Evaluation of Noninvasive Biomarkers in Staging of Hepatitis B-Related Fibrosis

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ABSTRACT:

BACKGROUND:

Infection with hepatitis B virus (HBV) is a public health problem worldwide; it is the main cause of fibrosis, cirrhosis and hepatocellular carcinoma (HCC). Early detection of significant fibrosis is essential for reaching antiviral therapy decisions. Considering the limitations of liver biopsy, noninvasive methods to identify significant fibrosis in chronically HBV infected patients are needed in clinical practice.

OBJECTIVE:

To evaluate the performance of aspartate aminotransferase (AST) to platelet ratio index (APRI) and fibrosis index based on four factors (FIB-4) as simple noninvasive markers for staging of liver fibrosis in chronic hepatitis B in comparison with liver biopsy and to compare between the diagnostic performance of APRI and FIB-4 in staging of liver fibrosis.

PATIENTS AND METHODS:

A combined retrospective (38 case) and prospective (2 cases) study of the records of 40 patients with chronic hepatitis B (CHB) who attended the Gastroenterology and Hepatology Teaching Hospital in Baghdad during the period from (January 2016 - July 2019). All patients had a percutaneous liver biopsy for staging of liver fibrosis. The cases were divided into two groups, non significant fibrosis and significant liver fibrosis, according to histopathology stage of fibrosis.

Serum AST, alanine aminotransferase (ALT) levels and platelet counts were obtained from the results of blood samples taken on the same day of liver biopsy. Calculation of APRI and FIB4 indices was done for each pateint of the study .

RESULTS:

the diagnostic performance of both indices in significant and non significant fibrosis was determined by Area Under Receiver Operating Curve (AUROC) .The value of AUROC for APRI index was 0.992, ,P=0.0001, specificity 100%, sensitivity 95.8% at cut-off value 0.41 and the value of AUROC for FIB4 index was 0.997, P=0.0001, specificity 100%, sensitivity 95.7% at cut value 0.73.

CONCLUSION:

Both indices APRI and FIB-4 show good performance with high sensitivity and specificity as simple noninvasive markers for staging of liver fibrosis in CHB.

KEYWORDS: CHB, FIB4, APRI**,** liver fibrosis.

INTRODUCTION:

Infection with hepatitis B virus (HBV) is a public health problem worldwide, two hundred fifty seven million people estimated to experience persistent HBV infection and it is the main cause of cirrhosis and hepatocellular carcinoma (HCC) [1,2].

Cirrhosis develops following long periods of chronic liver disease and is characterized by a decrease in hepatocyte proliferation, indicating an exhaustion of the regenerative capacity of the liver.[3] This is associated with an increase in fibrous tissue and a destruction of liver cells, which provides the environment for development of cancerous nodules[4]. Because liver cirrhosis can have a significant impact on liver reserve and is often an integral part of the morbidity and mortality associated with HCC, the presence and severity of cirrhosis must be defined in all patients in order to assess prognosis and make treatment recommendations.[5]

Liver biopsy is the gold standard for the detection of liver fibrosis, but has limitations such as invasive procedure, high cost, risk of rare but potentially life-threatening complications[5], pain, hypotension, biliary injuries and intraperitoneal bleedings and a number of drawbacks, as false positive or negative results and inter- and intraobserver variability being the most important[6].

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Serum fibrosis model based on routine laboratory tests might be a noninvasive method for the detection of liver fibrosis. Among them, aspartate transaminase (AST)-to-platelet ratio index (APRI) and fibrosis index based on four factors [FIB-4=(Age x AST) / (platelet count x √ALT)],could be a cheap and simple way to assess liver fibrosis.[7,8]

PATIENTS AND METHODS:

This cross sectional combined retrospective (58 cases) and prospective (2 cases) study of medical records of patients with chronic hepatitis B who attended the Gastroenterology and Hepatology Teaching Hospital during the period from (January 2016 - July 2019).

Only 40 of them (35 male and 5 females, mean age 33±8 years) were included in the study with inclusion criteria: HBsAg- positive for more than 6 month**,** not receive antiviral therapy and exclusion criteria: Hepatitis C virus infection**,** human immune deficiency virus infection, hepatitis E, A virus infection, superinfection or coinfection with hepatitis D virus, alcoholic hepatitis**,** autoimmune hepatitis, hepatocellular carcinoma, alcoholic steatosis, pregnancy, current immunosuppressive drug and liver transplantation.

The patients were classified according to histopathological Ishak staging system from 0-V stage, (20 cases were excluded including Ishak stage VI because incomplete informations of their records) ,also the cases were classified according to histopathology findings into presence and absence of fatty changes.

Blood analysis for liver enzymes (serum AST and ALT) and platelet count at the same day of biopsy, were included in the study.

The biochemical parameters (AST and ALT) were done using (Siemens Dimension X Pand plus integrated chemistry system). Blood counter (celltac α MEK 6510) was used for platelet count

Liver biopsy: Specimens were obtained by a percutaneous needle puncture under ultrasonography guidance. Core biopsies are 1 - 3 cm by 1 - 2 mm, preserved in 10% buffered formaline, processed and stained with H &E, then examined. Special stain for staging of fibrosis (trichrom or Van Gieson stain) was used. Staging done by Ishak staging system.

RESULTS:

Table 1 shows forty cases were included in the study, 16(40%) of cases were with fatty changes and 24(60%) cases no fatty changes, for purpose of comparison with other studies, they were divided into two groups[9], a fibrosis score of F0- F1Metavir staging (equivalent to Ishak stage 0=3cases, I=8 cases, II=6 cases) means that no significant fibrosis is present (which was 42.5% of cases), while a score of greater than or equal to F2 Metavir (Ishak III=14 cases, IV=6 cases, V=3 cases) indicates significant fibrosis (which was 57.5% of cases).

Table 1: Baseline characteristics of the study population.

Characteristics		Number	%	
Age (years)	<30y	16	40.0	
	30-39	12	30.0	
	≥40y	12	30.0	
Fatty changes	Yes	16	40.0	
	No	24	60.0	
Liver Fibrosis Stage Ishak staging	Stage 0	3	7.5	F0-F1 metavir
	Stage I	8	20.0	
	Stage II	6	15.0	
	Stage III	14	35.0	F2-F3 metavir
	Stage IV	6	15.0	
	Stage V	3	7.5	
liver Fibrosis Stage	Significant fibrosis	23	57.5	
	Non-significant fibrosis	17	42.5	

The mean± SD of platelet, AST, ALT, APRI and FIB4 were [235±45.7 U\L], [39±14 U\L], [43.8±9 U\L], [0.5±0.26], [0.87±0.47] respectively (table 2)

Table 2: Mean±SD of platelets, ALT, AST, APRI, FIB4 scores) of patients.

	Mean±SD
Platelets (x10^9)	235±45.7
ALT (U/L)	43.8±9
AST (U/L)	39±14
APRI	0.5±0.26
FIB4	0.87±0.47

No significant difference was found in the mean level of platelet count, ALT, AST, APRI, FIB4 in the presence or absence of fatty changes with P value of >0.05 (table 3)

Table 3: Mean± 1SD and P value for measured parameters in the presence and absence of fatty changes.

	Fatty changes (n=16)	No fatty changes (n=24)	P value
Platelet count (x10^9)	247±41.7	227±47.2	>0.05
ALT (U/L)	42±9.7	44.9±8.8	>0.05
AST (U/L)	35.38±12.3	41.21±14.4	>0.05
APRI	0.42±0.19	0.55±0.29	>0.05
FIB4	0.76±0.4	0.95±0.5	>0.05
-Data were presented as Mean±SD (Range)			
*Significant difference between two independent means using Students-t-test at P=0.05 level			

The results show significant difference in level of platelet, AST, APRI and FIB4 between significant and non significant fibrosis with P value of 0.0001 for each, while no significant difference in ALT level between both groups was seen, P value 0.8. (table 4)

Table 4: Mean±SD and P value of blood biomarkers in significant and non significant liver fibrosis.

	Liver Fibrosis Stage		P value
	Significant fibrosis (n=23)	Non-significant fibrosis (n=17)	
Platelets (x10^9)	201±24.3	281.8±16.7	0.0001*
ALT (U/L)	44±8.9	43.4±9.7	0.8
AST (U/L)	47.74±10.5	26.88±6.8	0.0001*
APRI	0.66±0.23	0.27±0.07	0.0001*
FIB4	1.19±0.38	0.45±0.09	0.0001*
-Data were presented as Mean±SD (Range)			
*Significant difference between two independent means using Students-t-test at 0.05 level			

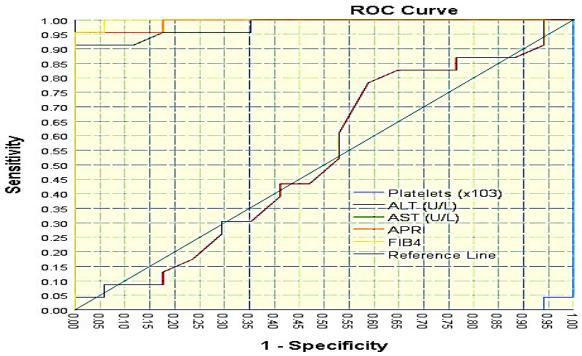

Figure 1: Receiver Operating Curve of blood biomarkers .

Table 5: Area Under Receiver Operating Curve of biomarkers for liver fibrosis.

Test Result Variable(s)	Area	Std. Error	P value	95% Confidence Interval	
				Lower Bound	Upper Bound
Platelets ($x10^3$)	0.99	0.004	0.0001*	0.98	1.00
ALT (U/L)	0.52	0.097	0.784	0.33	0.71
AST (U/L)	0.97	0.019	0.0001*	0.94	1.00
APRI	0.99	0.009	0.0001*	0.97	1.00
FIB4	0.99	0.004	0.0001*	0.98	1.00

The best cut-off values with highest specificity and sensitivity for determination of significant fibrosis for platelet ALT, AST, APRI, FIB4 were ($253x10^{9,}$ 100%, 94%), (39U\L, 41%, 78%), (38 U\L, 100%, 91%), (0.41, 100%, 95.8%) and (0.73, 100%, 95.7%) respectively. Below these cut off values non significant liver fibrosis is considered.

Table 6: The performance of biomarkers with best cut-off value (blue color).

Cut-off value	Sensitivity	Specificity
Platelets ($x10^9$)		
240	100.0	95.7
250	94.1	95.7
253	94.1	100.0
ALT (U/L)		
39.5	78.3	41.2
40.5	60.9	47.1
41.5	52.2	47.1
AST (U/L)		
29.5	100.0	64.7
36.5	91.3	88.2
38.5	91.3	100.0
APRI		
0.36	100.0	82.4
0.39	95.7	94.1
0.41	95.8	100.0
FIB4		
0.61	100.0	94.1
0.63	95.7	94.1
0.73	95.7	100.0

DISCUSSION:

In this study, the prevalence of fatty change was 40%, no significant difference in the mean of ALT, AST, platelet count, APRI, FIB4 score was seen in the presence and absence of fatty changes, which is in agreement with a study done by Baclig, et al.[10], who found the prevalence was 41%. While in a study done by Peleg, et al [11], the prevalence of steatosis was 46%, only platelet count show difference between two groups, no significant difference in AST, ALT, APRI , FIB4 mean level.

The mechanisms underlying the association between steatosis and HBV replication are not clear; few studies have suggested that fat deposition in HBV-infected hepatocytes may reduce HBV replication directly or by inducing hepatocyte apoptosis [12]. Others suggest that HBV induce fatty liver by affecting mitochondria and release of ROS. It might be considered as metabolic disease related to obesity, insulin resistance, and dyslipidemia as incidental finding not related to CHB infection.

The results (for significant fibrosis *vs.* non significant fibrosis respectively) were in agreement with that of Qiang ,et al.[13] with higher sensitivity and specificity in predicting fibrosis than that of Qiang , et al. In comparison with Tunisian study by Ayed , et al.[14]APRI index was significantly higher in patients with SF (1.1 ± 0.7 vs. 0.48 ± 0.26; $P < 0.001$).

APRI predicted accurately SF with an AUROC of 0.7 (CI 95% 0.62–0.77; P <0.001).
At a threshold of 0.5, APRI had a sensitivity of 62%, a specificity of 68%, which was lower than the present study. In Iraqi study done by Raghad J. Al-Akayshee [15] ,that show lower results than the present study, for APRI score the cut-off value for SF was (0.28), AUROC (0.59), sensitivity (53%), specificity(67%), P (0.35), while the values for FIB4 were (0.8, 0.54, 53%, 67%, 0.67 respectively).
The differences in results of this study and other studies may be related to sample size and number of patients in each stage, as increase in percentage of cases of SF to that of non SF will affect AUROC results and leads to increase the sensitivity and specificity of APRI and FIB-4 index Another cause is related to the differences in level of ALT, AST, platelet count (on which the indices are dependent) which might be due to difference in method and instruments used for measurement and difference in ULN and the duration of the disease. As fibrosis progresses, ALT activities typically decline, AST is often higher than ALT this might be due to release of mitochondrial AST with more severe damage and the ratio of AST to ALT gradually increases [16]
Low platelet count is a good indicator of advancing liver fibrosis because Platelet production can be decreased due to depressed thrombopoietin (TPO) production by fibrotic liver and direct bone marrow suppression.[17]
Combination of these markers in a form of APRI and FIB4 show a higher sensitivity and specificity than each marker alone

CONCLUSION:

Both indices AST to platelet ratio index (APRI) and fibrosis index based on four factors (FIB-4) show good performance as simple noninvasive markers for staging of liver fibrosis with sensitivity of (95.8%, 95.7%) and specificity of (100%, 100%) respectively .Above a cut-off value of 0.4 for APRI index and 0.7 for FIB4 index a significant liver fibrosis should be considered in chronic HBV infection.

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