SHASTA can determine the liver fibrosis Stages in Viral Hepatitis B Patients

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Abstract

Background: Chronic liver diseases may cause inflammation and progressive scarring, over time leading to irreversible hepatic damage (cirrhosis). Current study correlated between new noninvasive markers levels and the stages of liver fibrosis.

Methods: Ten ml blood samples were drawn from a total of 75 healthy volunteers and 75 patients with chronic hepatitis B virus (HBV) were enrolled according to the METAVIR classification. Hyaluronic Acid level was measured by commercially available ELISA kits. Serum HBV DNA was quantified by using RT-PCR assay. For non-invasive assessment of liver fibrosis we used specific scoring systems (APRI, FIB-4, NAFLD and SHASTA).

Results: The current study indicated SHASTA including HA biomarker and another liver fibrosis scoring systems significantly distinguished fibrosis patients from non-fibrosis group. These markers results discriminate early F1/F2 from F3/F4 ($p<0.001$).

Conclusions: We expect that combination of this novel biomarker and fibrosis scoring system could be applied clinically to predict the stages of liver fibrosis without the need of liver biopsy.

Keywords: Noninvasive Biomarkers; Hepatitis B; Liver fibrosis; METAVIR; Fibrosis Scoring Systems

Introduction: The clinical need for accurate, noninvasive alternatives to liver biopsy was driven by the growing burden of chronic liver disease worldwide. Unlike other major causes of mortality, rates of chronic liver disease are increasing rather than declining. [1]Chronic liver disease results from a wide range of etiological factors including hepatitis B virus (HBV). Histological activity of liver disease and stage of fibrosis are important predictors of disease progression and treatment outcome. Their precise verification is an obligate prerequisite before initiation of antiviral therapy[2].

Liver biopsy is considered the gold standard for assessing the grade of liver injury and stage of liver fibrosis in patients with chronic hepatitis. Liver biopsy, as a clinical tool, has some major limitations**.** Most famous limitations of liver biopsy are: fibrosis staging systems (with this assumption that there is a linear increase in the severity of fibrosis between stages) [3]**.** The METAVIR scoring system was designed specifically for patients with hepatitis B using a sum of experience-based opinions of 10 pathologists augmented by subsequent stepwise discriminated analysis[4]. While numerous researchers use Ishak system to assess liver histology in chronic hepatitis studies[5, 6], other researchers mostly from Europe prefer the METAVIR system[7].

Serum markers of liver fibrosis offer an attractive, cost effective alternative to liver biopsy for both patients and clinicians. Moreover, measurements may be performed repeatedly, thus, allowing for a dynamic monitoring of fibrosis[8].

HA is a high-molecular-weight glycosaminoglycan, which is an essential component of extracellular matrix in virtually every tissue in the body[9]. Currently, it has been introduced as one of the best available markers of hepatic fibrogenesis in chronic viral hepatitis[10].

The most frequently included indirect markers are platelets count, ALT, AST, ALB, Fasting blood glucose, the ratio index of AST to platelets (APRI), NAFLD, FIB-4 and SHASTA. Until now, the accuracy of these indirect markers is controversial[11]. Moreover, a major limitation of all these non-invasive liver tests is the absence of uniformly established and validated cut-offs for fibrosis stages[12]. The rate of adoption of different direct and indirect non-invasive biomarkers in prediction of liver fibrosis differs from country to country, but remains limited[13].

We aim to assess the efficiency and the performance of a panel of non-invasive marker including (Hyaluronic acid) and four indirect markers, APRI, NAFLD, FIB-4 and SHASTA to predict fibrosis stage in our patients with chronic HBV.

Serum Samples of patients: Ten ml blood samples were drawn by vacutainer needle after an overnight fast from totally 150 blood samples from 75 patients of chronic HBV infection and 75 healthy volunteers were obtained from Outer Clinics of Mansoura University hospitals in Mansoura, Egypt. These Ten ml blood was divided into one ml for platelet count test and nine ml blood was centrifuged and serum separated for another tests. The retrospective analysis covered the period between November 2016 and July 2017 within the laboratories of Mansoura University hospital.

Liver biopsy: Method was implemented for Liver Histopathological staging (F); Liver biopsy was obtained applying Menghini's technique aspirating needle set. Biopsies were examined and scored by an expert histopathologist, who was blinded to patient clinical characteristics and serum measurements. The stages were determined according to METAVIR classification.

Routine lab methods were used for the testing including platelets, as well as for serum levels of AST, ALT, albumin and fasting blood glucose.

Serum HBV RNA was quantified by using real-time PCR assay (Roche Diagnostics).

Serum fibrosis markers: Serum Hyaluronic Acid Level was measured by commercially available ELISA Sandwich kits.

Non-invasive indexes of liver fibrosis (Fibrosis Scoring Systems)

APRI: *AST-to-Platelet Ratio Index* is calculated as (AST/upper limit of normal range)/platelet count (10^9/L) × 100.

FIB-4: score which combines platelet count, ALT, AST and age,

NAFLD: is a relatively easy to use panel that includes age, hyperglycemia, body mass index, platelet count, albumin, and AST/ALT

SHASTA Index*:* including serum Hyaluronic acid (HA), AST, and albumin

BMI Kg/M^2: weight (in kilograms) over height squared (in meters)

IGF: - WHO criteria: fasting plasma glucose level (6.1: 6.9) mmol/l equal (110:125) mg/dL.

Statistical analysis of the data: Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. **(**Armonk, NY: IBM Corp**).** The

Kolmogorov- Smirnov, Shapiro and D'agstino tests were used to verify the normality of distribution of variables; Comparisons between groups for categorical variables were assessed using **Chi-square test. Mann Whitney test** was used to compare between two groups for abnormally distributed quantitative variables. **Receiver operating characteristic curve (ROC)** was used to determine the diagnostic performance of the markers. Area more than 50% gives acceptable performance and area about 100% is the best performance for the test. Significance of the obtained results was judged at the 5% level.

Results:

Fig. 1: ROC curve showed that the Accuracy of special studied markers tests for discrimination of patients with liver fibrosis vs. control. The areas under the receiver operating characteristic curve (AUROC) HA (0.988), APRI (1.000), SHASTA (1.000), NAFLD (1.000) and FIB-4 (1.000) in diagnosing different stages of fibrosis. the accuracy of HA is (94). In comparison of the AUC of the serum marker panels (APRI, NAFLD, FIB4 and SHASTA) to the AUC of the serum HA level, the AUC of these parameters were calculated and the results are presented in **table (1)**. Thus, the good accuracies of the studied markers were observed.

Fig. 2: ROC curve showed that the Accuracy of special studied markers tests for discrimination of patients with primary stages of liver fibrosis (F1 and F2) vs. advanced liver fibrosis (F3 and F4). Hence, the figure showed that the areas under the receiver operating characteristic curve (AUROC) HA (0.866), APRI (0.844), SHASTA (0.725), NAFLD (0.893*) and FIB-4 (0.880) in diagnosing different stages of fibrosis. The cut off for HA (>123), and the accuracy is (78.79). In comparison of the AUC of the serum marker panels (APRI, NAFLD, FIB4 and SHASTA) to the AUC of the serum HA level, the AUC of these parameters were calculated and the results are presented in **table (2)**.

Table (1): Agreement (sensitivity, specificity and Accuracy) from ROC curve to diagnose liver fibrosis in HBV group from control

	AUC	p	95% C.I		Cut off	Sensitivity	Specificity	PPV	NPV	Accuracy
			LL	UL						

H.A	0.988*	<0.001*	0.976	0.999	>36	100.0	89.33	89.2	100.0	94.33
APRI	1.000*	<0.001*	1.000	1.000	>0.3	100.0	100.0	100.0	100.0	100.0
SHASTA	1.000*	<0.001*	1.000	1.000	>-2.1	100.0	100.0	100.0	100.0	100.0
NAFLD	1.000*	<0.001*	1.000	1.000	>-1.645	100.0	100.0	100.0	100.0	100.0
FIB-4	1.000*	<0.001*	1.000	1.000	>0.9	100.0	100.0	100.0	100.0	100.0

Table (2): Agreement (sensitivity, specificity and Accuracy) from ROC curve to diagnose F3+F4 from F1+F2 in HBV group

	AUC	**p**	**95% C.I**		**Cut off**	**Sensitivity**	**Specificity**	**PPV**	**NPV**	**Accuracy**
			LL	**UL**						
H.A	0.866*	<0.001*	0.783	0.949	>123	90.62	67.65	72.5	88.5	78.79
APRI	0.844*	<0.001*	0.745	0.942	>2.32	87.50	82.35	82.4	87.5	84.85
SHASTA	0.725*	0.002*	0.601	0.849	>1.22	93.75	50.0	63.8	89.5	71.21
NAFLD	0.893*	<0.001*	0.817	0.969	>1.208	78.12	91.18	89.3	81.6	84.85
FIB-4	0.880*	<0.001*	0.797	0.963	>3.92	87.50	82.35	82.4	87.5	84.85

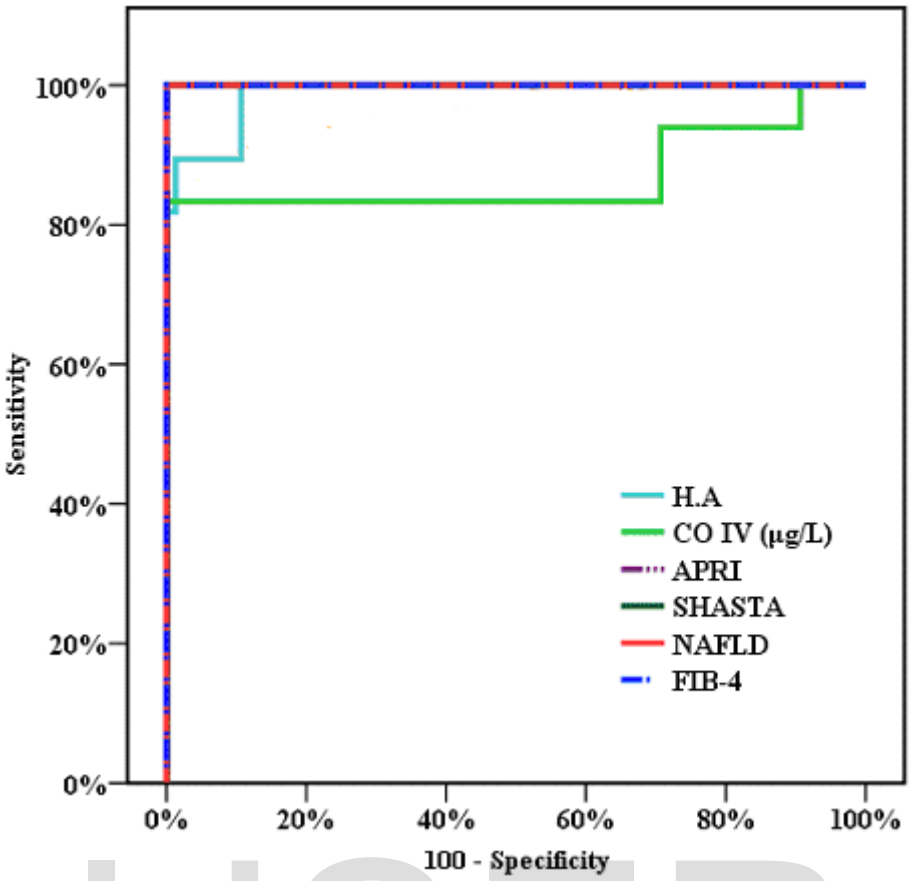

Figure (1): ROC curve to diagnose liver fibrosis in HBV group from control

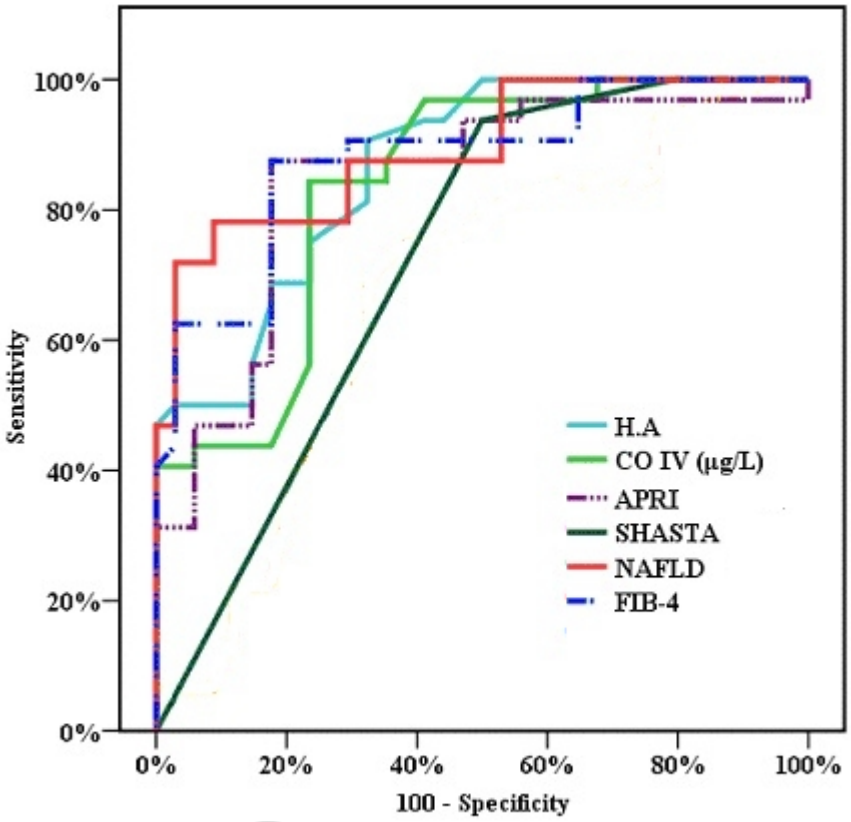

Figure (2): ROC curve to diagnose F3+F4 from F1+F2 in HBV group

Discussion the Patients with significant fibrosis or cirrhosis should be considered for antiviral therapy, which can potentially reverse cirrhosis and reduce complications[14]. APRI, NAFLD, FIB-4 and SHASTA are such noninvasive markers gaining increasing acceptance in clinical practice. These markers may reduce the need for liver biopsy and may help to monitor the efficacy of treatment[15].

The Fib-4 score was subsequently validated for detection of the moninfectious HBV. It showed AUCs of 0.85 and 0.81 for the detection of severe fibrosis, for isolated HBV infection, respectively[16]. Fib-4 showed a better performance in NAFLD compared with the APRI, and NAFLD fibrosis score (NFSA) [17]. These last studies matched our current study.

In the current study, using area under the ROC curve, APRI provided the best accurate results of discrimination ability to exclude patients without fibrosis from those HBV with early fibrosis changes parallel to METAVIR score of at least F0/1(AUC, 0.844) in comparison to the other two non-invasive liver fibrosis tests, HA

AUC is (0.866). In a study of NAFLD-related fibrosis of the liver, HA was found to be the best class I biomarker of fibrosis, being associated with an area under curve (AUC) of 0.97[18]. Furthermore, the negative predictive value of HA is much higher (98-100%) than its positive predictive value (61%), its main utility in its ability to rule out advanced fibrosis and cirrhosis[19].

Several noninvasive methods of detecting advanced fibrosis and cirrhosis in patients with NAFLD have been reviewed recently[20]. The most well studied and validated serum-based model of distinguishing patients with and without advanced fibrosis is the NAFLD fibrosis score[21]. The NAFLD fibrosis score uses 6 variables and was developed in an initial study including 75 patients for purposes. The test performed well with AUROC of 1.000 and 0.893 in the estimation and validation groups, respectively. In the HBV group it could exclude advanced fibrosis with a NPV of 81.6% for patients with fibrosis and diagnose advanced fibrosis with a PPV of 89.3%. Using these cutoffs, a biopsy could be avoided in 89% of patients tested with only a 10% false prediction rate. APRI, FIB-4, and SHASTA have all been studied as well with varying AUROC for detecting advanced fibrosis (0.844-1.000, 0.880-1.000 and 0.725–1.000 respectively). Thus, noninvasive fibrosis scoring systems tests (APRI, SHASTA, NAFLD and FIB-4) are excellent for excluding advanced fibrosis with good accuracy (84.85, 71.21, 84.85 and 84.85 respectively) for excluding or detecting milder forms of fibrosis.

SHASTA index is based on serum Hyaluronic acid, AST, and albumin. In a study of 75 HBV co-infected patients, an index showed a sensitivity of 100.0% and a negative predictive value of 100.0%, to diagnose liver fibrosis from control and showed a sensitivity of 93.75% and a negative predictive value of 89.5% for detection liver fibrosis stages F1+F2 from F3+F4 of patients[22].

Finally, we observed that all serum markers including HA are useful for predicting liver fibrosis in chronic hepatitis patients B compared to the healthy control, But we didn’t observe such a similar pattern for discrimination between patients with mild fibrosis and those with severe fibrosis and only HA performed better at excluding advanced fibrosis than mild fibrosis and patients with liver fibrosis than healthy individuals. Therefore, serum HA, SHASTA, APRI and FIB-4 measurement together with these tests could be used for predicting of liver fibrosis as alternate to liver biopsy, when liver biopsy is damaging.

In summary, SHASTA including HA marker performed as well as NAFLD, APRI, and FIB-4 scoring systems in estimation the stages of liver Fibrosis in HBV patients as a sensitive non-invasive are easy to perform biomarker of liver fibrosis.

ETHICAL APPROVAL: The study protocol was approved by the outer Clinics of Mansoura University hospitals in Mansoura, Egypt.

Availability of data and materials: all materials and data are available and sharing is available.

COMPETING INTERESTS: The authors declare that they have no competing interests.

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CONTRIBUTORSHIP: Authors completed the study protocol and were the main organizer of data collection drafting and revising the manuscript. Tamer Addissouky wrote the article and guarantees the paper. All authors contributed to the discussion and reviewed the manuscript and helped in designing the study and protocol and engaged in a critical discussion of the draft manuscript. All authors agreed on the final version of the manuscript.

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Abbreviations

ECM (extracellular matrix), **CHB** (chronic hepatitis B), **AST** (aspartate aminotransferase), **APRI** (AST to Platelet Ratio Index), **FIB-4** (Fibrosis-4 score), **PLT** (platelets), **ROC** (receiver operating characteristic), **AUC** (area under the curve), **ALT** (alanine aminotransferase), PPV (positive predictive value), NPV (negative predictive value), **BMI** (Body mass index**), NAFLD(** Non-Alcoholic Fatty Liver Disease**), HBV (**hepatitis B virus**), HSC (**hepatic stellate cells**), HA** (Hyaluronic Acid).

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