EVALUATION OF THE DIAGNOSTIC AND PREDICTIVE PERFORMANCE OF NON-INVASIVE MODELS FOR ASSESSING LIVER FIBROSIS IN PATIENTS WITH CHRONIC HEPATITIS B VIRUS INFECTION

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ABSTRACT

The performance of non-invasive models in the diagnosis and prediction of liver fibrosis have not been evaluated in all populations. This study evaluated the accuracy of gamma-glutamyl transpeptidase-to-platelets ratio index (GPRI), and S-index in the diagnosis and prediction of liver fibrosis in patients with chronic hepatitis B virus infection (CHBV). Fifty patients with CHBV and 40 control were recruited into this case-control study. Albumin (ALB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), bilirubin, gamma-glutamyl transpeptidase (GGT) and platelet count (PLT) were determined by colorimetric methods and Sysmex XS-10000 haematology automated analyzer respectively. GPRI and S-index were computed. Data were analyzed using ANOVA, Pearson's correlation and ROC curve at $p < 0.05$. ALP, ALT, AST, GGT, GPRI and S-Index were higher ($p < 0.05$), while ALB was lower ($p < 0.05$) in patients with liver fibrosis (LF), compared to patients with CHBV. ALP, GGT, GPRI and S-index were higher ($p < 0.05$), while ALB and PLT were lower ($p < 0.05$) in patients with CHBV compared to the controls. ALP, ALT, AST, GGT, GPRI and S-index were higher ($p < 0.05$), while ALB was lower ($p = 0.000$) in patients with LF compared to the controls. The area under the curve (AUROC) for S-index and GPRI were (AUC=0.835, P=0.000), GPRI (AUC=0.778, P=0.003). This study shows that both S-index and GPRI are good test instruments for evaluating liver fibrosis in patients with chronic hepatitis B. S-index was a better marker than GPRI for predicting liver fibrosis.

KEYWORDS: chronic hepatitis B virus, liver fibrosis, noninvasive models, diagnostic, predictive performance

INTRODUCTION

Chronic hepatitis B virus infection is a major public health challenge, as the foremost cause of liver fibrosis, cirrhosis and hepatocellular carcinoma.

Gamma glutamyl transpeptidase to platelets ratio index (GPRI) and S-index are new models for assessment of liver injury in patients with chronic hepatitis B virus infection but their diagnostic and predictive performance in hepatitis B related liver

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fibrosis have not been determined in all populations. Liver biopsy has its limitations. Transient elastography and fibroscan are not available for all patients. Improvement in patients' outcomes may require evaluation and refinement of noninvasive models for diagnosis and accurate prediction of fibrosis development using simple score of routine laboratory tests. This will reduce unnecessary biopsy and delineate patients who will benefit from immediate medical treatment to reduce the risk of progression to liver cirrhosis and hepatocellular carcinoma. The utmost significant objective in the treatment of hepatitis is to evade chronic liver disease and its complications (Leoni *et al.,* 2022). Regardless of an extensive vaccination program worldwide and availability of antiviral agents over the past three decades, a functional HBV cure with sustained, undetectable HBsAg and HBV DNA in serum with or without seroconversion to hepatitis B surface antibody (anti-HBs) following a finite course of treatment, resolution of residual liver injury and a decrease in risk of HCC over time, is rare and occurs in only 1% of all HBsAg-positive individuals yearly (Moini & Fung 2022). A complete sterilizing cure with undetectable HBsAg in serum and eradication of HBV DNA including intrahepatic cccDNA and integrated HBV DNA is not possible with current treatment agents (Lok *et al.,* 2017, Jeng and Lok, 2023). Even with a combination of two or more therapies, these agents are still not effective against covalently closed circular DNA and the integrated HBV DNA. Also, HbsAg is derived from both covalently closed circular DNA and the integrated HBV DNA, hence complete loss of HBsAg seems impossible (Feitelson *et al.,* 2022).

Gamma-glutamyl transpeptidase (GGT) is an enzyme that can be isolated from hepatocytes, duct cell and gall bladder epithelium. A number of hepatobiliary diseases can cause GGT elevation, including viral hepatitis, non-alcoholic fatty liver disease, alcoholic liver disease, drug use, viral hepatitis, toxins (aflatoxin B) and obesity (Zhao *et al.,* 2022, Xing *et al.,* 2022., Saini *et al.,* 2021). The GGT levels are used with other liver enzyme tests to diagnose liver disease. Both ALP and GGT levels become elevated when there is bile ducts or certain liver diseases, but only ALP will be elevated if there is bone disease. Therefore, high level of GGT and ALP, may be considered an indicator of significant fibrosis in CHB patients. GPRI contains two simple serological markers of GGT and platelet count, and has been shown to predict liver injury in chronic hepatitis B virus infection. Gamma glutamyl transferase activity (GGT) has served as a proxy for total ALP elevations attributable to the hepatic isoform given that both are membrane-bound proteins with a shared mechanism of release (Geno *et al.,* 2021).

Alkaline phosphatase (ALP) refers to a group of phosphomonoesterases that hydrolyze phosphate esters with optimum in vitro activity at a pH of 10. Levels of serum alkaline phosphatase have been reported to be significantly higher among those with chronic hepatitis B compared with healthy individuals. Platelets are produced with the aid of a hormone thrombopoietin synthesized by the liver (Hu *et al.,* 2019). Even though platelet count may provide clues regarding the severity of liver disease, there are currently no available data supporting the utility of the platelet count to evaluate the degree of liver injury in patients with chronic hepatitis B virus (HBV) infection (Yang *et al.,* 2020).

Albumin is synthesized in the liver and is the most abundant circulating protein with multifunctional properties, such as transport protein, oncotic pressure maintenance, immune modulation, endothelial stabilization and detoxification (Wong *et al.,* 2021). Low serum albumin level is a cardinal feature and prognostic biomarker of decompensated cirrhosis in patients with Child–Pugh cirrhosis scores B and C (Jeng, *et al.,* 2021).

Gamma-glutamyl-transpeptidase to platelet ratio index (GPRI) is a novel liver fibrosis model, which is reported to be more accurate than earlier noninvasive models such as fibrosis index based on the four factors (Fib-4) and aspartate transaminase (AST) to platelet ratio index (APRI) for detecting significant fibrosis and cirrhosis in patients with CHBV infection (Purkayastha *et al.,* 2023). S-index (GGT-PLT-albumin index) is a simple model comprising of routine laboratory tests for predicting liver fibrosis in patients with chronic hepatitis B virus (HBV) infection.

The connection between hepatitis B virus-related liver fibrosis, chronic hepatitis B virus infection and serum albumin, alkaline phosphatase, gamma glutamy transferase and platelet count gamma-glutamyl transferase to platelets ratio index (GPRI), and S-index is not fully understood. This study investigated the levels serum albumin, alkaline phosphatase, serum bilirubin, alanine aminotransferase, aspartate amino transferase gamma glutamyl transpeptidase and platelet count, to deduce noninvasive markers such as gamma-glutamyl transpeptidase to platelets ratio index (GPRI), and S-index and their predictive accuracy in patients with chronic hepatitis B virus infection and hepatitis B related liver fibrosis, using liver biopsy as the gold standard.

MATERIALS AND METHODS

Study design: This case-control study was conducted in Calabar Metropolis, from June 2022 to February 2023, to determine the association between serum levels of albumin (ALB), alkaline phosphatase (ALP), gamma glutamytranspeptidase (GGT), serum bilirubin, alanine aminotransferase (ALT), aspartate amino transferase (AST) and platelet count (PLT). Deduced noninvasive markers such as gamma-glutamyl transpeptidase to platelets ratio index

(GPRI), and S-index and their diagnostic and predictive performance among patients with chronic hepatitis B virus infection and hepatitis B related liver fibrosis, attending clinics in the University of Calabar Teaching Hospital (UCTH), Calabar, Southern Nigeria.

Study setting/subject selection: The study population consists of patients with hepatitis B virus infection and associated complications in Calabar Metropolis, comprising of residents of Calabar South and Municipal Local Government Areas, Southern, Nigeria. With the help of the attending physicians, 90 subjects were selected for this study. Fifty (50) patients with CHBV infection attending the University of Calabar Teaching Hospital, Calabar, aged 20-50 years and 40 apparently healthy aged matched controls residing in the same geographic location were recruited into the study. The hepatitis B virus infected subjects were further categorized based on the pathologic condition of the liver as confirmed by biopsy result into those with significant liver fibrosis (LF, n=13) and chronic hepatitis B virus infection without fibrosis (CHBV, n=37). All the participants were screened for HIV and HCV infections. Patients without a history of smoking, alcohol consumption and substance abuse, or any known chronic organ or systemic illness or medication were recruited into the study. In contrast, individuals whose ages were outside the selected age range, those with a history of smoking, alcohol consumption and substance abuse, diabetes or any known chronic organ or systemic illness or medication were excluded from the study. Socio-demographic data, (age, marital status, education, work), family and medical history of past illness, current medication use, social lifestyle (smoking habit, alcohol use, drug addiction, and substance abuse) were obtained from each participant using a structured interviewer questionnaire.

Sample collection: A standard venepuncture method was used to obtain 7 mL of blood from all the participants. Three milliliters of blood was dispensed into K_2 EDTA samples bottle for platelet estimation and 4mls into plain bottles, allowed to clot and then centrifuged at 3 000 rpm for 5 mins at 25°C. The sera were separated immediately into aliquots using sterile Pasteur pipettes and stored at −20 °C in the UCTH laboratory and analysed for the targeted biochemical indices within 7 days.

LABORATORY METHODS:
Determination of platelet count
Platelet count was determined by a 5 parts Sysmex XS-10000 haematology automatic analyzer. The sysmex-XS-1000 can analyse and output the results for 32 parameters of blood samples. It utilizes technology of fluorescence flow cytometry to quantitate the standard five part differential, immature granulocytes (metamyelocytes, myelocytes and promyelocytes), nucleated red blood cells (NRBC), reticulocyte count, immature reticulocyte fraction and "optical" fluorescent platelet count. The combination of side scatter (inner complexity of the cell), forward scatter (volume) and fluorescence intensity of nucleated cells gives a concise but precise image of each cell detected in the peripheral blood. A well-defined physical description of the different leucocyte populations (clusters) is obtained. Abnormal and immature cells, with their larger nuclear volume show much higher fluorescence intensity than normal cells, and are easily distinguishable in the DIFF scattergram.

Determination of serum alkaline phosphatase (ALP)
Serum ALP was determined by kinetic colorimetric Diethanolamine buffered method (pH=10.0), using a kit produce by Randox Laboratories Ltd, United Kingdom, that optimized standard method according to the recommendation of the Deutsche Gesellschaft fur klinische Chemie. Alkaline phosphatase catalysis the hydrolysis of p-nitrophenylphosphate, at pH=10.0, to produce phosphate and p-nitrophenol (Rec, 1972)

Determination of Gamma-glutamyl transferase
Gamma-glutamyl transpeptidase was determined by Kinetic colorimetric method according to Szasz 1969, using a kit obtained from AGAPPE DIAGNOSTICS LTD, Kerala, India. Gamma-glutamyl transferase present in the sample catalyzes the transfer of the glutamyl group from the substrate γ-glutamyl-3-carboxy-4-nitroanilide to glycylglycine forming glutamyl glycylglycine and 5-amino-2-nitrobenzoate. The rate of formation of 5-amino-2-nitrobenzoate is proportional to the activity of GGT present in the sample and can be measured kinetically at (400-420) nm.

GGTL-γ-glutamyl-3-carboxy-4-nitroanilide + glycylglycine —> L-γ-glutamyl-glycylglycine + 5-amino-2-nitrobenzoic acid (Szasz, 1969)

Determination of albumin
Albumin was determined by the Bromocresol green (BCG) colorimetric method using a reagent kit obtained from Atlas medical, Cambridge, CB4 4WX, UK. The method is based on the specific binding of bromocresol green (BCG), an anionic dye, and the protein at acid pH (4.3) produce a color change of the indicator from yellow –green to green –blue with the resulting shift in the absorption wavelength of the complex. The intensity of the color formed is proportional to the concentration of albumin in the sample, measured at 630 nm. (Doumas *et al.,* 1971).

Determination of serum aspartate aminotransferase (AST) and alanine aminotransferase (ALT
Serum AST and ALT were determined by commercial colorimetric kit produce by Randox Laboratories Ltd, United Kingdom according to the method of Reitman and Frankel 1957 (Reitman & Frankel, 1957.)

Determination of serum bilirubin

Serum bilirubin was determined by colorimetric method of Jendrassik and Grof 1938, using a kit obtained from Randox Laboratories Ltd., 55 Diamond Road, Crumlin, Co. Antrim, United Kingdom (Jendrassik and Grof, 1938).

$$GPRI = \frac{[GGT/ULN] \times 100}{Platelet\ counts\ [\times 10^9/l].}$$

Computation of S-index (Parikh, *et al.,* 2017)

$$S\text{-index} = \frac{1000 \times GT}{(PLT \times albumin).}$$

Calculation of the predictive values of the S-index and GPRI

$$\textbf{Positive predictive value (PPV)} = \frac{TP}{(TP+FP)} \times 100$$

$$\textbf{Negative predictive value (NPV)} = \frac{TN}{(TN+FN)} \times 100$$

$$\textbf{The overall efficiency of a test} \quad \frac{TP+TN}{(TP+FP+TN+FN)} \times 100$$

Study size: Sample size was determined according to the method of Sullivan (2016), using the formula $\frac{(Z_a + Z_\beta)^2 . \bar{p}(1-\bar{p})}{(p0-p1)2}$ (Sullivan *et al.,* 2016). The power of 0.84 was calculated at beta error of 80%. The sample size of 50 patients was arrived at, while 40 apparently healthy individuals who served as controls were selected for the study.

STATISTICAL ANALYSES

Results generated were presented as mean ± standard deviation. Data were analyzed using the statistical package for social sciences (SPSS version 23.0, IBM, USA). One way analysis of variance (ANOVA) was used to test the variations within and among group means and Fisher's least significant difference (LSD) post-hoc analysis was used for the comparison of multiple group means. Pearson's correlation was used to determine the associations between variables. DeLong's test was done to compare the area under the receiver operating characteristic curve (AUROC) for the noninvasive markers using histology results as reference. The confidence interval was set to 95%. The significance level of the tests was set at α=0.05.

RESULTS

Among the subjects (50) with chronic hepatitis B virus, 34 % (n=17) were females while 66 % were males (n=33). Metavir score was used to determine the level of fibrosis. Thirteen (n=13) 26 % of the patients with chronic hepatitis B virus infection had significant fibrosis of stage F2 (n=6) and F3 (n=7) on biopsy. Subjects with chronic hepatitis B virus infection without fibrosis were thirty seven (n=37), those with F0 were twenty (n=20), those with F1 were seventeen (n=17).

Computation of gamma glutamyltranspeptidase to platelets ratio index (GPRI)

Gamma-glutamyl transpeptidase to platelets ratio index was computed using the formula. The GPRI is calculated based on the serum GGT value (and the upper limit of normal [ULN] value for the laboratory) and platelet counts using the following formula:

Variation of age, serum albumin, alkaline phosphatase, gamma glutamyl transpeptidase, platelet count, gamma-glutamyl transpeptidase to platelets ratio index (GPRI), and S-index among subjects with chronic hepatitis B, Liver fibrosis and controls

The comparison of age, serum albumin (ALB), alkaline phosphatase (ALP), serum total bilirubin (TB) and conjugated bilirubin (CB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transpeptidase (GGT), platelet count (PLT), gamma-glutamyl transpeptidase to platelets ratio index (GPRI), and S-index among subjects with chronic hepatitis B, liver fibrosis and controls is shown in table 1. Serum albumin, ALP, GGT, ALT, AST, PLT, TB, CB, GPRI, and S-index varied significantly (p<0.05) among patients with chronic hepatitis B virus infection, liver fibrosis and the controls, while age did not vary significantly (p>0.05) across the groups. Serum ALP, ALT, AST, PLT, TB, CB, GGT, GPRI and S-Index were significantly higher (p<0.05) in patients with liver fibrosis when compared with patients with chronic hepatitis B virus infection, while serum albumin was significantly (p<0.05) higher in patients with chronic hepatitis B virus infection than in patients with liver fibrosis. Serum albumin and platelet count were significantly (p<0.05) lower, while serum ALP, ALT, AST, GGT, GPRI and S-index were significantly

(p<0.05) higher in patients with LF compared with the controls. Total bilirubin and conjugated bilirubin were significantly higher (<0.05) in patients with LF when compared to subjects with CHBV and controls. The distribution of GPRI values of subjects with chronic hepatitis B virus infection without fibrosis, patients with liver fibrosis and controls is presented as box plot in figure 1. The distribution of S-index values of subjects with chronic hepatitis B virus infection without fibrosis, patients with liver fibrosis and controls presented as box plot in figure 2.

Association between serum albumin, alkaline phosphatase, total and conjugated bilirubin, gamma-glutamyl transpeptidase, platelet count, gamma-glutamyl transpeptidase to platelets ratio index (GPRI), and S-index among subjects with chronic hepatitis B and Liver fibrosis.

A correlation plot of Log transformed GPRI against Log transformed S-index in patients with chronic hepatitis B virus infection is represented in figure 3. S-index correlated positively and significantly with GPRI (r= 0.806, P= 0.000) in patients with chronic hepatitis B virus infection.

Diagnostic and predictive performance of gamma-glutamyl transpeptidase to platelets ratio index (GPRI), and S-index among subjects with chronic hepatitis B and Liver fibrosis as determined from the area under the curve (AUC) receiver operator curve.

The area under the curve (AUC) receiver operator characteristic curve of gamma glutamyltranspeptidase to platelets ratio index (GPRI), and S-index among subjects with chronic hepatitis B and Liver fibrosis is represented in **figure 4.** S-index (AUC=0.835, SE=0.059, P=0.000, CI=0.721-0.951), GPRI (AUC=0.778, SE=0.065, P=0.003, CI= 0.666-0.918). The positive and negative predictive values of S-index were PPV=74 % and NPV=82 % respectively, while those for GPRI were 70 % and 69 % respectively. The overall test efficiency for S-index and GPRI were 78 % and 70 % respectively.

Table 1: Comparison of age, serum albumin, alkaline phosphatase, total bilirubin, conjugated bilirubin, gamma-glutamyl transpeptidase, platelet count, gamma-glutamyl transpeptidase to platelets ratio index (GPRI), and S-index among subjects with chronic hepatitis B, hepatitis B related liver fibrosis and controls

Parameters	CHBV without fibrosis (n=37)	LF (n=13)	Controls (n=40)	Cal. F	p-value
Age (years)	40.86±9.67	38.80±8.99	38.67±7.56	1.457	0.190
ALP (IU/L)(21-92)	159.81±60.81	201.41±48.90[a]	77.87±29.21	46.332	<0.0001
ALT (IU/L)(5 – 12)	10.37±2.96	24.93±13.57[b]	4.93±3.95	54.476	<0.0001
ST (IU/L) (5–12)	13.95±5.24	41.84±21.23[c]	9.00±4.74	64.548	<0.0001
TB (µmol/L)(2-17)	13.19±6.30	36.15±23.91[d]	10.58±4.04	32.676	<0.0001
CB(µmol/L)(2–7)	5.54±1.99	21.60±10.82[e]	4.63±2.08	26.450	<0.0001
PLT (100-300) 10^9/l	213.53±79.14	103.32±71.85	238.50±61.02[f]	4.213	0.018
GGT (U/L) (10-45)	72.82±38.81	124.95±28.91[g]	20.52±9.82	78.373	<0.0001
ALB (g/dl) (3.5-5.0)	2.50±0.41	2.10±0.41	4.05±0.43[h]	179.631	<0.0001
S-Index	172.93±127.00	332.84±187.39[i]	23.80±16.75	45.270	<0.0001
GPRI	1.02±0.58	1.66±0.71[j]	0.24±0.18	52.690	<0.0001

Key: results expressed as mean±SD. a, b, c, g, h, i & j = significant difference between patients with CHBV without fibrosis, liver Fibrosis and controls, d & e = significant difference between patients with CHBV without fibrosis and liver fibrosis, & between liver fibrosis and controls, f= significant difference between patients with liver fibrosis and controls. CHBV=chronic hepatitis B virus infection without fibrosis, LF = patients with liver fibrosis, AST=alanine amino transferase, AST, aspartate amino transferase, TB= total bilirubin, CB=conjugated bilirubin, ALP= alkaline phosphatase, PLT= platelets count, GGT, gamma-glutamyl transpeptidase, ALB= albumin, GPRI = gamma-glutamyl transpeptidase to platelets ratio index.
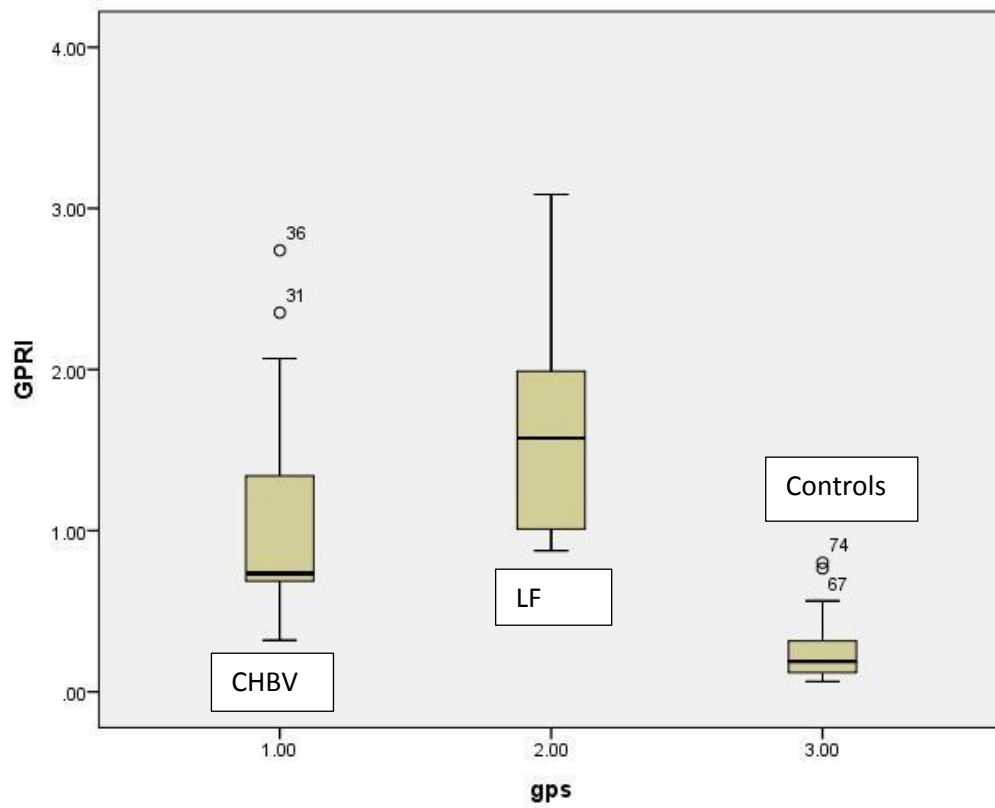
Figure 1, The distribution of GPRI values of subjects with chronic hepatitis B virus infection without fibrosis, patients with liver fibrosis and control presented as box and whisker plot.
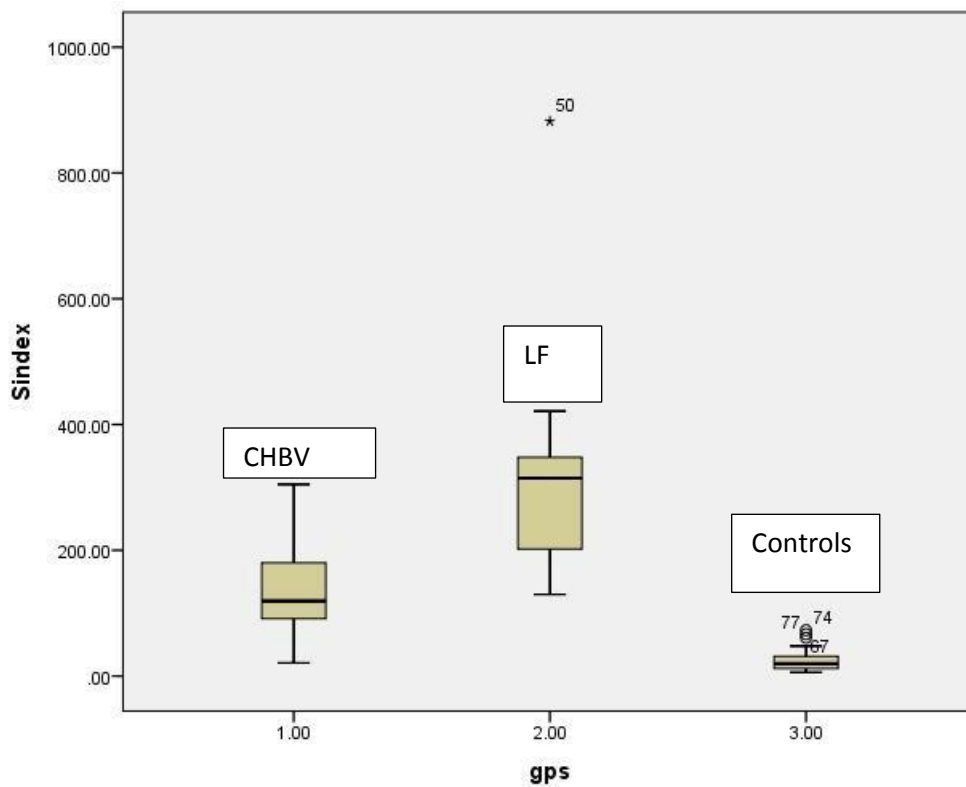

Figure 2, The distribution of S-index values of subjects with chronic hepatitis B virus infection without fibrosis, patients with liver fibrosis and controls presented as box and whisker plot.
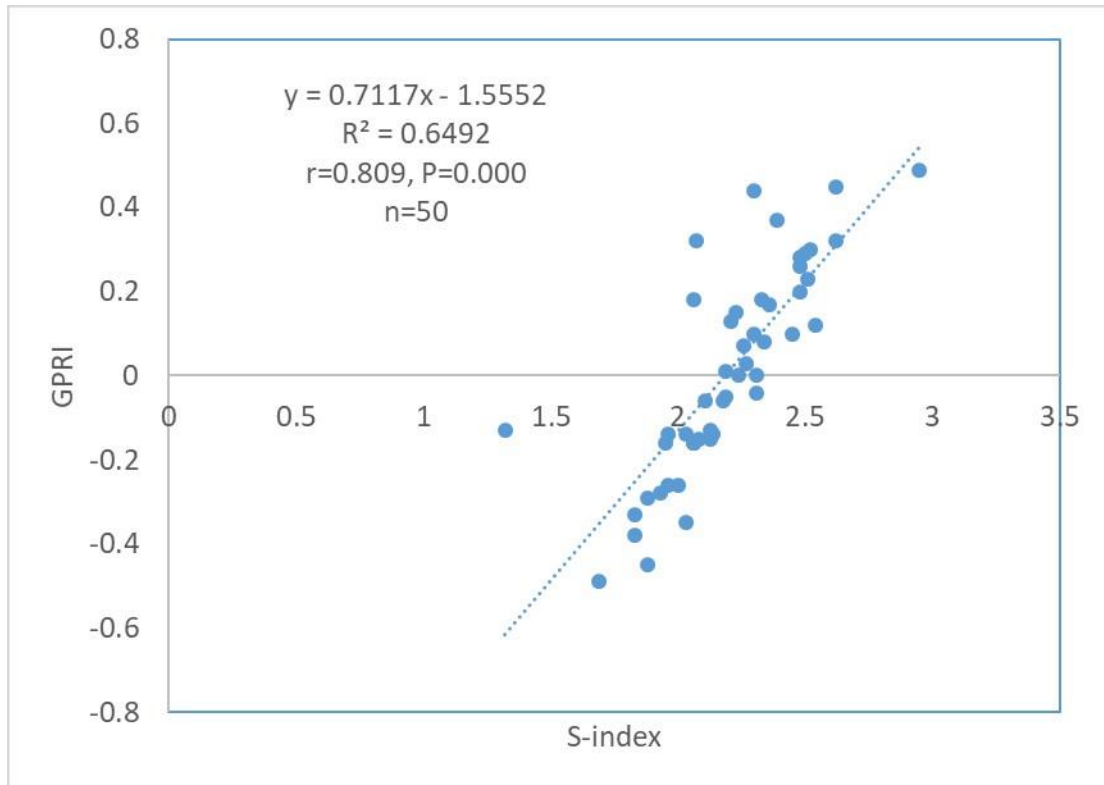
Figure 3, A correlation plot of Log_{10} GPRI values against Log_{10} S-index values of patients with chronic hepatitis B virus infection.
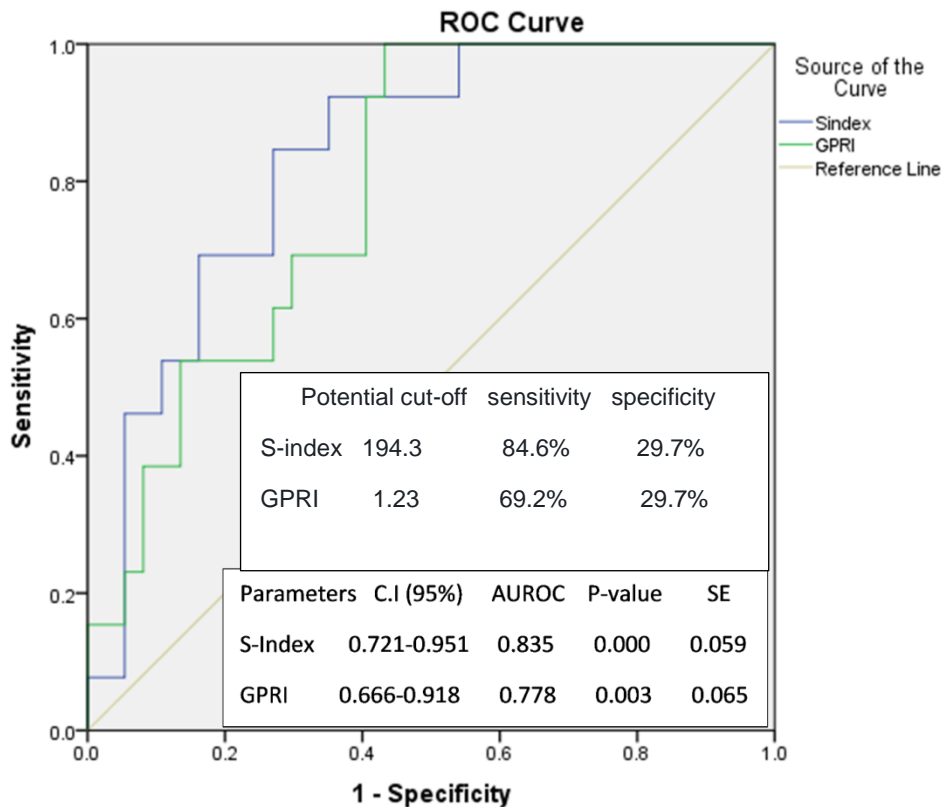

Figure 4, Receiver operator curve of GPRI and S-index in subject with chronic hepatitis B virus infection and liver fibrosis.

DISCUSSION

Chronic hepatitis B virus (HBV) infection represents one of the most significant global public health threats, regardless of availability of a very effective vaccine and antiviral regimes for more than three decades. A decisive improvement in patients' outcomes require continuous monitoring for treatment response and early detection of development of complications using proteins, enzymes produced in the liver and indirect biomarkers as overriding components of current treatment strategy. This study investigated the levels serum albumin, bilirubin (TB, CB), alkaline phosphatase (ALP), alanine aminotransferase (ALT), aspartate amino transferase (AST), gamma glutamyltranspeptidase (GGT) and platelet count (PLT) and to deduce noninvasive markers such as gamma glutamyl transpeptidase to platelets ratio index (GPRI) and S-index and their predictive accuracy in patients with chronic hepatitis B virus infection and hepatitis B related liver fibrosis.

The significantly higher levels of serum ALP, ALT, AST, GGT, gamma glutamyltranspeptidase to platelets ratio index (GPRI) and S-Index in patients with liver fibrosis when compared to patients with chronic hepatitis B virus infection, may suggest hepatitis B related chronic inflammation, hepatocytes death and nodular regeneration that affect the architectural integrity of the hepatic lobules. This leads to build-up of intrahepatic pressure, necrotic and toxic hepatocytes damage and the release of these enzymes from the hepatocytes and bile duct into circulation in patients with fibrosis. Hepatitis B virus related liver fibrosis patients present with a predominant rise in ALP and GGT commonly caused by biliary obstruction and hepatic congestion due to regenerating nodules and hepatitis B virus-related liver injury. This finding is similar to that of a previous study which observed that serum ALP levels increased gradually in all patients and separately in HBeAg (−) chronic hepatitis B patients and independently predicted significant fibrosis in all of the patients (Hu, et al., 2019). Similarly, Sun and colleagues, reported that high pretreatment serum GGT level significantly correlates with poor survival and unfavourable clinical and pathological features in HCC patients (Sun *et al.*, 2019). The higher GPRI and S-Index in patients with liver fibrosis may suggest progressive increase in serum levels of gamma glutamyl transpeptidase and a corresponding decrease in the platelet count and albumin with development of liver fibrosis. This observation is similar to that of Purkayastha, and colleagues, who stated that GPRI is a good predictor of liver fibrosis (Purkayastha *et al.,* 2023). Furthermore, Zeng and colleagues, recounted that S-index (GGT-PLT-albumin index), and GPRI have the best diagnostic performance for significant liver fibrosis and may serve as strong predictors of significant fibrosis in patients with chronic hepatitis B virus (Zeng *et al.,*

2015). Lower serum albumin in patients with liver fibrosis compared to patients with chronic hepatitis B virus infection suggest a progressive decline in the synthetic ability of the liver following the development of liver fibrosis. Platelet count were lower in patients with chronic hepatitis B virus infection and liver fibrosis but no significant difference were observed between the two groups.

In this study, serum albumin was significantly lower in patients with chronic hepatitis B virus infection compared to the controls. The lower albumin in the chronic hepatitis B virus patients than in the controls may suggest some level of malnutrition and impaired liver synthetic ability to produce albumin in these patients. The observed hypoalbuminemia is similar to that of Asghar and colleagues, who considered that serum albumin level below 3.5 g/dL is a known predictor of chronic liver disease and decreased survival (Asghar *et al.,* 2020). Thrombocytopenia observed in patients with chronic hepatitis B virus infection compared to the control may be due to hypersplenism, and enhanced destruction of platelets in the enlarged spleen as well as reduced thrombopoietin production from the failing liver which is required for the production of platelets in patients with chronic hepatitis B virus infection. This finding is in line with that of Yang *et al.,* who observed that platelet count is independently associated with moderate to severe liver function impairment and cirrhosis in patients with chronic HBV infection (Yang *et al.,* 2020). The platelet count may indicate the severity of liver injury and liver fibrosis. Accordingly, previous studies observed that worsening of fibrosis and increasing portal pressure are associated with the reduced production of thrombopoietin by hepatocytes and increased platelet sequestration within the spleen (Zeng *et al.,* 2015, Asghar *et al.,* 2020, Mitchell *et al.,* 2016). Likewise, previous studies, reported decline in platelet counts with disease duration and developing thrombocytopenia across different causes and pathophysiological mechanisms associated with development of chronic liver diseases (Lisman and Luyendyk 2018, Yoshida *et al.,* 2022). The positive correlation between ALP with GGT, suggesting a common source of origin for both enzymes. Elevated GGT is frequently used to clarify the origin of ALP elevation.

The area under the receiver operator characteristic curve (AUC) for gamma-glutamyl transpeptidase to platelets ratio index (GPRI), and S-index among subjects with chronic hepatitis B and Liver fibrosis shows that both S-index and GPRI are accurate predictors of liver fibrosis in patients with chronic hepatitis B virus infection. In this study, S-index performed better than GPRI in predicting significant liver fibrosis with a greater area under the curve even though the two models have good diagnostic and predictive performances. This finding is similar to that of reported by a study, which demonstrated that the S-index and GPRI correlated highest with

histopathological scores and had the finest diagnostic performance for significant liver fibrosis and were robust predictors of significant liver fibrosis in patients with chronic hepatitis B virus (Hu *et al.,* 2019.). Also, previous studies, have reported that the gamma glutamyltranspeptidase (GGT)-to-platelet ratio index (GPRI) serves as a simple and accurate index for predicting liver inflammation and fibrosis in patients with chronic hepatitis B virus (Lv *et al.,* 2017.). A favorable test model should have high sensitivity and high specificity characteristics with an AUROC of ≥0.900. S-index had an AUROC of 0.835 while GPRI had an AUROC of 0.778. Both models demonstrated good test instruments in predicting fibrosis. Using 194.3 as potential cut off value for S-index, a sensitivity of 84.6 % was obtained, suggesting that about 85 % of subjects with fibrosis will be correctly identified (diagnosed) by this model as positive. Also, a 1-specificity value of 29.7 % obtained indicates that about 30 % of subjects without fibrosis will be incorrectly classified as positive, that is, false positive. Using a cut off value of 1.23 for GPRI, a sensitivity of 69.2 % was obtained suggesting that about 70 % of subjects with fibrosis will be correctly identified (diagnosed) by this model as true positive while a 1-specificity value of 29.7 % suggest that about 30% of subjects without fibrosis (negative outcome) will be incorrectly classified as positive, that is, false positive. Balancing the brisk between sensitivity and specificity at these cut off values of 194.3 and 1.23 for S-index and GPRI respectively, the two models are good test instruments, with reliable sensitive in predicting fibrosis, but S-index performed better than GPRI in these group of patients studied. A high S-index and GPRI is associated with a positive outcome (fibrosis), that is, a high score indicates a stronger evidence for a positive actual state. S-index and GPRI showed the highest AUROC in assessing liver fibrosis compared to other methods such as AST to PLT ratio index (APRI model), age-platelet (PLT) index (API model), aspartate transaminase (AST) to alanine aminotransferase (ALT) ratio (AAR model), age-AST-PLT-ALT-international normalized ratio index (Fibro-Q model), age-AST-PLT-ALT index (FIB-4 model), as reported in a previous study (Zeng *et al.,* 2015). Liver biopsy remains the "gold standard" for assessing hepatic fibrosis. However, it has limitations, such as high cost, invasiveness, associated risk for complications and sampling variability. Liver biopsy clinical utility has lately been contested by the advent of novel noninvasive procedures, including serum markers of hepatic fibrosis, noninvasive models of predicting fibrosis and imaging techniques, including transient elastography (TE). However, the availability and cost of the equipment remains a concern resource-limited establishments. Also, it has been failed in individuals with high levels of ALT/bilirubin, obesity and/or ascites.

Serum albumin, platelet count and GGT are simple laboratory test scores that change with the severity of liver injury and are useful in deducing noninvasive diagnostic and predictive models for assessing significant fibrosis in patients with chronic hepatitis B virus infection. Levels of GGT vary in a number of conditions, such as alcohol consumption, inflammation, fibrosis, cholestasis. The foremost function of GGT is to enable the metabolism of glutathione (GSH) and glutathionylated xenobiotics, by catalyzing the transfer of a γ-glutamyl group from glutathione and other γ-glutamyl compounds to amino acids or dipeptides. This process results in oxidant activities that lead to tissue, cell, and DNA damage. Consequently, increased GGT activity in these patient may be directly related with liver injury. Albumin is produced in the liver, during chronic hepatitis B virus infection, plasma albumin gradually reduces due to diminished ability of the liver to synthesize more albumin and increased albumin use in binding and transportation of substances that are normally bio-transformed to harmless forms by the liver. During chronic hepatitis, deteriorating liver and development of fibrosis and increasing portal pressure are associated with diminished production of thrombopoietin and increased platelet confiscation within the spleen, leads to platelet destruction within the spleen and thrombocytopenia. The dynamics of these simple laboratory test score which may indicate worsening or an improving liver status, may be coupled in simple models useful in assessing chronic hepatitis B related liver fibrosis. The diagnostic and predictive performance values of S-index and GPRI in assessing liver fibrosis in patients with chronic hepatitis B virus infection are striking because they are simple tests that are readily accessible, and are cheap in the clinical laboratory or in an outpatient setting.

Limitations: This study has its limitations. The current study unit may not represent the general population with chronic hepatitis B infection, because of the limited small sample size, which did not permit robust comparison between patients categorized into the various stages of liver fibrosis F0, F1, F2, F4 using the Metavir system.

CONCLUSION: This study shows that both S-index and GPRI are good test instruments for evaluating liver fibrosis in patients with chronic hepatitis B. S-index performed better than GPRI in predicting liver fibrosis. The use of S-index and GPR as predictive models may represent simple and low-cost alternatives to liver biopsy in assessing liver injury in patients for whom transient elastography and liver biopsy are inaccessible. The clinical utility of S-index and GPR as predictive models may reduce both the need for unnecessary liver biopsies.

ETHICAL CONSIDERATION: This study was carried out in accordance with the ethical principles for Medical Research involving human subjects as outlined in the Helsinki Declaration in 1975 and subsequent revisions. The study protocol was approved by the Health Research Ethics Committee, University of Calabar Teaching Hospital, Calabar, Cross River State (Approval number: UCTH/HREC/33/VOL.111/040).

INFORMED CONSENT: Written informed consents were obtained from each of the study participants. The confidentiality of patient's information was preserved at all steps. The rights to withdraw from participation in the study at any point in time were respected.

CONFLICTING INTEREST: The authors declare that they have no conflicts of interest.

AUTHORS' CONTRIBUTIONS
R E E, ACNA, NAN, UAF and RUB performed methodology, investigation, and formal data Collection, processing and analysis. CCT, MCN, NAN, ARE, IME and O U E, wrote and prepared the manuscript and Interpretation of Results. R E E and A C N A conceived the study, supervised the study and approved the final Version to be published.

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