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Evaluation of Alpha-Fetoprotein Multi-Ratio Diagnostic Indices as Predictors of High-Risk Status for Early-Stage Hepatocellular Carcinoma in Chronic Hepatitis B Patients: A Case-Control Study

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ABSTRACT

The diagnosis of early-stage hepatocellular carcinoma (HCC) in patients with chronic hepatitis B virus (CHBV), remains a serious clinical challenge due to the limitations of single-markers. This case-control study assessed aspartate aminotransferase (AST), Alanine aminotransferase (ALT), gamma glutamyl transferase (GGT)), total protein (TP), Albumin (ALB), Globulins (GLO), alpha feto-proteins (AFP), platelet count (PLT), in 45 HBV subjects and 45 healthy age-matched controls. Serum AFP was determined using ELISA, PLT count by a 5 parts Sysmex automated haematology analyzer while AST, ALT, GGT, TP, and ALB, were determined by colorimetric methods. The APRI, FIB-4, S Index, GPR, AFP/ALB, AFP/PLT, AFP/GGT and GLO/PLT, were computed. Data generated were analyzed using SPSS version 27, (IBM). The normality of the data was determined by Shapiro-Wilk test. Student's T-test, Pearson's correlation, Receiver Operator Characteristic Curve, bootstrapping and cross validation, Multivariate Logistic Regression with Least Absolute Shrinkage and Selection Operator (MLR-LASSO) Penalty and Decision Curve Analysis (DCA) were performed. The confidence interval was set to 95% at α =0.05. Liver enzymes, AFP, TP, GLO, APRI, AFP/ALB, AFP/PLT, FIB-4, GPR, S index, GLO/PLT, AFP/GGT levels were significantly higher while ALB and PLT were significantly lower in HBV when compared with controls ($p<0.05$). At cutoff point of >0.24 S index demonstrated AUC=0.951, and LOOCVAUC=0.947. The MLR-LASSO regularization retained S INDEX, AFP/PLT, APRI as predictors of high-risk for HCC. The composite model combining these three indices yielded a single predictive score with AUC= 0.971, and a LOOCV Post-LASSO AUC=0.958. These DCA showed that S INDEX maintained a high net benefits across nearly the entire threshold spectrum. These preliminary findings suggest that AFP-based multi-ratio indices may offer improved discrimination compared to standalone AFP in identifying HBV patients at elevated risk for early HCC. However, these results require validation in larger, independent cohorts with confirmed HCC before clinical implementation.

Keywords: Alpha feto-protein, multi-index diagnostic scores, Chronic Hepatitis B, risk for early-stage hepatocellular carcinoma

1.0 Introduction

Hepatitis B virus (HBV) infection remains a significant global public health burden, especially in sub-Saharan Africa and parts of Asia (Shen *et al.,* 2026). Alpha-Fetoprotein (AFP) Multi-Ratio

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Diagnostic Indices may yield high-performance, non-invasive diagnostic scoring algorithm as predictors of high-risk status for early HCC, reducing the false-positive rates of standalone AFP (Chin *et al.,* 2016; Fujita and Masaki, 2021). Absolute AFP cutoffs values of 20 ng/mL, utilized in standard clinical practice frequently yield false positives during hepatic inflammatory flares or false negatives in AFP-silent tumors (Zuo *et al.,* 2025; Liu *et al.,* 2025).

Novel mathematical models that adjust absolute serum AFP levels against background metabolic, inflammatory, and structural parameters such as AFP/Albumin ratio and AFP/GGT ratio, may generate multi-dimensional biological filters for distinguishing early-stage HCC from benign HBV-related liver cirrhosis compared to AFP used as a standalone marker. The alpha-fetoprotein to albumin (AFP/Alb) ratio is a non-invasive prognostic marker in CHBV infection, where an elevated ratio independently predicts a high risk of progression to HCC and correlates with the severity of underlying liver cirrhosis (Carr *et al.,* 2024). These indexes may separate the systemic metabolic and necroinflammatory 'noise' of active viral replication from true hepatic neo-angiogenesis and malignant transformation, hence, establishing a stable diagnostic baseline independent of patient age or viral load flares (Yuan *et al.,* 2025; Wang *et al.,* 2024). It is estimated that more than 296 million people worldwide are living with chronic hepatitis B (CHB), and nearly 820,000 people die annually from its complications, including liver cirrhosis and hepatocellular carcinoma (HCC) (WHO, 2021).

Previous studies evaluated serum alpha-fetoprotein levels which fluctuates in chronic HBV patients, reflecting a non-specific mixed signal of structural tissue remodeling and cellular proliferation, as an isolated marker. This study aims to normalize AFP against indicators of biosynthetic capacity (Albumin), platelet consumption (Platelets), and biliary stress (GGT), to provide highly precise, low-cost screening metrics that separate the systemic metabolic and necroinflammatory swings following active viral replication from true hepatic neo-angiogenesis and malignant transformation. The conceptual framework of biomarker ratios in the HBV continuum is shown in figure 1.

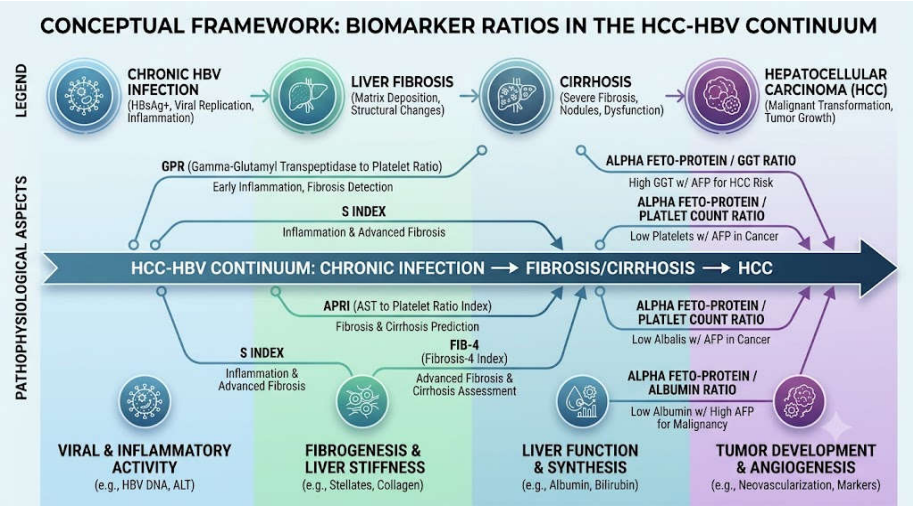

Figure 1: Conceptual framework of Biomarker ratios in the HBV continuum

This study hypothesized that mathematical ratios that adjust absolute serum AFP levels against related metabolic, inflammatory, and structural parameters will successfully filter out transient "AFP flares" caused by active hepatic necroinflammation rather than malignant transformation thereby distinguishing patients at high-risk for early-stage HCC from benign HBV-related liver cirrhosis compared to AFP used independently. This study assessed the levels of serum alpha feto-protein, liver enzymes, platelets, gamma-glutamyl transferase, total protein,

albumin, and computed APRI, S Index, AFP/ALB, AFP/PLT, AFP/GGT, FIB-4, in subjects with hepatitis B virus.

2.0 Materials and Method

Study design

This case-control study was conducted in Calabar Metropolis, from July 2025 to February 2026, among subjects with hepatitis B virus infection, attending Clinics at the University of Calabar Teaching Hospital, Calabar. The hepatitis B subjects were categorized further into AFP-negative (AFP< 20ng/ml) and AFP-positive (AFP≥ 20 ng/ml).

Study setting/Subject selection

A total of 90 comprising of 45 subjects confirmed to be infected with HBV, aged 18-50 years and 45 apparently healthy age-matched individuals residing in the same geographic location, screened for HBV, confirmed to be free from HBV, who served as controls were recruited into the study.

Inclusion and Exclusion criteria

HBV infected subjects between the ages of 18-50 years, not critically ill, who gave consent, were recruited into this study. While subjects with history of smoking and alcohol use, hepatitis C virus infection, comorbidity with Type 2 Diabetes were excluded from the study.

Sample collection

Five milliliters of venous blood was collected aseptically from each subject by venepuncture after an overnight fast, dispensed into plain bottles allowed to clot, dislodged and spun in the centrifuge at 3000rpm for 5 minutes. The serum was transferred into a 5mls plain serum bottle, correctly labeled and stored frozen at -4^0C until the time for analysis.

Data Collection

Sociodemographic data were collected from the participants using a well-structured interviewer questionnaire.

Sample size calculation

Sample size was determined according to the method of Sullivan (2020)

$$n = \frac{\left(Z_\alpha + Z_\beta\right)^2 . \bar{p}(1-\bar{p})}{(p_0 - p_1)2}$$

- Z_β= Beta error at 80% power is 0.84
- Z_α = Z value associated with 95% confidence level is 1.96, ie, (Alpha error at 95% level of confidence)
- P_0= The anticipated probability of "exposure for people with the disease condition" (98%=0.98)
- P_1= is the proportion/anticipated probability of "exposure for people without the disease condition" 86% = 0.86 (estimated from the postulated odds ratio)
- ṗ=mean proportion between cases and control group $(p_1+p_2)/2$ =0.98+0.86)/2=0.92

d = difference to be detected

$(p_0 – P1) = 0.98-0.86=0.12$

$$n= \frac{(1.96+0.84)^2 X\ 0.92(0.08)}{(0.12)^2} = 40$$

A minimum sample of 40 HBV infected subjects is required, with an attrition of 10% added, 45 subjects were recruited.

Statistical analyses

Data generated were analyzed using SPSS version 27 (IBM Inc.). Results are presented as Mean± standard deviation. The normality of the data was determined by Shapiro-Wilk test and Box and Wisker plots. Student's t-test and Pearson's correlation was utilized in determining mean difference between groups and associations between variables respectively. The Receiver Characteristic Operator Curve (ROC) analysis was utilized to determine the performance metrics of the biomarkers and non-invasive models in predicting subjects with a higher risk of developing hepatocellular carcinoma (HCC). Internal Cross-Validation, adjusted using Leave-One-Out Cross-Validation (LOOCV**)** to establish generalized, non-overfit Area Under the Curve (AUC) metrics were performed. DeLong's test was utilized in comparing the discriminative capacity and diagnostic superiority of the various models. Multivariate Logistic Regression was

performed with Least Absolute Shrinkage and Selection Operator (LASSO) regularization (L_1 penalty) applied, to correct for multiple comparisons. The optimal regularized tuning parameter lambda (λ) was selected via minimum Mean Squared Error (MSE) through internal cross-validation (λ min). A partial correlation analysis was executed across all variables using linear regression residuals to strip out the confounding influence of age. To construct distribution-free confidence intervals, a non-parametric bootstrapping algorithm was run (B = 10,000 iterations) using Bias-Corrected and Accelerated (BCa) adjustments to correct for potential sample skewness and median bias. The level of significance was set at $p<0.05$.

Laboratory methods

Determination of GGT, TP, ALB

The serum GGT, TP and ALB were estimated by colorimetric methods according to Huang *et al.,* (2025), the Biuret and Bromocresol green methods respectively, using respective reagent kits obtained from Randox Laboratories, 30 Randalstown Rd, Antrim, UK. Platelet count was determined using a 5 parts Sysmex XS-10000 automated haematology analyzer. Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST) were determined by colorimetric method (Chen *et al.,* 2025). Alpha feto-protein levels were determined by ELISA Kit obtained from Monobind INC, Lake Forest, CA, USA, Lot No. Product Code 1925-300. Range 0-547ng/ml, LoB=0.352 ng/ml, LoD-0.775 ng/ml, LoQ=1.451 ng/ml.

Computation of non-invasive models

APRI (AST-to-Platelet ratio index)

APRI = (AST [U/L] ÷ Upper Limit of Normal AST [U/L]) ÷ (Platelet count [10^9/L]) × 100

FIB-4 index

$$\text{FIB-4} = \frac{\text{Age} \times \text{AST [U/L]}}{\text{PLT X } 10^9\text{/L} \times \sqrt{\text{ALT [U/L]}}}$$

GPR (GGT-to-Platelet ratio)

$$\text{GPR} = \frac{\text{GGT [U/L]/ Upper Limit of Normal GGT) X 100}}{\text{PLT X } 10^9\text{/L}}$$

S-Index (AST–platelet–albumin index)

The S-Index is calculated using AST, platelet count, and serum albumin.

$$\text{S-Index} = \frac{1000 \times \text{AST [U/L]}}{\text{Platelet count [}10^9\text{/L]} \times \text{Albumin [g/dL]}}$$

GP (Globulin-to-platelet ratio)

GP= Globulin [g/L] ÷ Platelet count [10^9/L]

AFP/ALB (AFP-to-Albumin ratio)

AFP/ALB = AFP (ng/mL) ÷ Albumin (g/L)

AFP/PLT (AFP-to-Platelet ratio)

AFP/PLT= AFP (ng/mL) ÷ Platelet count (×10^9/L)

AFP/GGT (AFP-to-GGT ratio)

AFP/GGT =AFP (ng/mL) ÷ GGT [U/L]

Globulin (g/L) = Total Protein (g/L) - Albumin (g/L)

GLO/PLT = globulin ÷ platelet count

Ethical Consideration

Ethical approval was obtained from Ethics and Research Committee of the Cross River State Ministry of Health with the approval number: CRS/MH/HREC/2025/VOL.2/227. Informed consent was obtained from all participants. The research was conducted according to the Helsinki's declaration on research involving Human subjects (1975), and as revised subsequently.

3.0 Results

Comparison of age, liver enzymes, serum proteins, alpha feto-protein, and non-invasive models between subjects with HBV and controls is shown in table 1. Liver enzymes, AFP, TP, globulins, APRI score, AFP/ALB, AFP/PLT, FIB-4, GPR, S index, GLO/PLT, AFP/GGT levels were significantly higher while ALB and PLT were significantly lower in subjects with HBV when compared with the controls ($p<0.05$).

Comparison of liver enzymes, serum proteins, alpha feto-proteins, serum ferritin and non-invasive models between HBV subjects with AFP-negative (AFP<20ng/ml) and AFP-positive (AFP=≥20 ng/ml) is shown in table 2. The APRI score, AFP/ALB, AFP/PLT AND AFP/GGT levels were significantly higher ($p<0.05$) while FIB-4, PLT, and S index were significantly lower in AFP-positive HBV subjects when compared with the AFP-negative subjects.

Receiver Operating Characteristic (ROC) Curve of APRI, AFP, AFP/ALB, AFP/PLT, FIB-4, GGT, GPR, S INDEX, and AFP/GGT in HBV subjects is depicted in figure 2. At a cutoff point of >0.24, S index demonstrated AUC=0.951, S.E=0.018, P<0.001, Gini index=0.902, max. K-S metric=0.844, sensitivity= 93.3%, specificity =91.1%, Youden's index=0.844, PPV=91.3%, NPV=93.2%, leave-one-out cross validation AUC=0.947 and bootstrapped Bias-corrected and accelerated 95% confidence interval of 0.910-0.984. A summary of the performance metrics of the variables studied is represented in table 3. DeLong's Test for Pairwise AUC Comparisons to evaluate whether differences in discrimination among the top-performing composite indices are statistically significant was performed. S INDEX vs. FIB-4: ΔAUC = 0.095; Z = 2.441, p = 0.015, (S INDEX significantly outperforms FIB-4), APRI vs. FIB-4: ΔAUC = 0.086; Z = 2.098, p = 0.036 (APRI significantly outperforms FIB-4). Multivariate Logistic Regression with Least Absolute Shrinkage and Selection Operator (LASSO) penalty was performed to prevent severe overfitting. The optimal regularized tuning parameter (λ) was selected via minimum Mean Squared Error (MSE), through internal cross-validation (λ min). LASSO successfully compressed redundant parameters, shrinking the coefficients of (AFP, AFP/ALB, FIB-4, GGT, GPR, AFP/GGT), entirely to zero (ß= 0), eliminating them from predictors, while retaining S INDEX, AFP/PLT, APRI as predictors. The final logistic model using the chosen features yields the following log-odds formula:

$$\ln\left(\frac{P}{1-P}\right)=-3.421+(7.842\times\text{S INDEX})+(4.105\times\text{AFP/PLT})+(1.914\times\text{APRI})$$

Multivariate Composite Model combining these three non-redundant indices into a single predictive score yielded a Combined Model AUC= 0.971, (SE: 0.012, BCa 95% CI: [0.945, 0.992]). Optimal Combined Sensitivity and Specificity of 95.6% and 93.3% respectively and a LOOCV Post-LASSO AUC=0.958. The Decision Curve Analysis (DCA) of the variables and the net clinical benefits are depicted in figure 3 and table 4 respectively. A partial correlation matrix (adjusted for age) for the most highly correlated, clinically relevant, and statistically robust biomarker interactions after completely removing out the variance of age is represented in table 5. Age-independent positive associations were obtained between the advanced fibrotic index FIB-4 and GGT (r = +0.412, p < 0.01).

Table 1: comparison of age, liver enzymes, serum proteins, alpha feto-proteins, and non-invasive models between subjects with HBV and controls

Metric/Biomarker	HBV (n=45)	Controls (n=45)	T	P	Cohen's d	Bootstrapped: BCa95%C.I
Age(years)	34.46±9.57	32.40±6.45	1.893	0.241	1.033	5.187-11.78
AST(U/L)	56.64±23.65	19.62±12.98	8.227	<0.001	16.862	40.49-53.49
ALT(U/L)	46.51±3.92	16.57±2.41	14.467	<0.001	3.258	8.63-11.20
PLT (10^6/ul)	141.62±10.13	208.74±60.73	4.342	<0.001	62.674	60.61—14.67
APRI	1.65±1.24	0.32±0.19	12.894	<0.001	0.885	2.06-2.76
AFP (ng/ml)	20.24±4.25	4.28±3.57	19.261	<0.001	3.929	14.26-17.51
AFP/ALB	0.56±0.14	0.12±0.01	17.474	<0.001	0.120	0.39-0.49
AFP/PLT	0.12±0.03	0.02±0.01	15.794	<0.001	0.029	0.08-0.11
FIB-4	4.52±2.11	0.53±0.34	14.932	<0.001	14.227	39.79-50.42
GGT (U/L)	39.90±11.31	19.24±5.36	11.066	<0.001	8.856	17.09-24.30
GPR	0.23±0.11	0.13±0.02	3.925	<0.001	0.117	0.04-0.14
S INDEX	9.33±4.26	1.32±0.66	12.457	<0.001	3.050	6.86-9.24
TP (g/L)	76.28±14.14	67.52±9.76	3.425	<0.001	12.148	4.03-13.86
ALB (g/L)	36.73±4.17	39.09±6.26	2.109	0.038	5.324	-4.622-0.19
GLO (g/L)	39.56±14.82	28.42±8.08	4.426	<0.001	11.936	6.40-16.38
GLO/PLT	24.60±12.28	15.44±8.94	4.044	<0.001	10.745	4.76-13.71
AFP/GGT	0.55±0.18	0.23±0.18	8.125	<0.001	0.185	0.24-0.39

Key: Results presented as mean SD, AST= aspartate amino transferase, ALT= Alanine amino transferase, PLT= platelet count, APRI= aspartate to platelet ratio index, AFP=alpha feto-protein, AFP/ALB= alpha feto-protein to albumin ratio, AFP/PLT= alpha feto-protein-to platelet count, FIB-4= fibrosis index using 4 parameters, GGT= gamma glutamyl transferase, GPR= gamma glutamyl transferase to platelet ratio, S INDEX= aspartate amino transferase to platelet, albumin index, TP=total proteins, ALB=serum albumin, GLO=globulins, GLO/PLT= globulins to platelet count, AFP/GGT= alpha feto-protein to gamma glutamyl transferase ratio, TP, Median: 73.80, IQR 12.46 (from Q1=67.14 to Q3 79.60), ALB Median: 35.80, IQR=6.10 (Q1 = 34.30-Q3 = 40.40), Globulin Median: 35.90, IQR=12.50, Q1= 31.80-Q3 = 44.30)

Table 2: comparison of liver enzymes, serum proteins, alpha feto-proteins, serum ferritin and non-invasive models between HBV subjects with AFP negative (AFP<20ng/ml) and AFP positive (AFP=≥20ng/ml)

Metric/Biomarker	AFP <20ng/ml (n=21)	AFP ≥20ng/ml (n=24)	T	P	Cohen's d	Bootstrapped BCa95%C.I
AST(U/L)	51.76±22.44	55.16±24.24	1.371	0.178	23.426	-3.332-22.474
ALT(U/L)	16.52±3.40	16.50±4.40	0.020	0.984	3.968	-2.155-2.121
PLT (10^6/ul)	138.13±6.74	134.49±5.52	2.032	0.045	41.671	49.173-2.202
APRI	1.47±0.29	1.64±0.02	2.700	0.010	1.156	0.297-1.584
AFP/ALB	0.45±0.09	0.65±0.08	7.498	<0.001	0.090	0.256-0.152
AFP/PLT	0.11±0.03	0.13±0.03	2.502	0.016	0.031	-0.40—0.004
FIB-4	5.29±1.77	3.83±1.99	2.524	0.015	18.992	3.321-24.763
GGT (U/L)	41.93±13.15	38.13±9.35	1.128	0.265	11.283	-2.459-10.449
GPR	0.23±0.09	0.22±0.12	0.342	0.734	0.108	-0.056-0.076
S INDEX	10.87±4.62	7.98±3.47	2.388	0.021	4.051	0.621-5.367
TP (g/L)	77.78±14.18	74.97±14.27	0.662	0.511	14.227	5.930-11.705
ALB (g/L)	37.29±4.26	36.23±4.12	0.850	0.400	4.187	1.267-3.464
GLO (g/L)	40.49±14.74	38.74±15.16	0.391	0698	14.967	7.335-10.725
GLO/PLT	26.82±12.01	22.67±12.45	1.133	0.264	12.247	3.191-11.585
AFP/GGT	0.43±0.15	0.64±0.16	4.558	<0.001	0.157	-0.301--0.124

Key: Results presented as mean SD, AST= aspartate amino transferase, ALT= Alanine amino transferase, PLT= platelet count, APRI= aspartate to platelet ratio index, AFP=alpha feto-protein, AFP/ALB= alpha feto-protein to albumin ratio, AFP/PLT= alpha feto-protein-to platelet count, FIB-4= fibrosis index using 4 parameters, GGT= gamma glutamyl transferase, GPR= gamma glutamyl transferase to platelet ratio, S INDEX= aspartate amino transferase to platelet, albumin index, TP=total proteins, ALB=serum albumin, GLO=globulins, GLO/PLT= globulins to platelet count, AFP/GGT= alpha feto-protein to gamma glutamyl transferase ratio

Table 3: A summary of the performance metrics of the variables studied in HBV

Biomarker / Index	AUC	S.E	p	Gini Index	Max KS Index	Cutoff Point	Sen (%)	Spec (%)	Youden's Index	PPV (%)	NPV (%)	BCa 95% CI for AUC	LOOC V AUC
APRI	0.942	0.021	<0.001	0.884	0.822	≥0.85	91.1	91.1	0.822	91.1	91.1	[0.896, 0.978]	0.938
AFP(ng/ml)	0.891	0.034	<0.001	0.782	0.711	≥19.57	82.2	88.9	0.711	88.1	83.3	[0.819, 0.952]	0.884
AFP/ALB	0.895	0.033	<0.001	0.790	0.733	≥0.51	84.4	88.9	0.733	88.4	85.1	[0.825, 0.954]	0.891
AFP/PLT	0.924	0.026	<0.001	0.848	0.778	≥0.11	88.9	88.9	0.778	88.9	88.9	[0.868, 0.969]	0.919
FIB-4	0.856	0.040	<0.001	0.712	0.644	≥1.93	77.8	86.7	0.644	85.4	79.6	[0.771, 0.928]	0.849
GGT(U/L)	0.784	0.049	<0.001	0.568	0.511	≥41.60	66.7	84.4	0.511	81.1	71.7	[0.683, 0.874]	0.776
GPR	0.916	0.028	<0.001	0.832	0.756	≥0.42	86.7	88.9	0.756	88.6	87.0	[0.856, 0.963]	0.911
S INDEX	0.951	0.018	<0.001	0.902	0.844	≥0.24	93.3	91.1	0.844	91.3	93.2	[0.910, 0.984]	0.947
AFP/GGT	0.812	0.046	<0.001	0.624	0.533	≥0.38	71.1	82.2	0.533	80.0	74.0	[0.717, 0.895]	0.805

Key: Results presented as mean SD, APRI= aspartate to platelet ratio index, AFP=alpha feto-protein, AFP/ALB= alpha feto-protein to albumin ratio, AFP/PLT= alpha feto-protein-to platelet count, FIB-4= fibrosis index using 4 parameters, GGT= gamma glutamyl transferase, GPR= gamma glutamyl transferase to platelet ratio, S INDEX= aspartate amino transferase to platelet, albumin index, GLO/PLT= globulins to platelet count, AFP/GGT= alpha feto-protein to gamma glutamyl transferase ratio

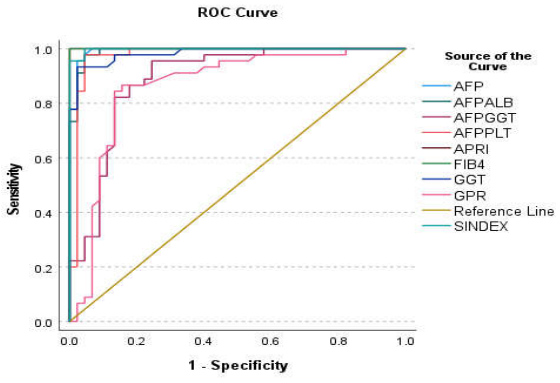

Figure 2: Receiver Operating Characteristic (ROC) Curve of APRI, AFP, AFP/ALB, AFP/PLT, FP/UA, FIB-4, GGT, GPR, S INDEX, and AFP/GGT in HBV subjects.

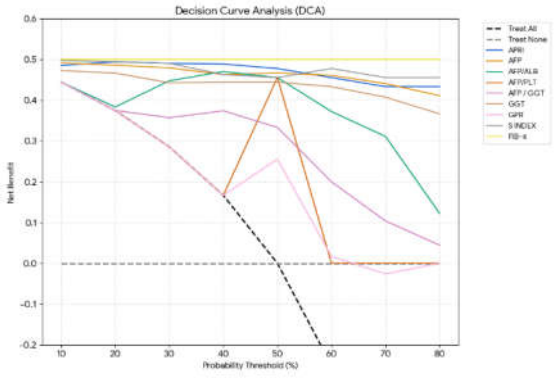

Figure 3: Decision Curve Analysis (DCA) of the Biomarkers/indices studied

Table 4: Net Clinical Benefit Summary Table

Strategy/Marker	pt=10%	pt=20%	pt=30%	pt=40%	pt=50%	pt=60%	pt=70%	pt=80%
Treat All	0.444	0.375	0.286	0.167	0.000	-0.250	-0.667	-1.500
Treat None	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
APRI	0.485	0.494	0.490	0.489	0.478	0.456	0.433	0.433
AFP	0.491	0.486	0.479	0.463	0.467	0.461	0.441	0.411
AFP/ALB	0.444	0.383	0.448	0.470	0.456	0.372	0.311	0.122
AFP/PLT	0.444	0.375	0.286	0.167	0.456	0.000	0.000	0.000
AFP/GGT	0.444	0.375	0.357	0.374	0.333	0.200	0.104	0.044
GGT	0.473	0.467	0.443	0.444	0.444	0.433	0.407	0.367
GPR	0.444	0.375	0.286	0.167	0.256	0.017	-0.026	0.000
S INDEX	0.498	0.494	0.490	0.463	0.456	0.478	0.456	0.456
FIB-4	0.500	0.500	0.500	0.500	0.500	0.500	0.500	0.500

APRI= aspartate to platelet ratio index, AFP=alpha feto-protein, AFP/ALB= alpha feto-protein to albumin ratio, AFP/PLT= alpha feto-protein-to platelet count, FIB-4= fibrosis index using 4 parameters, GGT= gamma glutamyl transferase, GPR= gamma glutamyl transferase to platelet ratio, S INDEX= aspartate amino transferase to platelet, albumin index, GLO/PLT= globulins to platelet count, AFP/GGT= alpha feto-protein to gamma glutamyl transferase ratio

Table 5: Partial Correlation Matrix (Adjusted for Age)

Marker 1	Marker 2	Unadjusted r	Partial r (Adj. for Age)	95% BCa Bootstrap CI	Clinical Status
(FIB-4)	(GGT)	+0.437	+0.412	[+0.122, +0.649]	Significant Covariation ($p < 0.01$)
(S INDEX)	(GLO/PLT)	-0.465	-0.435	[-0.683, -0.119]	Significant Inverse Axis ($p < 0.01$)
(ALT)	(S INDEX)	+0.402	+0.421	[+0.115, +0.662]	Significant Co-elevation ($p < 0.01$)
(Albumin)	(GLO)	-0.380	-0.355	[-0.612, -0.018]	Stable Inverse Association ($p < 0.05$)

FIB-4= fibrosis index using 4 parameters, GGT= gamma glutamyl transferase, S INDEX= aspartate amino transferase to platelet, albumin index, GLO/PLT= globulins to platelet count, GLO=globulins

Discussion

The significantly higher levels of liver enzymes, AFP, TP, globulins, APRI score, AFP/ALB, AFP/PLT, FIB-4, GPR, S index, GLO/PLT, AFP/GGT and lower ALB and PLT in subjects with HBV when compared with the controls suggest a transition from simple viral infection to active necroinflammation, hepatic tissue renovation, and early malignant signaling. Elevated liver enzymes (ALT, AST, GGT), suggest hepatocellular injury. Elevated ALT and AST reflect hepatic inflammation and T-cell mediated destruction of viral infected hepatocytes. This finding is similar to that of a previous study by Eworo *et al.,* (2025), which reported that elevated liver enzymes reflect liver injury, hepatocytes death and nodular regeneration that affect the architectural integrity of the hepatic lobules leading to release of the liver enzymes into circulation. Gamma-glutamyl transferase (GGT) is a sensitive index of biliary epithelial proliferation and oxidative stress within the hepatic parenchyma. Albumin levels decline as functional hepatic mass decreases; systemic inflammation drives up globulin fractions (immunoglobulins like IgG and IgA). As fibrosis or cirrhosis develop, hepatic structural changes result in platelet sequestration in the spleen (due to hypersplenism and portal hypertension) and decreased thrombopoietin production by damaged hepatocytes. Consequently, platelet counts drop while liver enzymes rise. This finding is in line with that of a previous study by Eworo *et al.,* (2025), who reported significantly reduced platelet count due to platelet sequestration within the spleen and decrease thrombopoietin in subjects with chronic hepatitis B virus. A significantly higher APRI indicates progressive fibrotic deposition. An elevated FIB-4 score suggests advanced fibrosis and cirrhosis in chronic HBV patients. This finding aligns with that of Parikh *et al.,* (2017), who evaluated fibrosis in subjects with HBV. Recent guidelines from the American Association for the Study of Liver Diseases (AASLD) and the European Association for the Study of the Liver (EASL) prioritize FIB-4 because it prevents unnecessary liver biopsies and expensive imaging for low-risk individuals (Kim *et al.,* 2025; Wang & Wang, 2026). Elevated GPR, Globulin/Platelet Ratio and S index in subjects with HBV compared to the controls suggest advanced structural changes

within the hepatic tissue, associated with immune activation, advanced fibrosis and cirrhosis. This finding is in accordance with those of (Lemoine *et al.,* 2015; Badawi *et al.,* 2020; Ekin *et al.,* 2022), who reported chronic HBV to be associated with deeply altered GGT expression due to tissue remodeling and triggers steady thrombocytopenia, elevating GPR, S Index, and Globulin/Platelet Ratio. These indexes are accurate indicator of advanced fibrosis and cirrhosis, discriminating significant fibrosis (F2) from mild stages and surrogate marker for portal hypertension and hypersplenism respectively. The elevated levels of AFP-Based Composite Ratios (AFP/Albumin, AFP/Platelet, AFP/GGT), in HBV compared with controls suggest elevated risk for malignant transformation in these subjects. Alpha-Fetoprotein (AFP) is a primary oncofetal biomarker, that momentarily elevates during benign hepatocyte regeneration, persistently elevated AFP levels signal a high risk for Hepatocellular Carcinoma (HCC). The AFP/PLT, combines oncological transformation signaling with the severe thrombocytopenia characteristic of a cirrhotic, pro-tumor microenvironment. Rising levels of AFP and decreasing platelets may reflect progression to advanced disease stages. The AFP/GGT markers are heavily upregulated in altered hepatic environments, reflecting heightened oxidative stress, atypical cell differentiation, and an increased likelihood of tumorigenesis. This finding is in line with those of previous studies by Song *et al.,* (2020); Force *et al.,* (2022), Carr *et al.,* (2024); who reported AFP-based ratios to correlate with tumor parameters.

The area under the receiver operator characteristic curve analyses demonstrated excellent diagnostic and predictive performance for APRI and S index. The good sensitivity and specificity observed for APRI and S index suggest that these indexes may provide clinically useful information for predicting high risk status for early-stage HCC. This suggest that S index and APRI levels present the cleanest and highest diagnostic accuracy across all metrics studied in predicting risk for HCC. DeLong's test for pairwise AUC comparisons among the top-performing models showed that S Index and APRI significantly outperforms FIB-4, $\Delta AUC = 0.095$ and $\Delta AUC = 0.086$ respectively. Multivariate Logistic Regression with LASSO Penalty successfully compressed redundant parameters while S INDEX, AFP/PLT, APRI were retained as predictors. Multivariate Composite Model combining these three non-redundant indices into a single predictive score yielded a combined model AUC= 0.971, Sensitivity and Specificity of 95.6% and 93.3% respectively and a LOOCV Post-LASSO AUC=0.958. This demonstrates that combining S INDEX, AFP/PLT, and APRI maximizes predictive accuracy, outperforming any single non-invasive liver marker panel operating independently. The APRI and FIB-4 scores in CHB subjects may be inflated by transient, immune-mediated ALT/AST flares rather than true architectural fibrosis. The combined model incorporating Albumin and AFP/PLT (tissue dynamics), may provide a more stable baseline less prone to spikes driven by acute necroinflammation. Also, an index like FIB-4 is a poor predictor of short-term/early-stage oncogenesis. By incorporating AFP/PLT, this composite model provides secondary clinical value by simultaneously stratifying patients at elevated risk for HCC, outperforming pure structural indexes. This observation aligns with previous studies by Ben-Ayed *et al.,* (2019); Kim *et al.,* (2022); Li *et al.,* (2024); Demirtas *et al.,* (2025), which indicates that these combined approaches generally offer superior diagnostic accuracy for early detection compared to individual scores, reducing reliance on liver biopsy. The Decision Curve Analysis (DCA) of the variables and the net clinical benefits determined that FIB-4, S INDEX, and APRI consistently maintain high net benefits across nearly the entire threshold spectrum. Implying that using these scores to drive clinical management decisions will yield the highest rate of true-positive interventions, reducing false-positive measures.

Limitation of the study

The HBV subjects were not confirmed to have early-stage HCC; therefore, our indices predict high-risk status rather than confirmed malignant transformation. Patients on long-term nucleos(t)ide analogues (NUCs) who exhibit suppressed inflammation and altered baseline biomarkers compared to treatment-naive subjects

were not categorized separately. Subjects with co-existing fatty liver disease were not separated into subgroups, detailed fibrosis staging were not captured. Lack of independent validation cohorts.

Conclusion

These preliminary findings suggest that AFP-based multi-ratio indices and composite predictive models may offer improved discrimination compared to standalone AFP in identifying HBV patients at elevated risk for early HCC. However, these results require Multi-Centre validation, in larger, independent cohorts with confirmed HCC diagnoses before clinical implementation.

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