-based Case-control study
Place and Duration of Study: Conducted at the Buea Regional Hospital, South West Region of Cameroon from February 2016 to December 2017
Methods: We enrolled HBV infected treatment naïve patients and "healthy" controls. All participants were subjected to alanine aminotransferase (ALT) and aspartate aminotransferase (AST) measurement, Full blood count (FBC), HBsAg, anti-HBc, HIV and HCV tests. Aspartate-platelet ratio index (APRI), fibrosis based on 4 factors (FIB-4), age-platelet index (API) and AST/ALT ratio (AAR) were generated from the test results. A questionnaire was administered to collect demographic data, alcohol consumption and history of liver/kidney disease or metabolic syndrome.
Results: A total of 202 cases and 202 controls were enrolled. Hematocrit (HCT) was significantly higher ($p<0.001$) in cases than controls. The controls had significantly higher mean values for platelet ($p=0.005$), neutrophil ($p=0.032$) and number of individuals with AST/ALT ratio (AAR) ≥1. Liver fibrosis was significantly associated with cases than controls based on APRI (OR:6.06, CI:3.59-10.24), FIB-4 (OR:5.35, CI:2.75-10.39) and API (OR:8.02, CI:1.81-35.55). Among the HBV infected cases, 69 (34.2%), 36(17.8%) and 8(4.0%) had results indicative of fibrosis from at least 2, at least 3 and all 4 NIMs respectively. AAR detected possible fibrosis in 136 HBV infected cases of which up to 77 (56.6%) were not detected as fibrosis by the other NIMs.
Conclusion: HBV infection affects neutrophil percentage, HCT, PLT, APRI, FIB-4 and API in our study population. AAR did not prove to be a reliable NIM. Using at least 3 NIMs for HBV infected patients can significantly scale up their reliability for determining liver fibrosis in clinical practice.

Keywords: HBV infection; APRI; API; FIB-4; AST/ALT ratio; non-invasive markers; hematological changes; liver fibrosis.

ABBREVIATIONS

AST-Aspartate Aminotransferase; ALT-Alanine Aminotransferase; AAR-AST/ALT Ratio; API-Age-Platelet Index; APRI-Aspartate-Platelet Ratio Index; FBC-Full Blood Count; FIB-4-Fibrosis Index Based on 4 Factors; Anti-HBc-Hepatitis B Core Antibody; HBsAg-Hepatitis B Surface Antigen; HBV-Hepatitis B Virus; HCC- Hepatocellular Carcinoma; HCT-Hematocrit; HCV-Hepatitis C Virus; HIV-Human Immunodeficiency Virus; NIM-Non-Invasive Markers; PLT-Platelets; RBC-Red Blood Cells; SPSS-Statistical Package for Social Sciences; SW-South West; ULN-Upper Limit Normal; WBC-White Blood Cells; OR-Odds ratio; CI-Confidence Interval.

1. INTRODUCTION

Hepatitis B virus (HBV) infection is known to be a major cause of liver cirrhosis worldwide. The risk of liver cancer is greatly increased once cirrhosis develops. Cirrhosis and liver cancer are now among the top ten causes of death worldwide [1,2]. Monitoring of chronic liver disease to identify and assess the stage of fibrosis is of paramount importance in preventing cirrhosis.

Liver biopsy is known to be the gold standard method for assessing liver fibrosis [3] although it is costly, invasive, has increased risk of complication, requires hospitalization, could lead to underestimation of fibrosis stage and has inter- and intra-observer discrepancies [4–8]. The rapid development of new medications for the treatment of liver diseases (including chronic hepatitis B) increases the requirement for more frequent evaluation of liver fibrosis to assess treatment response. Liver biopsies are not ideal for frequent evaluations and thus cannot be regularly used for this purpose. HBV infected patients who require treatment usually have a higher Aspartate-Platelet Ratio Index (APRI) score as compared to those who don't require treatment [9]. This justifies the need to as well rely on non-invasive markers (NIMs) in evaluating hepatic fibrosis and determining treatment necessity [10].

NIMs for hepatic fibrosis must be simple, readily available, reliable, inexpensive, safe, and well

validated in different forms of chronic liver disease [11]. Some NIMs include Aspartate-platelet ratio index (APRI) score [12,13], Age-platelet index (API) [14], Fibrosis index based on 4 factors (FIB-4) [15,16] and AST/ALT ratio (AAR) [17,18].

The use of APRI and FIB-4 as non-invasive markers (NIMs) have been validated [12] but still not very much used in Cameroon despite the high prevalence of HBV infection. Liver biopsy is not commonly done in most parts of the country due to limited number of specialists who can carry out the procedure. As a result, liver fibrosis assessment in Cameroon happens to be a significant loop hole in the management of HBV infected patients.

Most of the studies that have been done so far to investigate the use of non-invasive markers actually enrolled just HBV infected patients with a recent liver biopsy result which was used as the gold standard to compare with and ascertain the use of the non-invasive markers. These studies did not address the possibilities of excluding or including fibrosis in HBsAg negative people using NIMs. In order to investigate how well these non-invasive markers could be used in excluding fibrosis or liver damage especially of hepatitis B origin, we compared the scores of some non-invasive markers (APRI, API, FIB-4 and AAR) between HBV infected cases and "healthy" controls.

The complex nature of HBV infection and the fact that it affects the liver gives the infection the potentials to indirectly account for extrahepatic manifestations causing blood related conditions like anemia [19–21] and significant changes in white blood cells (WBC) [22]. This study also investigated the effects of HBV infection on some hematological parameters so as to identify hematological changes that may occur in HBV chronically infected patients in the South West Region of Cameroon.

2. MATERIALS AND METHODS

2.1 Study Design and Sampling Technique

This was a hospital-based case-control study that enrolled HBV infected individuals and "healthy" controls all ≥ 18 years of age. The study was conducted in Buea, the capital city of the South West region of Cameroon. The cases were HBV treatment naïve hepatitis B surface antigen (HBsAg) positive people who were negative for HIV and HCV with no history of kidney disease or metabolic syndrome. The controls were physically strong and "healthy" looking people negative for HIV, HCV, HBsAg and hepatitis B core antibody (anti-HBc) with no history of liver disease, kidney disease or metabolic syndrome. Alcohol consumers as well as pregnant women were excluded from the study. The participants were enrolled during an HIV, HCV and HBV free screening exercise we conducted in the Buea Regional Hospital. People who came to donate blood and tested positive for HBsAg were as well enrolled as cases.

2.2 Sample Size Calculation

Case-control study (80% power desired with an Odds ratio of ≥2.0). We used the formula in equation 1 below:

$$n = \left(\frac{r+1}{r}\right)\frac{(p)(1-p)(Z_{\beta}+Z_{\alpha/2})^2}{(P_1-P_2)^2} \quad [23] \quad (1)$$

Where

n=minimum sample size to achieve 80% power

r=ratio of controls to cases (1)

Z_{β}= the desired power (typically .84 for 80% power).

Zα/2=the desired level of statistical significance (typically 1.96).

p(1-*p*) = A measure of variability (similar to standard deviation)

P_1=Proportion of controls with elevated ALT: 21.7% [24]

P_2=Proportion of HBV infected cases with elevated ALT

$$P_2 = \frac{OR_{Pcontrol\,exp}}{Pcontrol\,exp\,(OR-1)+1} = \frac{2.0(0.22)}{0.22(2.0-1)+1} = 0.36$$

Therefore

$$n = 2\frac{0.29\,(1-0.29)(0.84+1.96)^2}{(0.3\ 6-0.22)^2} = 164.7$$

Therefore, at least 165 controls and 165 HBV infected cases were considered to achieve the desired power (80%).

2.3 Data Collection and Clinical Evaluation

A standard questionnaire [25] was administered (interview mode) to all participants to obtain demographic information as well as to find out if

the participants consume alcohol and if they have any history of liver disease, kidney disease or metabolic syndrome. A medical doctor clinically examined the participants for signs of metabolic syndrome and/or kidney disease.

2.4 Sample Collection

Using the vacutainer system, a phlebotomist collected 10 ml of blood from each participant into 2 tubes: one containing Potassium Ethylenediaminetetraacetic acid (K_3EDTA) anticoagulant and the other with no anticoagulant (dry tube).

2.5 Laboratory Analysis

2.5.1 Full Blood Count (FBC) and WBC Differential Count

The sample in the K_3EDTA tube was used to perform full blood count (FBC) using an Auto Hematology Analyzer (Mindray model BC-2800, Mindray Bio-Medical Electronics, Nanshan, Shenzhen, P.R. China) following manufacturer's instructions. We considered the reference ranges as stated in the Mindray model BC-2800 operator's manual. WBC differential count was done manually for each blood sample in K_3EDTA tube following a standard procedure [26]. We considered reference ranges as 20-40% and 40-80% for lymphocyte and neutrophil respectively [26].

2.5.2 Immunochromatographic qualitative tests

The samples in the dry tubes were centrifuged at 1000g for 5 minutes to obtain sera which was first of all used to screen for HIV (Abbot Determine, USA), HCV antibodies (Acon® Laboratories Inc., USA) and HBsAg (Diaspot Inc. USA). HBV serologic profile was done using a qualitative panel kit (Blue Cross Bio-Medical Co. Beijing) for the detection of HBsAg, anti-HBs, HBeAg, anti-HBe and anti-HBc following manufacturer's instructions

2.5.3 Liver Aminotransferase measurement

With the sera, liver aminotransferase was measured for each participant using AST-(GOT)-Human reagent and ALT-(GPT)-Human reagent by spectrophotometry (Mindray® BA-88 Biochemistry analyser) following the manufacturer's instructions. ALT reference ranges were <32 IU/ml for females and <42 IU/ml for males while AST reference ranges were <31 IU/ml for females and <37 IU/ml for males as per the reagent used.

2.6 Calculation and Interpretation of Non-invasive Markers (NIMs)

2.6.1 APRI score

This was calculated by inserting the corresponding AST level, Upper limit normal (ULN) AST value and Platelet count in the following formula (equation 2):

$$APRI = \frac{AST\ level/ULN*}{Platelet\ cou\ nt(10^9/L)} \times 100 \quad [27] \qquad (2)$$

*ULN is the upper limit normal of AST for the reagent used.

An APRI score <0.5 and ≥0.5 was used to exclude and include possible fibrosis respectively [12].

2.6.2 FIB-4 score

FIB-4 was calculated by inserting age, AST value, ALT value and platelet count in the following formula (equation 3):

$$FIB\ 4 = \frac{Age \times AST}{Platelet\ cou\ nt \times \sqrt{ALT}} \quad [28] \qquad (3)$$

A FIB-4 value of <1.45 and ≥1.45 was used to exclude and include possible fibrosis respectively [28,29]

2.6.3 API score

Age-platelet index was obtained using age grouped as follows: <30 years old; 0 points, 30-39 years old; 1 point, 40-49 years old;2 points, 50-59 years old; 3 points, 60-69 years old; 4 points and ≥70 years old: 5 points. The platelet count (10^3/µl) was grouped as follows: ≥225; 0 points, 200-224;1 point, 175-199; 2 points, 150-174;3 points, 125-149; 4 points and <125; 5 points [14]. It was then calculated using the following formula (equation 4):

$$API = (points\ for\ age) + (Points\ for\ Platelet) \quad [14] \qquad (4)$$

An API score ≥6 indicated significant histologic findings (necroinflammatory lesions, septal fibrosis and/or cirrhosis). Scores <6 showed no fibrosis [14,30].

2.6.4 Aspartate Aminotransferase/Alanine Aminotransferase Ratio (AAR)

This was calculated using the formula (equation 5):

$$AAR = \frac{AST}{ALT} \quad [31] \qquad (5)$$

AAR<1 and AAR ≥ 1 was used to exclude and include significant fibrosis respectively [30].

2.7 Statistical Analysis

Data was entered in MS excel and analyzed in SPSS (Statistical Package for Social Sciences) version 21. Data were presented using frequency distribution tables and summary statistics. Categorical variables were compared using the Pearson's Chi-square test or the Fisher's exact test. Continuous variables were compared using the student t-test. A logistic regression analysis was fitted for cases that recorded p value < 0.05 in the crude Odds ratio. A p value < 0.05 was considered significant for all analyses.

3. RESULTS

After matching for age and sex, a total of 404 participants (202 HBsAg positive individuals and 202 "healthy" controls) were enrolled. Mean ages of 31.39±8.3 and 31.89±8.5 were recorded for the cases and controls respectively. Cases had 100(49.5%) females and 102 (50.5%) males while controls had 104 (51.5%) females and 98(48.5) males. All the participants were aged between 18 and 65 years (Table 1).

Table 1. Age and gender distribution

Groups (n)	Mean age (years)	Age range (years)	Gender	n (%)
HBV infected cases (202)	31.39±8.3	18-62	Female	100 (49.5)
			Male	102 (50.5)
Healthy controls (202)	31.89±8.5	18-65	Female	104 (51.5)
			Male	98 (48.5)

Table 2. Mean comparisons of liver enzymes, hematological parameters and non-invasive markers (NIMs) between HBV infected cases and controls

Test group	Parameter	Status	Mean±SD	95% CI*	P-value
Liver enzymes	ALT (U/L)	Cases	32.6±18.8	8.3 to 14.0	**<0.001**
		Controls	21.5±7.7		
	AST (U/L)	Cases	35.5±23.4	7.2 to 14.0	**<0.001**
		Controls	24.9±7.8		
Hematological parameters	HGB (g/dl)	Cases	14.2±2.1	1.08 to 1.48	0.759
		Controls	14.4±9.0		
	WBC (10^3/µl)	Cases	5.0±1.4	-0.25 to 0.25	1.000
		Controls	5.0±1.1		
	RBC (10^6/µl)	Cases	5.0±0.6	0.02 to 0.22	0.095
		Controls	4.9±0.6		
	Lymphocyte (%)	Cases	44.7±10.7	0.76 to 2.96	0.247
		Controls	43.6±8.2		
	Neutrophil (%)	Cases	50.0±11.8	0.20 to 4.41	**0.032**
		Controls	52.3±9.6		
	HCT (%)	Cases	42.9±6.2	1.01 to 3.19	**<0.001**
		Controls	40.8±4.9		
	PLT (10^3/µl)	Cases	220.3±62.4	5.08 to 28.92	**0.005**
		Controls	237.3±59.4		
Non-invasive markers of fibrosis	APRI	Cases	0.50±0.32	0.12 to 0.22	**<0.001**
		Controls	0.33±0.12		
	FIB-4	Cases	0.97±0.6	0.09 to 0.29	**<0.001**
		Controls	0.78±0.4		
	AAR	Cases	1.18±0.4	-0.04 to 0.10	0.394
		Controls	1.21±0.3		
	API	Cases	2.06±1.8	0.03 to 0.67	**0.030**
		Controls	1.71±1.4		

**confidence interval*

Table 3. Comparing the interpretation of liver enzymes and hematological parameters between HBV infected cases and controls

Test group	Parameter	Status	Interpretation			P-value
			Low	Normal	High	
Liver enzymes	ALT (U/L)	Cases	-	150 (74.2)	52 (25.8)	**<0.001**
		Controls	-	189 (93.6)	13 (6.4)	
	AST (U/L)	Cases	-	135 (66.8)	67 (33.2)	**<0.001**
		Controls	-	183 (90.6)	19 (9.4)	
Hematological parameters	HGB (g/dl)	Cases	25 (12.4)	177 (87.6)	-	0.426
		Controls	34 (16.8)	168 (83.2)	-	
	WBC (10^3/µl)	Cases	31 (15.3)	171 (84.7)	-	0.421
		Controls	37 (18.3)	165 (81.7)	-	
	RBC (10^6/µl)	Cases	4 (2.0)	139 (68.8)	59 (29.2)	0.773
		Controls	3 (1.5)	144 (71.3)	55 (27.2)	
	Lymphocyte (%)	Cases	2 (1.0)	73 (36.1)	127 (62.9)	0.398
		Controls	2 (1.0)	82 (40.6)	118 (58.4)	
	Neutrophil (%)	Cases	102 (50.5)	95 (47.0)	5 (2.5)	**0.041**
		Controls	67 (33.2)	133 (65.8)	2 (1.0)	
	HCT (%)	Cases	33 (16.3)	161 (79.7)	8 (4.0)	0.049
		Controls	44 (21.8)	157 (77.7)	1 (0.5)	
	PLT (10^3/µl)	Cases	27 (13.4)	174 (86.1)	1 (0.5)	**<0.001**
		Controls	3 (1.5)	193 (95.5)	6 (3.0)	

Table 4. Comparing the interpretation of non-invasive markers for fibrosis between HBV infected cases and controls

Non-invasive markers	Status	NIM interpretation		Crude odds ratio (CI)	P-value	Adjusted odds ratio (CI)	P-value
		Possible fibrosis	No fibrosis				
APRI	Cases, n (%)	86 (42.6)	116 (57.4)	6.06 (3.59-10.24	<0.001	1.77 (1.47-2.13)	<0.001
	Controls, n (%)	22 (10.9)	180 (89.1)	1		1	
FIB-4	Cases, n (%)	51 (25.2)	151 (74.8)	5.35 (2.75-10.39)	<0.001	6.06 (2.92-12.50)	<0.001
	Controls, n (%)	12 (5.9)	190 (94.1)	1		1	
API	Cases, n (%)	15 (7.4)	187 (92.6)	8.02 (1.81-35.55)	0.006	7.58 (1.54-37.04)	0.013
	Controls, n (%)	2 (1.0)	200 (99.0)	1		1	
AAR	Cases, n (%)	136 (67.3)	66 (32.7)	0.48 (0.30-0.76)	0.002	0.52(0.32-0.83)	0.006
	Controls, n (%)	164 (81.2)	38 (18.8)	1		1	

(APRI <0.5=No fibrosis, APRI ≥0.5=Possible fibrosis: FIB-4 <1.45=No fibrosis, FIB-4≥1.45 =Possible fibrosis: API<6= No fibrosis, API ≥6=Possible fibrosis: AAR<1=No fibrosis, AAR≥1=Possible fibrosis)

A statistically significant difference (P<0.05) was recorded for the mean values of ALT, AST, neutrophil, HCT, PLT, APRI, FIB-4 and API between cases and controls (Table 2). Cases recorded a significantly higher number of participants with abnormal results for ALT, AST, neutrophil and platelet as seen in Table 3. The mean AAR (p=0.394) did not show any statistically significant difference between cases and controls (Table 2) although there were significantly more controls (p=0.002) with AAR≥1 (Table 4). Liver fibrosis was significantly more associated with cases than controls based on APRI (OR:6.06, CI:3.59-10.24), FIB-4 (OR:5.35,

CI:2.75-10.39) and API (OR:8.02, CI:1.81-35.55) both in the crude and adjusted Odds ratio (Table 4).

Considering APRI ≥0.5 and FIB-4 ≥1.45 as the low cut-offs to denote fibrosis [12,28] for our HBV infected participants, 86 (42.6% of 202) and 51 (25.2% of 202) of them were considered to have some form of fibrosis at least based on APRI and FIB-4 respectively. AAR recorded 136 (67.3% of 202) cases with possible fibrosis while API had 15 (7.4% of 202). The 4 NIMs excluded fibrosis in 27 (13.4%) of the 202 HBV infected cases as seen in Fig. 1. Seventy-seven (38.1%) and 29 (14.4%) of the cases showed evidence of fibrosis based on AAR only and APRI only respectively.

Sixty-nine (34.2%) of the cases had a result indicative of possible fibrosis from at least 2 of the different NIMs (Fig. 1). Thirty-six (17.8%) had an interpretation indicative of possible fibrosis from at least 3 of the different NIMs while 8(4.0%) had an interpretation indicative of fibrosis from all the 4 different NIMs (Fig. 1).

4. DISCUSSION

HBV is known to affect mainly the liver but the inflammatory and immune response in HBV infected patients can affect the clinical, serological and hematological outcome of the patient as the severity of the viral disease depends on the immune system's ability to attack infected hepatocytes [32]. Our study looked at possible changes in some hematological parameters (HB, RBC, WBC, PLT, HCT, Neutrophil and lymphocyte) that may have been brought about by HBV infection. Cases recorded a significantly lower mean neutrophil percentage as compared to controls. Some other studies showed a statistically significant difference in the mean Hemoglobin measurements of cases and controls [33] while others did not have a statistical difference for neutrophil but rather had a statistical difference for lymphocytes [22] with the mean lymphocyte percentage of HBV infected cases significantly higher than that of the controls. This may look different from our findings but the clinical implication and interpretation is similar because during viral infections, a rise in lymphocyte percentage and a drop in neutrophil percentage is usually noticed [34].

Our study showed that the mean platelet count in HBV infected cases was significantly lower than that of the controls as seen in other studies [35,36]. Thrombocytopenia (deficiency of platelets in the blood) is a common complication in liver disease. The major mechanisms for thrombocytopenia in liver cirrhosis are (1) platelet sequestration in the spleen; and (2) decreased production of thrombopoietin in the liver. More platelets are destroyed in the enlarging spleen as a result of fibrosis and aggravated portal hypertension [37].

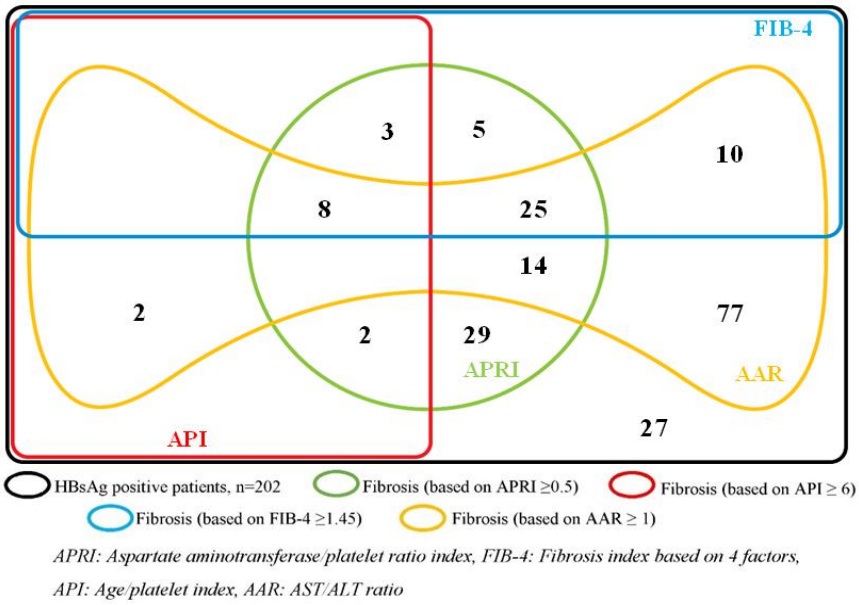

Fig. 1. Relationship and overlap between different non-invasive markers of liver fibrosis in the HBV infected cases (n=202)

HBV infected cases recorded a significantly higher HCT as compared to controls in our study. This does not corroborate with previous findings [22,33,38]. We did not notice any statistically significant difference with the mean values of hemoglobin and RBC between cases and controls. Although some studies [19,20] have shown that HBV infection can cause anemia, the infection is rarely linked to hematological changes in erythrocyte or its indices. Most studies link it to changes in leucocytes and platelets in blood [38].

Over the past decade several non-invasive markers of fibrosis have been proposed and validated following research. A good number of them are quite inexpensive and does not require a lot of skills like the liver biopsy which is presently still considered as the gold standard despite all its short comings. The mean APRI, FIB-4 and API scores of HBV infected cases were significantly higher than that of the controls in our study. Similar findings were reported in previous studies with APRI [39].

AAR (AST/ALT ratio) has been widely utilized as a predictor of hepatic fibrosis [40]. We did not notice any statistically significant difference between the mean AAR of HBV infected cases and controls (Table 4). Interestingly there was significantly more "healthy" controls with AAR≥1 (indicative of possible fibrosis) than HBV infected cases (Table 4). AST is seemingly more abundant in cells (as compared to ALT) because it can be found in the heart, liver, kidney, brain, RBC and muscle tissues whereas ALT is mainly found in liver cells [41]. Moreover, even at the level of the liver, ALT is only found in the cytoplasm of hepatocytes whereas AST can be found in the cytoplasm as well as in the mitochondria of hepatocytes [42]. Elevation of AST above ALT (which would probably result to AAR≥1) may be seen in several different conditions. It could be suggestive of a non-hepatic source of AST which may occur due to release of AST from blood cells during hemolysis or reduction in AST clearance [43]. AST may as well be released into the circulation following injury to non-liver cells in the body (muscle, heart, kidney, brain etc) particularly cells that contain mitochondria [42]. This lack of specificity probably explains why our study recorded a significant proportion of HBsAg negative people with AAR≥1.

Some researchers now criticize and contest the use of AAR (AST/ALT ratio) as a marker of fibrosis especially in chronic HBV infection [44,45]. AST/ALT ratios below 1.0 (AAR<1) are typical of chronic viral hepatitis (e.g. hepatitis B and C). However ratios slightly above 1.0 may be found in chronic viral hepatitis but this is particularly when progression to fibrosis and cirrhosis is present [42]. Our Study couldn't agree less as we recorded a higher number of HBV infected cases with AAR<1[66 (32.7%)] as compared to controls with AAR<1[38 (18.8%)]. Some studies have shown that AAR is a better marker for fibrosis in Alcoholic hepatitis and not viral hepatitis [41,46].

Based on the fact that cut off values for many non-invasive markers are still under debate, a high cut off with high specificity (i.e. fewer false-positive results) and a low cut off with high sensitivity (i.e. fewer false-negative results) have been generated for APRI and FIB-4 [12,47] to include and exclude fibrosis respectively. This controversy on cut off values actually questions the reliability of NIMs in determining liver fibrosis. In order to bypass this problem, we tried to compare the relationship and/or overlap between 4 different NIMs under the assumption that if all the NIMs or most of the NIMs share the same conclusion for a patient, then its most likely the true clinical picture of the patient as far as possible presence or absence of liver fibrosis is concerned. We realized that there was no individual who had a FIB-4 or API score indicative of fibrosis based on FIB-4 or API only unlike APRI (independently showed possible fibrosis in 29 cases) and AAR (independently showed possible fibrosis in 77 cases). The fact that APRI alone identified possible fibrosis in 29 cases which were not identified as such by the other NIMs further confirms previous findings which showed that the <0.5 cut off mark for APRI was not very reliable in ruling out fibrosis [47–49] as compared to the <1.45 cut off for FIB-4. In our study, 34.2%, 17.8% and 4.0% of our HBV infected cases had results indicative of possible hepatic fibrosis with at least 2 NIMs, at least 3 NIMs and all 4 NIMs respectively. A systematic review that assessed the proportion of HBV patients with liver fibrosis in Sub Saharan Africa showed that over 15% have significant fibrosis/cirrhosis[50]. A study conducted in Cameroon recorded 13.4% of HBV patients with fibrosis [51]. Another study conducted in the Gambia had 17.5% prevalence of fibrosis among HBV infected individuals screened at a blood bank. These proportions of HBV infected patients with liver fibrosis are similar to the 17.8% of patients we had with fibrosis based on

at least 3 different NIMs. Although this may not be a solid foundation to validate the use of NIMs, it could ascertain their reliability in our setting if at least 3 of them are considered before ruling in or ruling out fibrosis for every HBV infected patient on clinical assessment. Unfortunately, liver biopsy was not done in this study which could have been used to further justify the use of NIMs in our setting.

5. CONCLUSION

HBV infection affects neutrophil, HCT, PLT, APRI, FIB-4 and API in our study population. AAR did not prove to be a reliable NIM in detecting fibrosis of hepatitis B origin due to lack of specificity. Using more than two NIMs for an HBV infected patient at a given time can by-pass the problem of contradictory cut off values and significantly reduce the possibilities of arriving at a wrong conclusion when determining fibrosis in clinical practice. Moreover, most NIMs are inexpensive and may not pose that much of a problem financially if the doctor demands for more than one at a given time. Besides some of the NIMs share similar parameters and as such, data generated for one may as well be used for another like APRI and FIB-4.

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CONSENT

A signed informed consent form was obtained from every participant prior to enrolment. A trained counsellor verbally explained the study protocol to those who could not read nor write and counselled each participant prior to testing.

ETHICAL APPROVAL

The National Ethics Committee of Research for Human Health (NECRHH) in Cameroon approved the study protocol.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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