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Novel Prognostic Indicators to reflect The Grade of Liver Fibrosis in Hepatic Patients

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Abstract

Background: Liver fibrosis is the result of a sustained wound healing response to chronic liver injury. The main reasons include chronic hepatitis C virus (HCV), hepatitis B virus (HBV) infection, and Non-alcoholic fatty liver disease (NAFLD). Non-invasive methods of assessing fibrosis are often used in clinical practice as a safer, more accessible, and less costly way than liver biopsy to stratify individuals by risk.

The current study early diagnosis of liver fibrosis in patients with hepatitis B, C, and NAFLD using indirect non-invasive biomarkers and evaluating the diagnostic impact of the Study applied on 250 patients divided into five equal groups: HBV Group, HCV Group, HBV + HCV Group, NAFLD Group and Control Group, for each with the same inclusion and exclusion criteria in order to monitor the prevalence of Apolipoprotein B (ApoB) (g/L), Lipoprotein A (mg/dL), Serum Amyloid A (SAA) (mg/L) and Insulin-like growth factor 1 (IGF1) (ng/ml) as biomarkers reflecting the grade of liver fibrosis in hepatic patients. Results: In the terms of Alanine aminotransferase ALT ($p<0.05$), Gamma-glutamyltransferase GGT ($p<0.001$), Glycosylated hemoglobin HbA1C ($p<0.001$), and Apo B($p<0.001$), in the NAFLD group, had the highest value. Meanwhile, the terms of Aspartate aminotransferase AST ($p<0.001$), Aspartate aminotransferase to platelet ratio index APRI ($p<0.001$), total bilirubin ($p<0.05$), and Serum Amyloid A SAA ($p<0.001$)in the HCV group had the greatest value. In ddition, the HBV+HCV group had the highest AST/ALT value ($p<0.001$), 2 macroglobulin($p<0.001$) values. platelets ($p<0.001$), haptoglobin ($p<0.001$), lipoprotein A ($p<0.001$), and Insulin-like growth factor 1 IGF1($p<0.001$) were all greatest in the control group. The biomarkers Lipoprotein (A) in HBV and NAFLD; Apolipoprotein B (ApoB) in HCV and HBV+ HCV group were found to be predictive of early fibrosis using Receiver Operator Characteristics (ROC) curves.

Conclusion: The estimation of non-invasive biomarkers (ApoB), (IGF1), (SAA), and Lipoprotein A were the most predictive of early fibrosis in hepatic patients with significant accuracy. These biomarkers can be used as liver biopsy alternatives to support the early diagnosis of liver fibrosis

Keyword: Apolipoprotein Biomarker,Hepatitis, Liver fibrosis, Serum Amyloid A, Aspartate aminotransferase to platelet ratio index, Insulin-Like Growth Factor 1

I. : Introduction:

Liver fibrosis is a clinically significant discovery that has a significant effect on patient morbidity and mortality. The mechanism of fibrosis includes multiple various cellular pathways, but the primary cell type involved appears to be hepatic stellate cells. Numerous liver diseases, such as hepatitis B, hepatitis C, and fatty liver, could result in continuous damage to liver cells, resulting in liver fibrosis [1]. Invasive liver biopsy is the gold standard method for managing pain and complications. The biomarkers used to detect liver fibrosis include direct markers of extracellular matrix conversion and indirect markers of the level of liver dysfunction[2]. Common serum and ultrasound-based screening tests were used to assess fibrosis involved aspartate aminotransferase to platelet ratio index score, fibrosis 4 score, FibroTest / FibroSure, non-alcoholic fatty liver fibrosis score, standard ultrasound, and transient elasticity Imaging. Generally speaking, non-invasive tests are most helpful in identifying patients with no fibrosis, mild fibrosis, or advanced fibrosis[3]. The FibroTest was approved as a biomarker for the diagnosis of the stages of fibrosis in non fatty liver disease (NAFLD) with results similar to those in chronic hepatitis C, B, and alcoholic liver disease

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[4]. Serological markers such as the aspartate transaminase to platelet ratio (APRI), fibrosis-4 scores (FIB-4) were utilized to assess liver fibrosis and have proved to be effective[5]. However, they are controversial in assessing the stage of liver fibrosis [6]..Hepatic fibrosis resulting from liver damage, ALT and AST released from the liver tissue into the circulation should indicate the degree of liver injury and function [7]. Numerous previous studies showed the relationship between IGF-1 and liver fibrosis markers and histological liver fibrosis in (NAFLD) patients [8]. Serum amyloid A (SAA) mRNA transcription has been shown to be significantly increased in mouse models of hepatic fibrosis caused by an injection of carbon tetrachloride and ligation of the bile ducts[9]. Reduced apolipoprotein AI is a serum and tissue marker of hepatic fibrosis independent of steatosis, alcoholic hepatitis, hepatic function tests ,and diet parameters[10].

Our study aims to Prognostic Indicators study early diagnosis of liver fibrosis in patients with hepatitis B, C and NAFLD using indirect non-invasive biomarkers and evaluated the diagnostic efficiency of these biomarkers to reflect the degree of liver fibrosis in liver patients.

II. Materials and methods:

The present study included 250 patients attending The Egyptian Company for blood transfusion services, Egyptian Holding Company for Biological Products & Vaccines, Donor Unit, and Clinical pathology unit of the blood bank, medical sector units. They were divided into five equal groups: HBV group (n = 50), HCV group (n = 50), HBV + HCV group (n = 50), NAFLD group (n = 50) and control group (n = 50), with the same inclusion and exclusion criteria. The exclusion criteria included patients with clinical signs of infection or other chronic inflammation. The study was approved by the Ethics Committee VACSERA (NO. 00778552V).

Patients were diagnosed with complete clinical tests and laboratory investigations .

We aim to monitor apolipoprotein B (ApoB) (g/l), lipoprotein A (mg/dl), serum amyloid A (SAA) (mg/l) and the prevalence of insulin-like growth factor 1 (IGF1) (ng/ml) is used as a biomarker to reflect the degree of liver fibrosis in liver patients. Liver function tests aspartate aminotransferase (AST) and alanine aminotransferase (ALT), Gamma-Glutamyl Transferase (GGT), total bilirubin, and HbA1c were measured on an automated biochemistry analyzer. - ADVIA Chemistry PT Systems, Siemens Healthcare Diagnostics Products GmbH. The U.S.A. Platelet count was determined by KX-21 Sysmex automated hematology analyzer (Sysmex Corporation, Japan). Lipoprotein (a), Serum Amyloid (A) Apolipoprotein B, (Apolipoprotein A1, α2 macroglobulin,, Haptoglobin, were estimated by means of particle-enhanced immunonephelometry using the BN II and BN ProSpec® System. “Siemens Healthcare Diagnostics Products GmbH. U.S.A.Insulin-like growth factor 1(IGF-1) was measured by IMMULITE 2000 XPi IGF-I assay “Siemens Healthcare Diagnostics”. Immulite 2000 IGF-I is a solid-phase, enzyme-labeled chemiluminescent immunometric assay. Hepatitis B serology (hepatitis B surface antigen, antibody to hepatitis B surface antigen), hepatitis C serology (hepatitis C virus antibody) were determined (The ADVIA Centaur® XPT Immunology analyzer). Liver fibrosis was staged from F0 to F4 according to the Metavir staging system. Fourdiagnostic targets were defined as follows: F0, normal connective tissue; F1, focal perivenular or pericellular fibrosis in zone 3; F2, perivenular or pericellular fibrosis confined to zones 2 and 3 with portal/periportal fibrosis; F3, bridging or septal fibrosis; and F4, cirrhosis [11]. APRI (AST to platelet ratio index) score was calculated as AST (IU/l)/platelet count (× 109/l) × 100. The cut-off was adopted as follows: APRI < 0.5 to identify fibrosis-free liver, APRI > 0.5 for liver fibrosis [12].

Fibro-Test score

The Fibro-Test score is calculated from the results of a six-parameter blood test, combining six serum markers with the age and gender of the patient: Alpha-2 macroglobulin, Haptoglobin, Apolipoprotein A1, Gamma-glutamyltranspeptidase (GGT), Total bilirubin, and Alanine transaminase (ALT).

Formula	Equation
FibroTest	4.467 × log[A-2M (g/L)] − 1.357 × log[Haptoglobin (g/L)] + 1.017 × log[GGT (IU/L)] + 0.0281 × [Age (years)] + 1.737 × log[TBil (µmol/L)] − 1.184 × [Apo A-1 (g/L)] + 0.301 × Gender (Female = 0, Male = 1) − 5.540 (www.biopredictive.com)

III. Statistical analysis

The recorded data were analyzed using the statistical package for social sciences, version 23.0 (SPSS Inc., Chicago, Illinois, USA). The quantitative data were presented as mean± standard deviation and ranges when their distribution was parametric (normal) while non-normally distributed variables (non-parametric data) were presented as median with inter-quartile range (IQR). Also, qualitative variables were presented as numbers and percentages. Data were explored for normality using Kolmogorov-Smirnov and Shapiro-Wilk Tests. Receiver operating characteristics (ROC

curve) analysis was used to find out the overall productivity of parameters and to find out the best cut-off value with detection of sensitivity and specificity at this cut-off value. Spearman's rank correlation coefficient (rs) was used to assess the degree of association between two sets of variables if one or both of them was skewed. Multivariate logistic regression analysis: Odds ratios (OR) with 95% confidence intervals were computed to assess the overall association between each possible factor and the occurrence of early fibrosis.

IV. Results:

The results of the present study are demonstrated in the following tables and figures.Monitoring the prevalence of Apolipoprotein B (ApoB) (g/L), Lipoprotein A (mg/dL), Serum Amyloid A (SAA) (mg/L) and Insulin-like growth factor 1 (IGF1) (ng/ml) as biomarkers reflecting the grade of liver fibrosis.

Table (2). There was a highly statistically significant difference between the NAFLD Group compared to other groups according to ALTwith p-value ($p<0.05$). The highest value was found in NAFLD group. Additionally, there was a highly statistically significant difference between (HCV group, HBV+HCV group, and NAFLD group) compared to (HBV group and control group) according to AST and AST/ALT ratio and APRI and GGT and Platelets with p-value ($p<0.001$). Meanwhile, there was a highly statistically significant difference between Control group and other groups according to Total bilirubin with p-value ($p<0.05$). Also, there was a highly statistically significant difference between groups according to α2 macroglobulin with a p-value ($p<0.001$). The highest value was found in HBV + HCV Group and HCV Group followed by NAFLD Group. Whenever there was a highly statistically significant difference between the control group compared to other groups according to Haptoglobin with p-value ($p<0.001$). The highest value was found in the Control Group followed by HBV Group . In addition, there was a highly statistically significant difference between NAFLD group compared to other groups according to HbA1C with p-value ($p<0.001$). The highest value was found in NAFLD Group followed by HBV Group. there was none statistically significant difference between the groups as regards Apolipoprotein A1 with p-value ($p=0.900$).

Table (3) showed the comparison between the studied groups according to the studied biomarkers. There was a highly statistically significant difference between the groups according to (ApoB) with p-value ($p<0.001$). The highest value was found in NAFLD Group (0.94±0.22). Additionally, there was a highly statistically significant difference between groups according to Lipoprotein A with a p-value ($p<0.001$). The highest value was found in the Control Group (14.60±1.97). Furthermore, there was a highly statistically significant difference between the groups according to (SAA) with a p-value ($p<0.001$). The highest value was found in HCV Group (8.62±3.97) and HBV + HCV Group (8.62±3.96). Meanwhile, there was a highly statistically significant difference between the groups according to (IGF1) with p-value ($p<0.001$). The highest value was found in the Control Group (158.64±23.10)

Table (1): Comparison between the studied groups according to demographic data

Demographic data	HBV Group	HCV Group	HBV + HCV Group	NAFLD Group	Control Group	Test	p-value
Age (years) mean ± SD	56.28±9.40	56.76±9.57	59.04±7.11	54.32±16.79	55.10±11.65	H=1.261	0.286
Female	10 (20.0%)	10 (20.0%)	6 (12.0%)	12 (24.0%)	9 (18.0%)	x2=2.515	0.642
Male	40 (80.0%)	40 (80.0%)	44 (88.0%)	38 (76.0%)	41 (82.0%)		

Our present results showed that there was no statistically significant difference between groups as regard age and gender with p-value (p=0.286; p=0.642).

Table (2): Comparison between the studied groups according to laboratory tests

Biomarker laboratory	HBV Group	HCV Group	HBV + HCV Group	NAFLD Group	Control Group	H=Test	p-value
ALT (U/L) mean ±SD	24.48±8.63B	25.46±14.16B	25.10±20.06B	35.56±23.89A	23.96±16.88B	3.866	0.005*
AST (U/L) mean ± SD	31.92±16.75B	51.64±23.61A	50.88±20.21A	49.32±21.21A	24.58±16.89B	19.870	<0.001**
AST/ALT ratio mean ±SD	1.29±0.48B	2.22±1.00A	2.52±1.21A	1.86±1.24A	1.05±0.15B	22.318	<0.001**
APRI mean ±SD	0.41±0.27B	0.84±0.47A	0.83±0.43A	0.73±0.43A	0.29±0.20B	23.209	<0.001**
GGT (IU/L)							

mean ±SD	26.74±10.80B	50.06±46.13A	41.46±29.62A	59.80±42.54A	26.42±13.90B	10.387	<0.001**
Total bilirubin(mg/dL) mean ±SD	0.57±0.32A	0.67±0.36A	0.62±0.33A	0.60±0.28A	0.43±0.22B	4.487	0.002*
Platelets mean ±SD	204.88±17.33A	168.86±31.22B	167.52±29.50B	185.78±34.27AB	214.18±5.46A	32.852	<0.001**
HbA1C mean ± SD	5.69±0.46B	5.27±0.09B	5.26±0.12B	8.22±1.33A	5.29±0.15B	202.806	<0.001**
α2 macroglobulin (g/L) mean ±SD	2.05±0.71C	3.10±0.79A	3.21±0.87A	2.26±0.80B	1.77±0.47C	37.579	<0.001**
Haptoglobin (g/L) mean ±SD	1.24±0.70B	1.21±0.53B	1.20±0.70B	1.10±0.64B	1.66±0.68A	5.430	<0.001**
Apolipoprotein A1 (g/L) mean ±SD	1.29±0.23	1.29±0.24	1.26±0.22	1.27±0.19	1.26±0.19	0.266	0.900

Table (3): Comparison between the studied groups according to biomarkers

Biomarkers	HBV Group	HCV Group	HBV + HCV Group	NAFLD Group	Control Group	H=Test	p-value
Apolipoprotein B (ApoB) (g/L) mean ±SD	0.70±0.19B	0.92±0.20A	0.92±0.17A	0.94±0.22A	0.56±0.01C	37.170	<0.001**
Lipoprotein A (mg/dL) mean ±SD	12.83±0.77B	10.05±2.38C	10.60±2.34C	11.69±2.36B	14.60±1.97A	39.250	<0.001**
Serum Amyloid A (SAA) (mg/L) mean ±SD	5.28±2.55B	8.62±3.97A	8.62±3.96A	6.69±4.11B	3.70±0.45C	20.789	<0.001**
Insulin-like growth factor 1 (IGF1) (ng/ml) mean ±SD	113.58±28.75B	84.38±31.38D	80.96±21.31D	97.28±34.83C	158.64±23.10A	62.232	<0.001**

1. **Discrimination results for Fibro test Assement in different studied groups:**

There was a highly statistically significant difference between groups according to fibro test with a p-value (p<0.001). The highest value was found in HBV + HCV Group (0.57±0.21) and HCV Group (0.55±0.24).

2. **Discrimination results for Fibro Grade Assement in different studied groups:**

There was a highly statistically significant difference between groups according to Fibro grades with a p-value (p<0.001). The highest value of advanced fibrosis was found in HBV + HCV Group (54%) and HCV Group (54%) followed by NAFLD Group (32%) while the lowest value was found in HBV Group (12%) followed by the Control Group (0%).**(Figure 2).**

3. **Results for Correlation between Fibro test with all parameters, using Spearman's rank correlation coefficient,**in

a) **HBV group**

A highly statistically positive significant correlation p-value (p<0.001) between Fibro test with AST, AST/ALT ratio, APRI, Total bilirubin, α2 macroglobulin, (ApoB), Lipoprotein A, (SAA) and statistically positive significant correlation p-value (p<0.05 S) between Fibro test with age, ALT, GGT but A highly statistically negative significant correlation p-value (p<0.001) with Platelets, Haptoglobin, Apolipoprotein A1, Lipoprotein A, (IGF1) and there was none statistically significant difference between fibro test as regard HbA1C, with p-value (p=0.321)

b) **HCV group**

A highly statistically positive significant correlation p-value (p<0.001) between Fibro test with ALT, AST, AST/ALT ratio, APRI, GGT, Total bilirubin, (ApoB), (SAA) and statistically positive significant correlation p-value (p<0.05 S) between Fibro test with age, α2 macroglobulin but A highly statistically negative significant correlation p-value (p<0.001) with Platelets, , Lipoprotein A, (IGF1)and statistically negative significant correlation p-value (p<0.05 S) between Fibro test with Haptoglobin and there were none statistically significant difference between fibro test as regard HbA1C and Apolipoprotein A1, with p-value (p=0.624, p=0. 750)

c) **HBV + HCV group**

A highly statistically positive significant correlation p-value (p<0.001) between Fibro test with ALT, AST, APRI, GGT, Total bilirubin, α2 macroglobulin, (ApoB), (SAA) and statistically positive significant correlation p-value (p<0.05 S) between Fibro test with AST/ALT ratio, but A highly statistically negative significant correlation p-value (p<0.001) with Platelets,

Haptoglobin , Lipoprotein A, (IGF1) and there were none statistically significant difference between fibro test as regard age, HbA1C and Apolipoprotein A1, with p-value (p=0.717, p=0. 526, p=0.728)

d) **NAFLD group**

A highly statistically positive significant correlation p-value (p<0.001) between Fibro test with age, AST, APRI, GGT, Total bilirubin, HbA1C, α2 macroglobulin (ApoB), (SAA) and statistically positive significant correlation p-value (p<0.05 S) between Fibro test with AST/ALT ratio, but A highly statistically negative significant correlation p-value (p<0.001) with Platelets, Haptoglobin , Lipoprotein A, (IGF1) and there were none statistically significant difference between fibro test as regard ALT, and Apolipoprotein A1, with p-value (p=0.479, p=0. 305)

e) **control group**

There was no statistically significant correlation between Fibro test with all parameters in control group, with p-value (p>0.05).

f) all groups

A highly statistically positive significant correlation p-value (p<0.001) between Fibro test with age, ALT AST, AST/ALT ratio, APRI, GGT , Total bilirubin, α2 macroglobulin, (ApoB), (SAA) but A highly statistically negative significant correlation p-value (p<0.001) with Platelets, Haptoglobin, Lipoprotein A, (IGF1) and statistically negative significant correlation p-value (p<0.05 S) between Fibro test with Apolipoprotein A1, and there was none statistically significant difference between fibro test as regard HbA1C, with p-value (p=0.679)

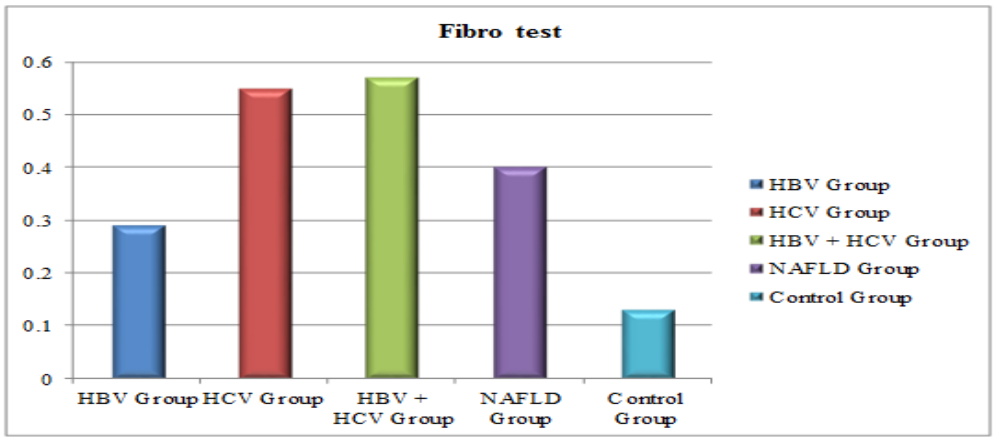

Figure (1): Comparison between the studied groups according to Fibro test

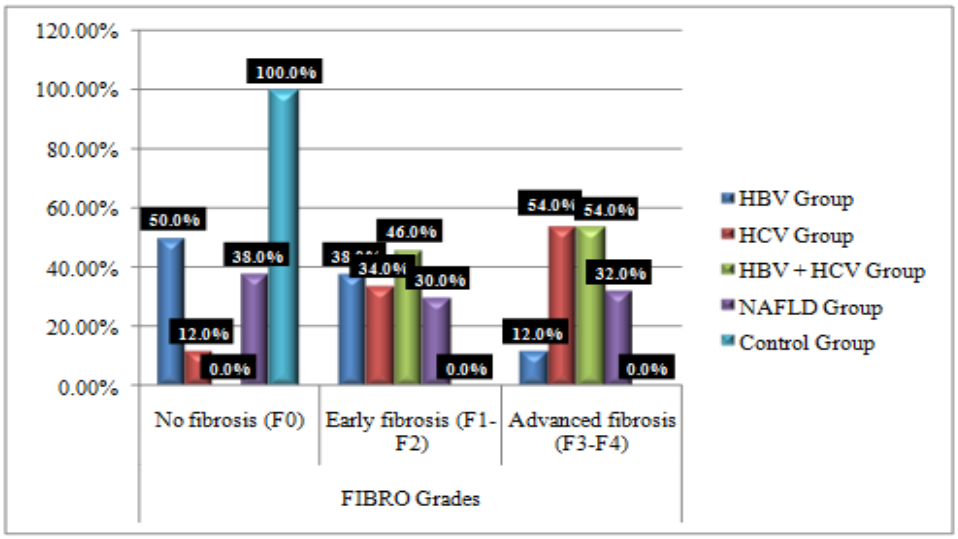

Figure (2): Comparison between the studied groups according to Fibro grades

4. Multivariate logistic regression analysis for independent predictors for early fibrosis , in all groups

Table (4): Multivariate logistic regression analysis for independent predictors for early fibrosis in HBV group

HBV Group	β	SE	OR	95% C.I. for OR	p-value

				Lower	Upper	
Age (years)	0.369	0.136	2.826	1.587	6.950	0.107
ALT (U/L)	0.429	0.236	1.695	0.684	4.209	0.171
AST (U/L)	2.561	0.691	1.914	0.377	4.405	<0.001**
AST/ALT ratio	0.548	0.148	5.459	5.200	5.731	<0.001**
APRI	0.977	0.362	5.382	3.155	9.177	<0.001**
GGT (U/L)	0.475	0.176	5.475	4.903	6.109	0.013*
Platelets	-1.721	0.809	7.094	5.499	9.144	<0.001**
Total bilirubin(mg/dL)	0.394	0.106	3.921	3.735	4.116	0.002*
HbA1C	-0.214	0.118	0.845	0.341	2.099	0.880
α2 macroglobulin (g/L)	1.471	0.691	6.063	4.700	7.815	<0.001**
Haptoglobin (g/L)	-0.461	0.124	4.587	4.370	4.816	0.004*
Apolipoprotein A1 (g/L)	-0.315	0.117	4.562	2.696	9.253	<0.001**
Apolipoprotein B (ApoB) (g/L)	3.047	0.823	2.277	0.449	5.242	<0.001**
Lipoprotein A (mg/dL)	-1.039	0.488	4.281	3.319	5.519	<0.001**
Serum Amyloid A (SAA) (mg/L)	0.341	0.126	3.932	3.521	4.388	<0.001**
Insulin-like growth factor 1 (IGF1) (ng/ml)	-0.291	0.160	2.223	0.567	7.449	<0.001**

AST, AST/ALT ratio, APRI, Platelets, α2 macroglobulin, Apolipoprotein A1 (ApoB), Lipoprotein A ,(SAA) and (IGF1) with p-value (p<0.001) but GGT, Total bilirubin, Haptoglobin with p-value (p<0.05 S), while age, ALT, , HbA1C, were in significant with p-value (p>0.05 NS).

Table (5): Multivariate logistic regression analysis for independent predictors for early fibrosis in HCV group

HCV Group	β	SE	OR	95% C.I. for OR		p-value
				Lower	Upper	
Age (years)	0.234	0.063	2.327	2.216	2.443	0.201
ALT (U/L)	1.021	0.480	4.209	3.263	5.426	0.135
AST (U/L)	0.690	0.255	3.801	2.228	6.481	<0.001**
AST/ALT ratio	0.531	0.196	7.687	4.544	15.593	0.005*
APRI	0.260	0.096	1.996	1.121	4.908	<0.001**
GGT (U/L)	0.335	0.124	3.866	3.462	4.314	0.147
Platelets	-0.375	0.139	5.428	3.209	11.011	<0.001**
Total bilirubin(mg/dL)	0.734	0.345	3.023	2.344	3.897	0.208
HbA1C	0.399	0.148	4.601	4.120	5.134	0.436
α2 macroglobulin (g/L)	0.286	0.106	3.305	2.959	3.687	<0.001**
Haptoglobin (g/L)	-0.821	0.304	4.523	2.651	7.712	0.711
Apolipoprotein A1 (g/L)	-0.280	0.076	2.437	1.397	5.514	0.409
Apolipoprotein B (ApoB) (g/L)	4.776	2.245	4.776	4.422	5.157	<0.001**
Lipoprotein A (mg/dL)	-0.237	0.088	2.730	2.445	3.046	0.233
Serum Amyloid A (SAA) (mg/L)	0.230	0.062	2.288	2.179	2.402	<0.001**
Insulin-like growth factor 1 (IGF1) (ng/ml)	-0.198	0.053	1.721	0.987	3.894	<0.001**

AST, APRI, Platelets, HbA1C, α2 macroglobulin, (ApoB), (SAA) and (IGF1) with p-value (p<0.001) but AST/ALT ratio with a p-value (p<0.05 S), while age, ALT, GGT ,Total bilirubin, Haptoglobin, Apolipoprotein A1, Lipoprotein A were none significant with p-value (p>0.05 NS).

Table (6): Multivariate logistic regression analysis for independent predictors for early fibrosis in HCV+ HBV group

HBV + HCV Group	β	SE	OR	95% C.I. for OR		p-value
				Lower	Upper	
Age (years)	-1.535	0.721	1.349	0.595	6.785	0.692

ALT (U/L)	1.519	0.410	1.136	0.224	2.614	0.004*
AST (U/L)	0.245	0.135	1.868	0.476	6.259	<0.001**
AST/ALT ratio	0.282	0.104	3.249	2.909	3.625	0.032*
APRI	1.163	0.430	6.405	3.754	10.921	<0.001**
GGT (U/L)	0.347	0.191	2.646	0.674	8.864	<0.001**
Platelets	-2.048	0.963	8.441	6.544	10.881	<0.001**
Total bilirubin(mg/dL)	0.265	0.098	3.833	2.266	7.776	0.002*
HbA1C	3.373	1.585	3.373	3.122	3.642	0.950
α2 macroglobulin (g/L)	1.808	0.488	1.351	0.266	3.111	0.019*
Haptoglobin (g/L)	-0.180	0.099	0.710	0.287	1.764	<0.001**
Apolipoprotein A1 (g/L)	-1.277	0.345	0.954	0.188	2.197	0.732
Apolipoprotein B (ApoB) (g/L)	0.469	0.127	4.666	4.444	4.899	<0.001**
Lipoprotein A (mg/dL)	-0.250	0.138	0.989	0.399	2.455	<0.001**
Serum Amyloid A (SAA) (mg/L)	0.241	0.089	2.777	2.487	3.098	<0.001**
Insulin-like growth factor 1 (IGF1) (ng/ml)	-3.663	1.722	3.220	1.420	16.192	<0.001**

AST, APRI, , GGT, Platelets, , Haptoglobin, (ApoB), Lipoprotein A , (SAA) and (IGF1) with p-value (p<0.001) but ALT, AST/ALT ratio Total bilirubin, α2 macroglobulin with p-value a (p<0.05 S), while age, HbA1C, Apolipoprotein A1, were insignificant with p-value (p>0.05 NS).

Table (7): Multivariate logistic regression analysis for independent predictors for early fibrosis in NAFLD group

NAFLD Group	β	SE	OR	95% C.I. for OR		p-value
				Lower	Upper	
Age (years)	0.298	0.164	1.177	0.475	2.922	0.091
ALT (U/L)	0.331	0.089	3.295	3.138	3.459	0.436
AST (U/L)	1.384	0.512	7.622	4.467	12.996	0.045*
AST/ALT ratio	0.387	0.105	3.855	3.672	4.047	0.442
APRI	1.215	0.571	5.009	3.883	6.457	0.003*
GGT (U/L)	1.827	0.859	1.606	0.708	8.075	<0.001**
Platelets	-0.273	0.074	2.722	2.593	2.858	<0.001**
Total bilirubin(mg/dL)	0.616	0.290	2.541	1.969	3.275	0.004*
HbA1C	0.303	0.167	1.197	0.483	2.972	<0.001**
α2 macroglobulin (g/L)	0.360	0.198	1.425	0.575	3.537	<0.001**
Haptoglobin (g/L)	-0.235	0.064	2.048	1.174	4.633	0.004*
Apolipoprotein A1 (g/L)	-0.721	0.339	2.973	2.304	3.832	0.537
Apolipoprotein B (ApoB) (g/L)	0.223	0.082	3.221	1.904	6.534	<0.001**
Lipoprotein A (mg/dL)	-2.174	1.022	1.911	0.843	9.609	<0.001**
Serum Amyloid A (SAA) (mg/L)	1.750	0.823	7.215	5.593	9.300	<0.001**
Insulin-like growth factor 1 (IGF1) (ng/ml)	-0.278	0.075	2.769	2.637	2.907	<0.001**

GGT, Platelets, HbA1C, α2 macroglobulin, (ApoB), Lipoprotein A , (SAA) and (IGF1) with p-value (p<0.001) but AST, APRI, Total bilirubin, Haptoglobin with a p-value (p<0.05 S), while age, ALT, AST/ALT ratio Apolipoprotein A1 were none significant with p-value (p>0.05 NS).

5. Receiver operator characteristics (ROC) curves as predictors of early fibrosis in all different studied groups

1) In the HBV Group.

Figure (3) (ApoB), Lipoprotein A, (SAA) and (IGF1) indices were significant discriminated as denoted by the significantly large area under the curves (AUCs); The AUROC of (ApoB) was 0.656(p<0.05)the sensitivity and specificity to predict early liver fibrosis were 48% and 100%,The AUROC of

Lipoprotein A was 0.960 (p<0.001) the sensitivity and specificity to predict early liver fibrosis were 100% and 96%,The AUROC of (SAA) was 0.719 (p<0.001) the sensitivity and specificity to predict early liver fibrosis were 80% and 58%,The AUROC of (IGF1) was 0.884 (p<0.001) the sensitivity and specificity to predict early liver fibrosis were 62% and 96%.This indicates that Lipoprotein A the most predictable of early fibrosis, followed by (IGF1), (SAA), and (ApoB) so Lipoprotein A being the most significant discrimination, with p-value <0.001 highly significant predictions of early fibrosis in the HBV group.

2) In the HCV Group.

Figure (4): (ApoB), Lipoprotein A, (SAA) and (IGF1) indices were significant discriminated as denoted by the significantly large area under the curves (AUCs); The AUROC of (ApoB) was 0.993 (p<0.001)the sensitivity and specificity to predict early liver fibrosis were 94% and 100%,The AUROC of Lipoprotein A was 0.960 (p<0.001) the sensitivity and specificity to predict early liver fibrosis were 100% and 96%,The AUROC of (SAA) was 0.885 (p<0.001) the sensitivity and specificity to predict early liver fibrosis were 78% and 100%,The AUROC of (IGF1) was 0.954 (p<0.001) the sensitivity and specificity to predict early liver fibrosis were 88% and 100%.This indicates that (ApoB) is the most predictable of early fibrosis, followed by Lipoprotein A , (IGF1), (SAA) and so (ApoB) being the most significant discrimination, with p-value <0.001 highly significant predictions of early fibrosis in the HCV group.

3) In the HBV+ HCV Group

Figure (5): (ApoB), Lipoprotein A , (SAA) and (IGF1) indices were significant discriminated as denoted by the significantly large area under the curves (AUCs); The AUROC of (ApoB) was 1.0 (p<0.001)the sensitivity and specificity to predict early liver fibrosis were 100% and 100%,The AUROC of Lipoprotein A was 0.960 (p<0.001) the sensitivity and specificity to predict early liver fibrosis were 100% and 96%,The AUROC of (SAA) was 0.945 (p<0.001) the sensitivity and specificity to predict early liver fibrosis were 84% and 100%,The AUROC of (IGF1) was 0.998 (p<0.001) the sensitivity and specificity to predict early liver fibrosis were 100% and 96%.This indicates that (ApoB) is the most predictable of early fibrosis, followed by (IGF1) , Lipoprotein A , (SAA), and so (ApoB) being the most significant discrimination, with p-value <0.001 highly significant predictions of early fibrosis in HBV+HCV group.

4) In the NAFLD Group.

Figure (6) (ApoB) , Lipoprotein A , (SAA), and (IGF1) indices were significant discriminated as denoted by the significantly large area under the curves (AUCs); The AUROC of (ApoB) was 0.960 (p<0.001)the sensitivity and specificity to predict early liver fibrosis were 86% and 100%,The AUROC of Lipoprotein A was 0.980 (p<0.001) the sensitivity and specificity to predict early liver fibrosis were 100% and 96%,The AUROC of (SAA)was 0.687 (p<0.001) the sensitivity and specificity to predict early liver fibrosis were 52% and 100%,The AUROC of (IGF1) was 0.918(p<0.001) the sensitivity and specificity to predict early liver fibrosis were 78% and 100%.This indicates that Lipoprotein A the most predictable of early fibrosis, followed by (ApoB), (IGF1), (SAA) ,and so Lipoprotein A is the most significant discrimination, with p-value <0.001 highly significant predictions of early fibrosis in theNAFLD Group.

5) In all patients group.

Figure (7,8) (ApoB) , Lipoprotein A ,(SAA) , and (IGF1) indices were significant discriminated as denoted by the significantly large area under the curves (AUCs); The AUROC of (ApoB) was 0.984 (p<0.001)the sensitivity and specificity to predict early liver fibrosis were 97.3% and 100%,The AUROC of Lipoprotein A was 0.947 (p<0.001) the sensitivity and specificity to predict early liver fibrosis were 97.3% and 90%,The AUROC of (SAA)was 0.975 (p<0.001) the sensitivity and specificity to predict early liver fibrosis were 83.3% and 100%,The AUROC of (IGF1) was 0.980 (p<0.001) the sensitivity and specificity to predict early liver fibrosis were 98.7% and 91%.This indicates that ApoB) is the most predictable of early fibrosis, followed by (IGF1) , (SAA), Lipoprotein A ,and so (ApoB) is the most significant discrimination, with p-value <0.001 highly significant predictions of early fibrosis in all patients among the study group.

V. Discussion:

Liver fibrosis has been shown to be reversible after the elimination of etiology, especially in the early stages. Therefore, early diagnosis of liver fibrosis is of crucial importance for clinical management. Liver biopsy remains the gold standard for both diagnosis and staging of fibrosis, but it is largely suboptimal due to its invasive nature and various associated complications. To overcome this, several non-invasive diagnostic methods based on serum biomarkers and imaging techniques have been developed[13]. Serum biomarkers exclude a lot of the concerns related to liver biopsy. In general, in addition to being inexpensive and minimally invasive, these tests are associated with very low sampling errors and intra / interobserver variability [14]].

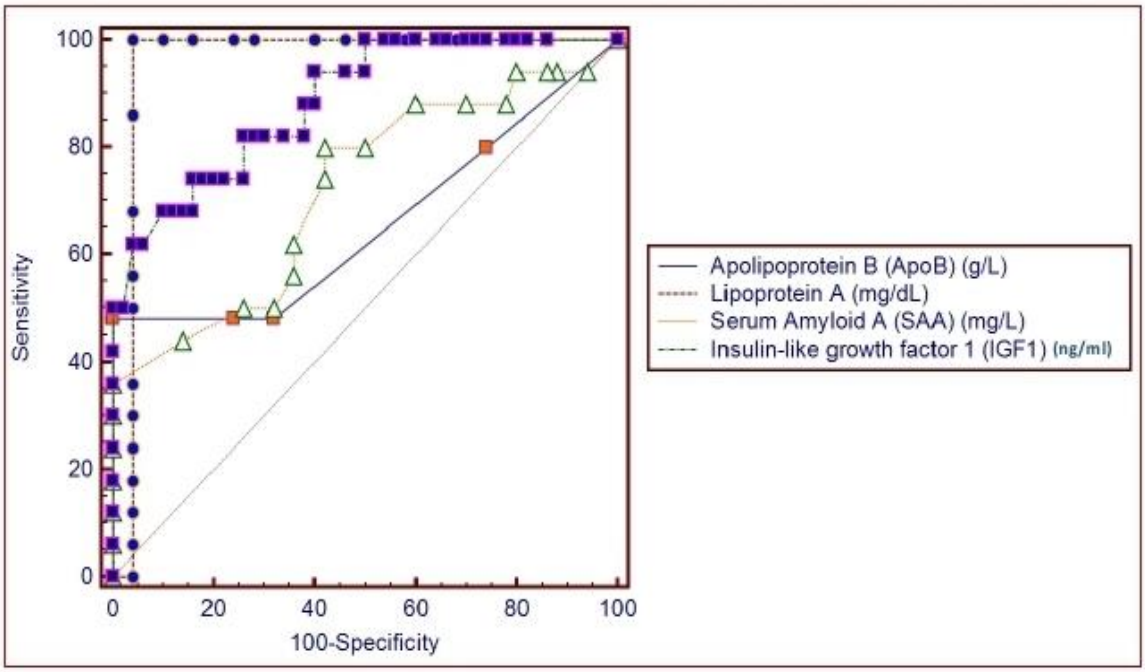

Figure (3): Receiver-operating characteristics (ROC) curves for prediction of early fibrosis using the biomarkers regarding (ApoB), Lipoprotein A, (SAA) and (IGF1) in HBV Group

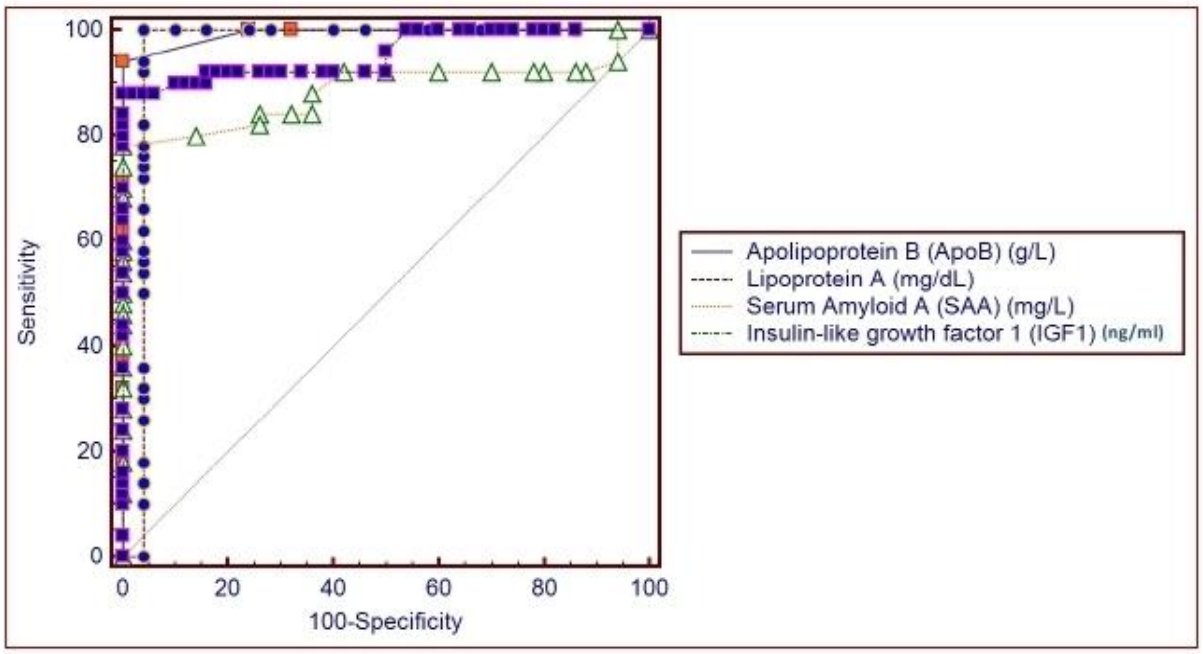

Figure (4): Receiver-operating characteristics (ROC) curves for prediction of early fibrosis using the biomarkers regarding (ApoB), Lipoprotein A, (SAA) and (IGF1) in HCV Group

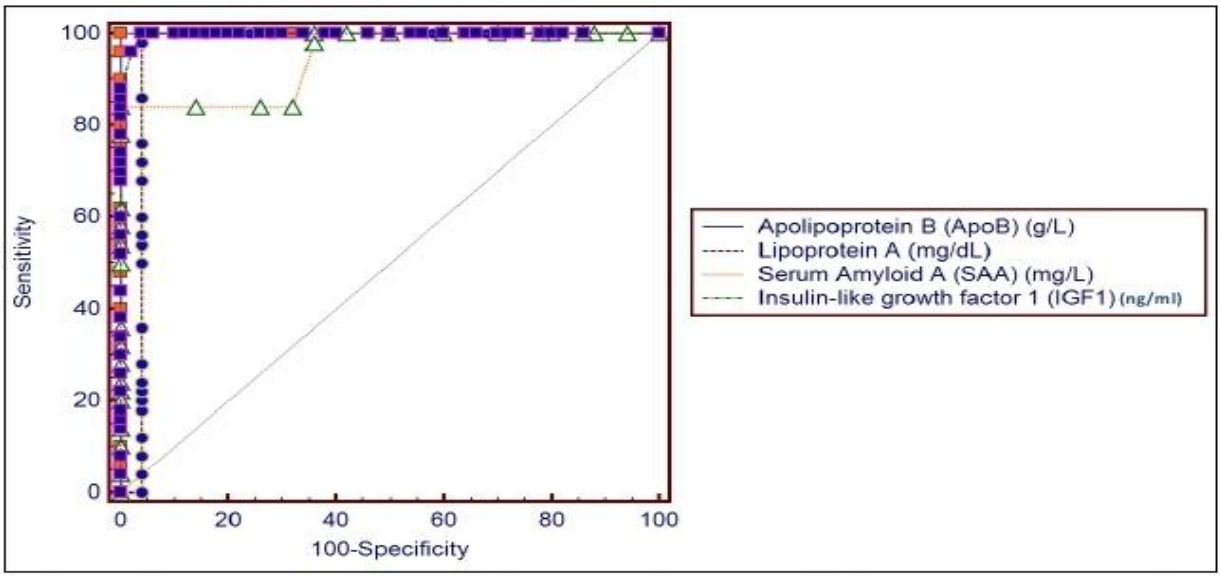

Figure (5): Receiver-operating characteristic (ROC) curve for prediction of early fibrosis using the biomarkers regarding (ApoB), Lipoprotein A, (SAA) and (IGF1) in HBV+ HCV Group

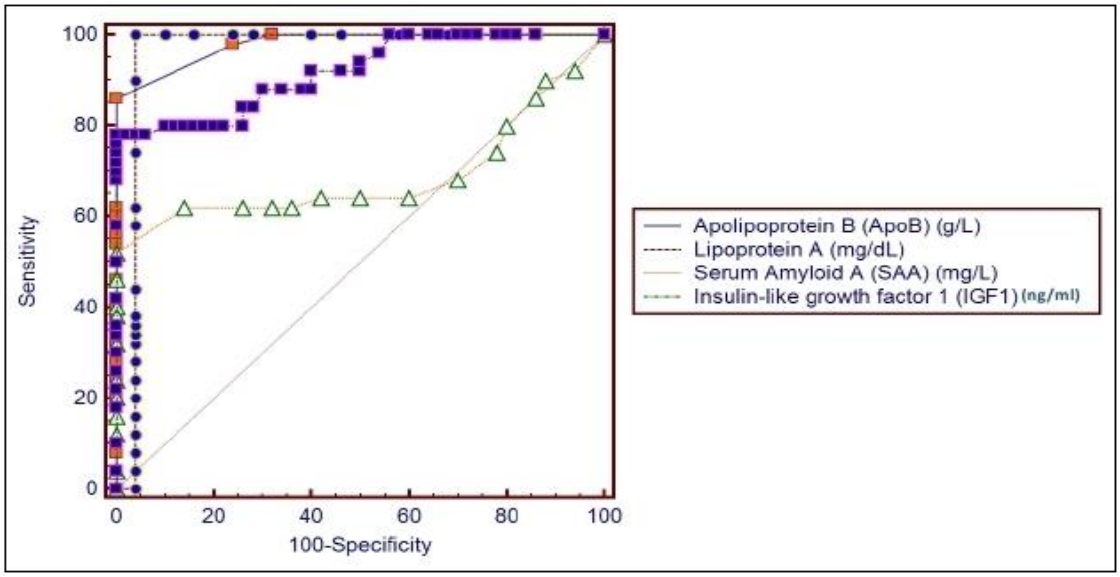

Figure (6): Receiver-operating characteristics (ROC) curves for prediction of early fibrosis using the biomarkers regarding (ApoB), Lipoprotein A, (SAA) and (IGF1) in the NAFLD Group

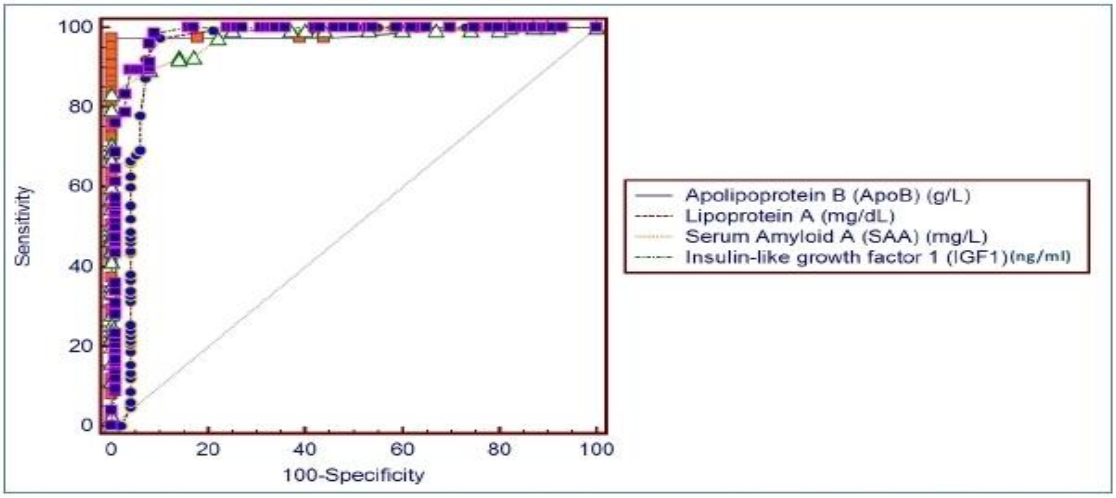

Figure (7): Receiver-operating characteristics (ROC) curves for prediction of early fibrosis using the biomarkers regarding (ApoB), Lipoprotein A, (SAA) and (IGF1) in all patients Group

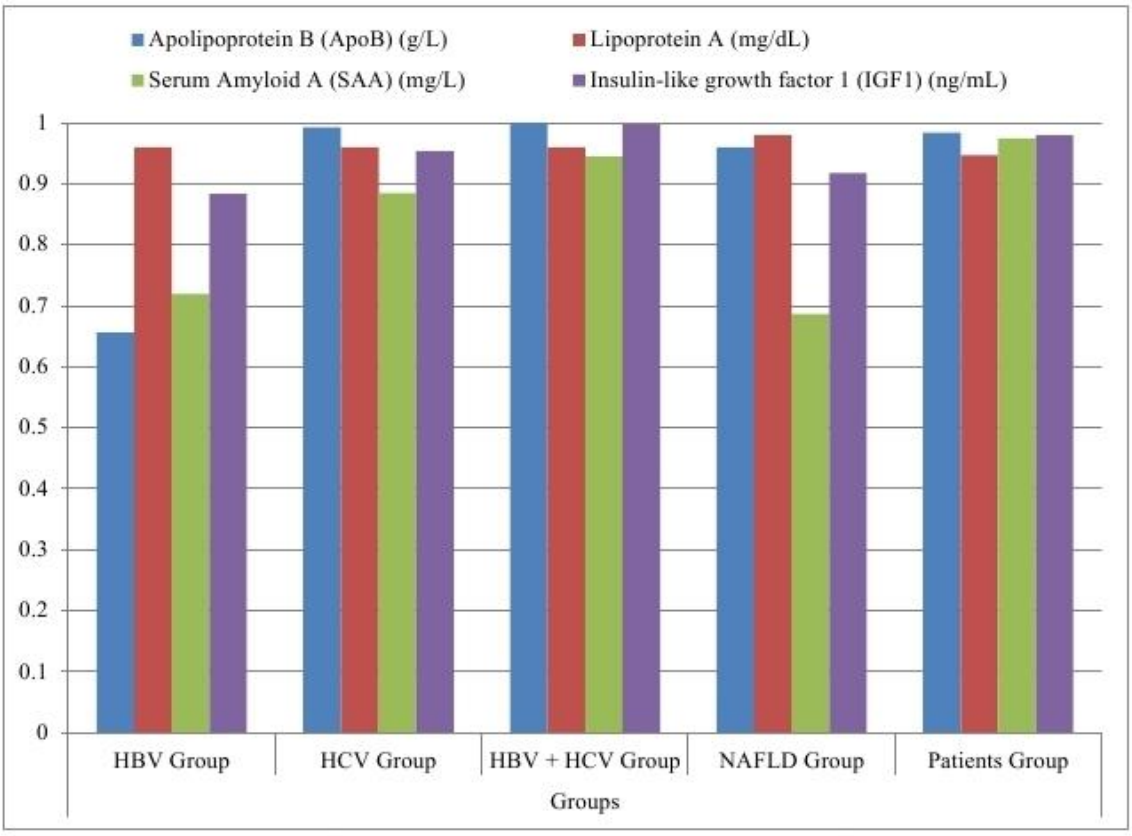

Figure (8): High value of area under the curve among study group

Our Results revealed that a highly statistically positive significant correlation p-value ($p<0.001$) between Fibro test with age, ALT AST, AST/ALT ratio, APRI, GGT, Total bilirubin, α2 macroglobulin, Apolipoprotein B (ApoB), Serum Amyloid A (SAA) but A highly statistically negative significant correlation p-value ($p<0.001$) with Platelets, Haptoglobin, Lipoprotein A, Insulin-like growth factor 1 (IGF1) and statistically negative significant correlation p-value ($p<0.05$ S) between Fibro test with Apolipoprotein A1, and there was none statistically significant difference between fibro test as regard HbA1C, with p-value ($p=0.679$) Identifying those affected with chronic liver disease is critical to containing disease progression and, more importantly, identifying people with end-stage liver disease who are at significant risk of decompensation. Identifying people with treatable diseases like HCV,

HBV can delay or even reverse fibrosis and early cirrhosis.. We tested liver fibrosis in the studied patient group by fibrotest; FibroTest is another proprietary test panel that combines direct and indirect markers of liver fibrosis. This test includes haptoglobin and apolipoprotein A1. Haptoglobin is negatively correlated with liver fibrosis. Hepatocyte growth factor, which is elevated in the stages of liver regeneration, including cirrhosis, stimulates an increase in α2M and a decrease in haptoglobin production. Apolipoprotein A1 is the main component of high-density lipoproteins (HDL) and is synthesized by the liver. , HDL levels are inversely proportional to liver fibrosis[15]. Results of the present study revealed the male predominance in each of the studied groups, in (HBV) group, (80%) were male and (20%) were females; also, in (HCV) group, (80%) were male and (20%) were females; in HBV+HCV group (88%) were male and (12%) were females; in NAFLD (76%) were male and (24%) were females and in the control group (82%) were male and (18%) were females.Men are at markedly increased risk of advanced liver disease in various disease etiologies; this supports the potential role of gender differences in exposure to risk factors, as well as gender-based biological differences in disease progression. This was attributed to the role of testosterone[16]. The mean age of the studied groups was (56.28±9.40), (56.76±9.57), (59.04±7.11), (54.32±16.79) and (55.10±11.65) in HBV, HCV, HBV+HCV, NAFLD, and control groups, respectively. In our study, advanced age was associated with liver fibrosis, which is consistent with the results of previous studies [17]. The pathophysiology of increased liver fibrosis in the elderly population has been suggested. The number and volume of individual hepatocytes decreases with age, which may increase liver fibrosis, and aging influences the molecular mechanisms that regulate liver regeneration [17Regarding the hepatitis B (HBV) group, the results of the present study showed that the highest value of platelets was found in HBV patients (204.88±17.33). In addition, the lowest values of fibrotest and Fibro grades (0.29±0.22) and (12%), respectively, All HBV patients in [18]. The study had well-preserved liver functions as reported in our study. Our results were agreed with [19]. results, where platelet counts in the chronic hepatitis B group were significantly higher than in the control group ((p<0.001)). This could be explained by the fact that thrombocytopenia in patients with hepatitis B was contributed to platelet destruction/sequestration caused by hypersplenism [20]. A decrease in peripheral platelet counts may indicate an increase in the degree of fibrosis in the course of chronic viral hepatitis B and C, and factors other than hypersplenism may influence this decrease in peripheral platelet counts[21]. As regards the fibrotest results, similar findings were found in the study of[22]. (7.7%) of HBV patients had FibroTest >0.74. Patients with chronic hepatitis B more often have a wide range of fluctuations in necroinflammatory activity and have macronodular cirrhosis, leading to a relatively lower fibrotic content than patients with chronic hepatitis C[23]. Fibrogrades recorded the lowest values in comparison to other patient groups. This could be explained by the fact that in the positive phases for HBeAg, many patients remain in the immune tolerance state for many years, which is characterized by normal levels of transaminases and is also classically associated with little or no liver damage [24]. There was a highly statistically significant correlation between the Fibro test with all parameters except HbA1C (p<0.05), our results were confirmed with [25]. who reported that FibroTest had excellent diagnostic accuracy for the identification of HBV-related significant fibrosis and cirrhosis.There are several categories of noninvasive markers that are used to predict the severity of fibrosis in HBVfrom these, surrogate markers use routine laboratory measurements, such as transaminases, synthetic markers of liver function (bilirubin), or other readily available indices related to the stage of liver disease (platelet levels, red blood distribution). Several studies with a combination of these parameters have shown useful non-invasive fibrosis scores [26], A comprehensive review of [27]. Showed that the precision and applicability of noninvasive methods varied between HBV and HCV patients, and some methods were found to be invalid in HBV patients. Therefore, recent studies have focused on developing several new non-invasive models to assess liver fibrosis, particularly in HBV-infected patients [28]. When we studied the significant predictors of early fibrosis in HBV; multivariate analysis showed that they were AST, AST/ALT ratio, APRI, Platelets, α2 macroglobulin, Apolipoprotein B (ApoB), Serum Amyloid A (SAA), and Insulin-like growth factor 1 (IGF1) with p-value (p<0.05 S).In agreement with our results, [29]. Also investigated the value of serum biochemical markers in liver fibrosis diagnosis in patients with hepatitis B and observed that levels of ALT, total bilirubin, alpha 2-macroglobulin, GGT, and Apo A-I were significantly correlated with the clinical staging of liver fibrosis. In contrast [30]. Noted that AST/ALT ratio was performed lower than other non-invasive blood-based algorithms in the estimate of the fibrosis phase in patients with HBV. Similarly, AST/ALT ratio's ability to diagnose significant fibrosis (F2F4) was poor in a US cohort of patients with HBV [31]. The main advantage of APRI over other non-invasive tests is that it is based on readily available blood tests and is easy to use. There are many studies looking at APRI in HBV patients [26]. The platelet count was also a strong predictor for liver fibrosis in HBV. The platelet counts as a simple, non-invasive index that could assess the degree of liver fibrosis in patients with HBV. It showed good performance in our results as well as previous studies. According to [32]. the median and mean rank of platelet

count decreased along with the aggravation of fibrosis. In addition, the usefulness of ApoB as an early fibrosis prediction biomarker was proved in our study results. Some clinical studies with HBV infected populations have significantly reduced concentrations of ApoB[33,34]. demonstrated that overexpression of the HBx protein in hepatocytes decreased ApoB secretion, increased intracellular levels of ApoB, TG, and cholesterol, and altered VLDL / LDL composition or secretion. As regards SAA, our findings also suggest the usefulness of this biomarker in HBV-caused hepatic fibrosis prediction. In previous studies, the performance of SAA in visualizing liver inflammation and fibrogenesis was tested in non-abscessed liver disease with a milder inflammatory state, such as chronic HBV. Serum SAA levels were found to be significantly higher in patients with active chronic HBV than in those with inactive chronic HBV[35]. Our results agreed with [36], serum IGF1 level was a useful indicator of liver function, as well as a prognostic marker of progression time and overall survival in HBV study. [37] evaluated IGF-1 in chronic liver diseases associated with HBV infection and described the effect of liver status on IGF-1 variables. Receiver operator characteristics (ROC) curves of early fibrosis prediction biomarkers in the HBV group showed that Lipoprotein A was the most predictable of early fibrosis, with p-value <0.001, denoted by the significantly large area under the curves (AUCs) (0.960), sensitivity (100%) and specificity (96%) followed by (IGF1), (SAA) and (ApoB).Regarding the hepatitis C (HCV) group, the results of the current study showed that the HCV patient group showed the highest value of AST (51.64±23.61), APRI (0.84±0.47, total bilirubin (0.67±0.36), SAA (8.62±3.97), (ApoB) (0.92±0.20), α2 macroglobulin (3.10±0.79). in addition to the lowest value of Lipoprotein A (10.05±2.38). These results could be attributed to steatosis present in patients with chronic hepatitis C, which can influence initial biochemical parameters, and micronodular cirrhosis[23]. These findings are in agreement with [38]; patients with advanced fibrosis had higher mean ALT and AST scores (p <0.005). However, platelet counts were lower in patients with advanced fibrosis. Also, recently [39]. the study revealed that the mean serum AST and ALT had increased in patients with chronic (HCV) infection, and platelet counts decreased, as the extent of hepatic fibrosis increased, whereas mean APRI did not increase until F3 and F4 fibrosis were reached. Regarding bilirubin values in HCV patients; our results agreed with [40]; they found that the higher grade of fibrosis was associated with higher levels of direct bilirubin with HCV infection. Lipoprotein estimation in HCV patients was of beneficial importance because the HCV assembly and secretion pathway are closely related to the production and secretion of lipoproteins, and the infectivity of HCV particles is highly dependent on the interaction of lipoproteins. Furthermore, the entry of HCV into hepatocytes is strongly influenced by lipoproteins. Therefore, apolipoprotein monitoring may be beneficial in understanding abnormal lipoprotein metabolism in chronic HCV infection[41]. In our study, there was a highly statistically significant correlation between the Fibro test with all parameters except HbA1C and Apolipoprotein A1 (p<0.05). In agreement with our findings, in Caucasian populations, FT was validated with chronic hepatitis C mainly and the results showed a good correlation with the stage of liver fibrosis[42,43]. Multivariate analysis revealed that the significant predictors of early fibrosis in HCV were AST, AST/ALT ratio, APRI, Platelets, α2 macroglobulin, Apolipoprotein B (ApoB), Serum Amyloid A (SAA), and Insulin-like growth factor 1 (IGF1) with p-value (p<0.05).In agreement with these results [44]. reported that the sensitivity and specificity of APRI were greater than 80% for predicting advanced fibrosis and cirrhosis in chronic hepatitis C patients . Different findings were achieved by[45]; they found that the most informative markers of liver fibrosis in hepatitis C patients were: alpha2 macroglobulin, alpha2 globulin (or haptoglobin), gamma globulin, apolipoprotein A1, gamma glutamyltranspeptidase, and total bilirubin. Receiver operator characteristics (ROC) curves of early fibrosis prediction biomarkers in the HCV group showed that Apolipoprotein B (ApoB) was the most predictable of early fibrosis, with a p-value <0.001, denoted by the significantly large area under the curves (AUCs) (0.993), sensitivity (94%) and specificity (100%) followed by (IGF1), Lipoprotein A and (SAA). Early prediction of HCV by (ApoB) in HCV patients in our study recorded the best diagnostic performance among the other studied biomarkers. This could be explained by the close interaction between HCV replication and lipoprotein production in the liver due to serum apo-B levels, a strong correlation with LDL cholesterol determined in part by amino acid changes in core proteins and NS5A[41]. Moreover, a recent study showed that the ApoB polymorphism rs1042034 was significantly correlated with the HCV infection status[46]. IGF1 was also one of the best predictors of HCV hepatic fibrosis. In agreement with these results [47]. recorded that low IGF-1 levels were associated with advanced stages of fibrosis and contributed to the progression of hepatic fibrosis in chronic hepatitis C. While, the gradual increase in HCV viral load corresponds to a decrease in circulating IGF1 levels, but did not reach statistical significance in the study of[48].

Regarding the HBV+HCV group, results of our study showed the highest value of AST (50.88±20.21), AST/ALT ratio (2.52±1.21), APRI (0.83±0.43), total bilirubin (0.62±0.33), α2 macroglobulin (3.21±0.87), Apolipoprotein B (0.92±0.17), SAA (8.62±3.96). In addition to recording the lowest value of HbA1C (5.26±0.12), Lipoprotein A (10.60±2.34), and IGF1 (80.96±21.31). Significant differences are known to be observed between HCV-positive patients and (HBV) -

positive patients, not only in etiology but also in relation to many other clinical parameters, including the natural course of the disease, laboratory parameters, and histology. However, the number of fibrosis marker reports in HBV-positive patients is much lower than in HCV-positive patients. In particular, little has been reported on the relationship between metabolic parameters and the histological grade of liver fibrosis in HBV-positive patients [49]. Regarding the value of HbA1C recorded in our patients; the results agreed with[49], this is explained by the fact that, in patients with chronic liver disease, hypersplenism shortens the lifespan of red blood cells, resulting in lower HbA1c levels relative to the level of blood glucose[49]. Reporting the lowest value of IGF1 in combined HBV and HCV infection was registered already in chronic liver disease patients as a result of a significant increase in plasma GH levels and a decrease in hepatic response to GH[50,51]. The results of the current study showed the highest value of fibro test (0.57±0.21), besides, the highest value of advanced fibrosis (54%). Also, there was a highly statistically significant correlation between the Fibro test with all parameters except age, total bilirubin, and Apolipoprotein A1 ($p<0.05$). This agreed with[52]; multivariate analysis confirmed the association between F3-F4 fibrosis and HCV infection in HBV-infected patients. Multivariate analysis revealed that the significant predictors of early fibrosis in HBV+HCV were AST, AST/ALT ratio, APRI, GGT, Platelets, Total bilirubin, α2 macroglobulin, Haptoglobin, (ApoB), Lipoprotein A, (SAA) and (IGF1), with p-value ($p<0.05$ S). While traditional blood tests, such as (ALT) levels, are useful as a measure of disease activity, they alone have been shown to be poor indicators of liver fibrosis[53]. Our results demonstrated the most significant liver fibrosis biomarkers in HBV+HCV patients; in agreement with our results [30]. investigated the relationship between five noninvasive models [AST/ALT ratio (AAR), aspartate aminotransferase to platelet ratio index (APRI), Bonacini cirrhosis discriminant score (CDS), age-platelet index (APind), and King's score] and the degree of hepatic fibrosis as determined by biopsy in patients with chronic hepatitis B and C. Four of the five noninvasive methods evaluated in this study can be used to predict advanced fibrosis in patients with hepatitis B and C.In contrast [54] of HBV/HCV co-infection showed that none of ALT and AST parameters were predictors of severe fibrosis.α2- macroglobulin also proved to be valuable as an early fibrosis predictor in HBV/HCV co-infection. Multiple studies have shown an increase in the level of α2-macroglobulin in the serum of patients with fibrosis or cirrhosis compared to healthy volunteers. α2- macroglobulin had been involved in many models as a parameter of noninvasive diagnostic fibrosis in chronic HBV[55], and chronic HCV[56], If the liver is inflamed or damaged, the increase in α2-macroglobulin inhibits the catabolism of matrix proteins and thus causes liver fibrosis[57]. Receiver operator characteristics (ROC) curves of early fibrosis prediction biomarkers in the HBV+HCV group showed that (ApoB) was the most predictable of early fibrosis, with p-value <0.001, denoted by the significantly large area under the curves (AUCs) (1.000), sensitivity (100%) and specificity (100%) followed by (IGF1), Lipoprotein A and (SAA). Viral hepatitis is a common cause of hepatic dysfunction, and numerous studies have demonstrated evident changes in serum lipids, lipoprotein, and apolipoprotein patterns in patients infected with either HBV or HCV [58]. And as reported in a previous study, HBV infection plays an inhibitory effect on apoB expression [59]. In addition, others have demonstrated that HCV nonstructural proteins, such as nonstructural protein 5A, inhibit ApoB secretion [60]. HBV+HCV co-infection seems important because HCV and HBV infection can have different effects on fibrosis progression and the presence of related markers.Regarding (NAFLD) group, results of the present study showed the highest value of ALT (35.56±23.89), GGT (59.80±42.54), HbA1C (8.22±1.33), (ApoB) (0.94±0.22), in addition to the lowest value of haptoglobin (1.10±0.64) also, (IGF1) (97.28±34.83).These results are in agreement with previous studies[61,62] they found that AST, ALT, GGT, triglycerides, glucose, insulin, and the homeostasis model assessment of insulin resistance were either significantly higher or showed a trend toward higher levels in NAFLD patients compared with healthy controls. Also, [63]. found that NAFL group had higher ApoB, ALT, and AST levels than the control group. We also demonstrated lower values of haptoglobin in NAFLD patients; also, NAFLD patients in [64]. The study had a much lower frequency of haptoglobin 1-1 genotype. According to[65] haptoglobin contributed to NAFLD progress. ALT enzyme reported the highest values among all studied patient groups. Some other research has suggested that the ALT enzyme is the best single marker for detecting fatty infiltration in the liver[66]. In contrast, a study conducted in untreated patients to examine the association between changes in liver enzymes and histological changes in the liver in NAFLD showed the lack of a clear association between the pattern of ALT levels and the changes in steatosis, inflammation, swelling of the hepatocytes, or the degree of fibrosis over time. The results of this study also showed that liver enzyme levels were an insensitive tool for tracking histological changes in the liver in NAFLD patients [67]. Histologically, NAFLD is characterized by excessive accumulation of liver fat in the absence of alcohol consumption [68]. VLDL particles produced by NAFLD patients may contain more triglycerides and may be larger than those produced by normal subjects. Each VLDL particle contains one molecule of apoB100,

necessary for the export of VLDL from the liver. The mechanisms responsible for the inappropriate export of hepatic VLDL triglycerides in patients with NAFLD are unknown [69]. Thus, it is important to monitor apoB levels and associations in our NAFLD population. Elevated serum apoB levels independently indicate an increased risk of incident NAFLD [70]. Genetic defects in apoB genes can cause NAFLD, as in familial hypobetalipoproteinemia [71]. Our results showed that the lowest serum IGF-1 levels in NAFLD patients; our results agreed with [72], where the mean serum IGF-1 was lower in subjects with a higher fibrosis stage. The current study results showed a highly statistically significant correlation between the Fibro test with all parameters except ALT and Apolipoprotein A1 ($p<0.05$). The FT has demonstrated high predictive values for advanced fibrosis in patients with NAFLD[73]. Results from this sample of NAFLD patients confirmed the previously observed significant performance for FibroTest[74,75]. Multivariate analysis revealed that the significant predictors of early fibrosis in (NAFLD) were AST, APRI, GGT, Platelets, Total bilirubin, HbA1C, α2 macroglobulin, Haptoglobin, (ApoB), Lipoprotein A, (SAA) and (IGF1), with p-value ($p<0.05$ S).In agreement with our results, (AST) was associated with significant hepatic fibrosis in NAFLD diabetic patients of [76] study. NAFLD is typically characterized by a hepatocellular pattern of liver-related enzymes with slight increases (12 times the upper limit of normal) in serum (ALT) and (AST) [77]. Despite the disease, up to 50% of NAFLD patients may have normal ALT and AST levels [78]. Therefore, various biomarkers have been suggested to aid diagnosis.IGF1 was one of the successful early predictors of fibrosis in NAFLD. IGF1 levels are down-regulated in NAFLD patients compared to healthy controls in the study of [79] , suggesting that IGF1 could be used as a potential biomarker and therapeutic target for NAFLD which agreed with our results. The direct effect of IGF1 on fatty acid metabolism in the liver is unlikely, as there are very few hepatic IGF1 receptors, but this possibility cannot be ruled out. Lower IGF1 levels in NAFLD may simply reflect decreased synthesis in the presence of liver disease. Low levels of IGF1 have been associated with fibrotic stages of nonalcoholic steatohepatitis (NASH) and have also been observed in patients with other causes of liver fibrosis [80]. ApoB was also one of the considered biomarkers in NAFLD; previous reports examined the diagnostic performance of apoB. In univariate analysis of [81] study NAFLD was associated with a higher prevalence of each apoB dyslipoproteinemia vs. subjects with an FLI < 60 ($P < 0.001$), except for low-density lipoprotein (LDL) dyslipoproteinemia. Additionally, each apoB dyslipoproteinemia was independently correlated with NAFLD in age- and sex-adjusted logistic regression analysis, including the apoB dyslipoproteinemias together ($P < 0.001$).Receiver operator characteristics (ROC) curves of early fibrosis prediction biomarkers in (NAFLD) group showed that Lipoprotein A was the most predictable of early fibrosis, with a p-value <0.001, denoted by the significantly large area under the curves (AUCs) (0.980), sensitivity (100%) and specificity (96%) followed by (IGF1) and (SAA). Lipoprotein metabolism is an integral part of hepatocellular lipid homeostasis and is involved in the pathogenesis, possible diagnosis, and treatment of NAFLD[82]. Insulin resistance may be the mediator of the relationship between Lp (a) and fatty liver disease[83]. A small number of studies have examined the relationship between the serum concentration of Lp (a) and NAFLD, but the results are conflicting. In logistic regression analysis after adjusting for various risk factors, the relationship between Lp (a) concentrations and the presence of NAFLD remained significant in[84]. study. Lp (a) concentrations decreased with the severity of NAFLD and the prevalence of NAFLD decreased with the tertiles of Lp (a). [85]. reported that NAFLD patients had a lower Lp (a) than the general population. However, after adjusting for various risk factors, the results were only found in men.Our results showed that the value of IGF1 is a recommended biomarker in NAFLD fibrosis. Other studies also take this into consideration. [86]. found that IGF-1 was lower with advanced fibrosis. They also found that the accuracy of serum IGF1 alone in distinguishing weak/mild fibrosis from advanced fibrosis further improves to 93% when IGF1 is combined with ferritin and INR. IGF1 in patients with moderate to severe fibrosis was significantly reduced, demonstrating that IGF1 can help distinguish advanced fibrosis in patients with NAFLD according to[87]. SAA1 may as a potential target for improving NAFLD. High-fat diet-induced hepatic SAA1 over-expression exacerbates steatohepatitis by promoting platelet aggregation in the liver[88]. Regarding the control group, results of the present study showed the lowest value of ALT (23.96±16.88), AST (24.58±16.89), AST/ALT (1.05±0.15), APRI (0.29±0.20), GGT (26.42±13.90), total bilirubin (0.43±0.22), α2 macroglobulin (1.77±0.47), platelets (214.18±5.46), haptoglobin (1.66±0.68), (ApoB) (0.56±0.01), Lipoprotein A (14.60±1.97), IGF1 (158.64±23.10), and SAA (3.70±0.45). Meanwhile, the lowest value of Fibro test was found in the control group (0.13±0.05), also the lowest value of fibro grades (0%). These results were expected due to the absence of liver damage and fibrosis found in the other studied patients' groups. Regardless of the etiology, prolonged inflammation leads to chronic liver damage, fibrosis, and often cirrhosis. Persistent liver damage leads to hepatocellular damage and excessive collagen deposition by activated hepatic stellate cells (HSCs). HSCs play the most important role in liver fibrogenesis and are the target of interest in future therapeutic modalities to prevent or reverse advanced liver fibrosis. As soon as the activated HSCs

release chemokines, the affected regions of the liver parenchyma undergo an unregulated deposition of the extracellular matrix, which leads to the development of fibrosis. Most clinicians associate both acute chronic liver disease and abnormal liver function tests. However, there are very few published data comparing changes in laboratory values and stages of fibrosis [89]. The pattern of liver enzyme changes is often the first piece of evidence to come to the attention. This is because common causes of liver disease show typical patterns. However, sometimes the details of the pattern are not fully explored[90].Regarding all patient groups, there was a highly statistically significant difference between groups regarding (ApoB), Lipoprotein A, (SAA) and (IGF1), fibro test, and fibro grades with p-value ($p<0.001$). There was a highly statistically significant correlation between the Fibro test with all parameters except HbA1C in all patient groups ($p<0.05$).On the basis of the available evidence, the fibro test was proven to be a perfect tool in our patients' population. Fibro Tests can be performed with comparable diagnostic accuracy for the noninvasive staging of liver fibrosis due to different etiologies. In a study in patients with chronic hepatitis C, fibro test showed a prognostic value similar to that of a liver biopsy for 5 years[91]. Furthermore, the fibro test was shown to accurately define the 4-year prognosis in patients with hepatitis B[92]. Receiver operator characteristics (ROC) curves of early fibrosis prediction biomarkers in all patient groups showed that (ApoB) the most predictable of early fibrosis, with p-value <0.001, denoted by the significantly large area under the curves (AUCs) (0.980), sensitivity (97.3%) and specificity (100%) followed by (IGF1), (SAA) and Lipoprotein A. ApoB In vitro data from cell culture systems have shown that the amount of ApoB protein synthesized by hepatocytes is much greater than the amount of ApoB that is secreted. This is due to the significant intracellular degradation of ApoB that occurs in hepatocytes. The interaction between ApoB biosynthesis, ER liver homeostasis, inflammatory pathways, and insulin signaling must be closely balanced to maintain a healthy state[93]. Our results had proved ApoB efficacy in HCV and HCV+HBV liver fibrosis early prediction.We are concerned about the relation between IGF1 and the early prediction of liver fibrosis regardless of the cause of liver fibrosis. Fibrosis and liver cirrhosis (end-stage) change the production and metabolism of proteins in the IGF system and suppress the proper functions of the body [94]. IGF1 was negatively correlated with the severity of liver disease in cirrhotic patients. Furthermore, IGF1 could distinguish early from advanced disease in cirrhotic patients. [95]. SAA has been shown to stimulate multiple pro-inflammatory and anti-apoptotic pathways. However, its role in liver damage and fibrogenesis is still unclear. SAA can mediate crosstalk between hepatocytes and HSCs in the injured liver. Future studies should define its target receptor and confirm the possible pro and antifibrogenic effects of SAA and as a prognostic biomarker of liver fibrosis [9]. [96] reported a significant increase in Lp (a) levels that occurred after treatment in patients with chronic active hepatitis. Only patients who responded fully showed a significant increase in Lp (a) values. These results suggested that elevated Lp (a) levels are an expression of improved liver function. Since the liver is the organ that synthesizes Lp (a), the decrease in Lp (a) levels in chronic liver disease can be attributed to the relative decrease in synthesis by a damaged liver. Therefore, it is conceivable that the state of liver cell damage, the various cytokines released during disease, and the hormonal environment influence the metabolic pathway of Lp (a). Plasma levels of Lp (a) are affected early if liver function is impaired, as the half-life of Lp (a) in human plasma is approximately 3.33.9 days[97].When comparing diagnostic accuracy between different studies, the effect of different scoring systems should be taken into account. In the current study, we utilized the Metavir scoring system; this system has the advantage of simplicity, reproducibility, and application to a large number of biopsies. Ishak and METAVIR are nearly identical; but, Ishak is of a wider scale.The degree of the disease is intended to reflect how quickly the disease progresses to the terminal stage. Grades can be viewed as a reference to the severity of the underlying liver disease, with characteristics that vary according to type and pattern of injury. Also, the sample size is important in reaching a convincing conclusion to assess the accuracy of the diagnosis. Previous studies performed analyses based on a relatively small sample size resulted in reducing the conviction of the conclusions.There were some differences between our results and other studies. This can be explained by the differences in the included markers and pathogenesis of liver fibrosis in each liver disease group. In NAFLD, the mechanism of fibrosis occurs due to, fat accumulation in the liver resulting in immune cell infiltration and swelling and eventually liver fibrosis[98]. In HCV, the fibrosis mechanism was due to the continuous induction of pro-inflammatory cytokine production by liver macrophages in response to infection; then the activation of inactive HSCs for ECM secretion and deposition in the liver, leading to the development of fibrosis[99]. HBV is a complex nature centered on the liver, and the interaction of viral proteins and the immune system is linked to the hepatocyte damage and tissue repair cycle. This repair involves repetitive deposition of the extracellular matrix that leads to advanced cirrhosis over time. The HBV X protein can also affect a certain fibrosis and carcinogenicity during the period [26].The value of age as a marker of fibrosis seems obvious since the progression of fibrosis in patients with HBV and HCV

is time-dependent. The hepatitis C virus slowly destroys the liver, with a median incidence of 20 years[100].
The major strength of current study was the use of more than one non-invasive biomarker in four different patient groups with different etiologies of hepatic fibrosis testing their efficacy and performance in the early prediction of liver fibrosis.

VI. **Conclusions:** The estimation of non-invasive biochemical markers can detect liver fibrosis in hepatic patients with significant accuracy. These biomarkers can be used as an alternative to liver biopsy and help with early diagnosis of liver fibrosis.

Recommendations:

Further studies are required with a larger sample sizes, because fibrosis noninvasive biomarkers will be key tools in the cost-effective management of hepatic fibrosis and the application of antifibrotic drugs.

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