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Efficacy of Biomarkers in Detecting Fibrosis Levels of Liver Diseases

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Abstract: By far, liver biopsy is regarded as an effective standard to estimate the fibrosis levels which is hurtful. Thereafter, craving for noninvasive biomarkers to determine fibrosis stages is an inevitable to alleviate clinical issues. Hyaluronic acid, collagen IV and COMP levels have been determined in patient's serum. Required samples were drawn from particular cases. Blood samples were collected from 68 healthy individuals as control in addition to 75 infected HCV patients. The biochemical results were compared to METAVIR classification and specific fibrosis scoring systems. The levels of biomarkers have been determined by specific kits. HCV RNA has been quantified in samples by RT-PCR assay. Current study has reported that Hyaluronic acid, COMP and CO-IV tests are significant in discriminating fibrosis patients from healthy individuals. By the results of studied markers, distinguish initial levels of fibrosis from advanced stages becomes available. Conclusions: We propose that the combination of these novel biomarkers: H.A, CO-IV and Comp tests could be implemented clinically to estimate the liver fibrosis stages without making liver biopsy.

Key words: COMP · Hepatitis C · Liver Fibrosis · SHASTA · Biopsy

INTRODUCTION

Biopsy of liver has practical role in estimation and diagnosis of fibrosis levels as well as assessment of another several diseases such as inflammation, steatosis and necrosis. Nevertheless, it is mostly a risk which causes suffering of severe pain and complication after operation which includes mortality in about 0.1% of cases in addition to long stay at hospital under observation in around 5% of patients [1]. Also, for implementation of biopsy, 1-2 pieces of 1 cm tissue as 1/50,000 of the volume of liver might be insufficient in estimation [2]. In advanced stage of live disease, sampling error in detecting cirrhosis is measured up to 20 percent of cases [3]. Most importantly, biopsy is beside its effective role in monitoring the response of patients to treatment and assessment of progression of fibrosis its repletion time is described as risk and impractical technique which leads to more complications to cases [4]. As a consequence for these reasons, looking for alternative techniques particularly noninvasive planning that can be repetitive and effective in assessment of liver fibrosis as well as screening treatment response and detecting early stages of diseases is required.

Liver fibrosis in cases that infected with HCV is a noticeable disease. Inflammation or injury of liver can cause liver fibrosis as a response of repair [5]. An implementation of biopsy for liver is still an effective indicator to evaluate the liver fibrosis [6]. Nevertheless, biopsy has harmful consequences resulting from sampling error and limited characters [7]. Currently, liver transplantation is the only practical solution for cirrhosis treatment, whilst initially estimation of liver fibrosis to be treated will lead to elimination of needing of liver transplantation [8]. The determination of fibrosis levels is unduly considered an efficient factor on treatment of hepatitis C virus patients [9].

By study of consequences of biopsy in several areas has resulted that disputation between pathologists as well as long stay of cases at hospital and increase of treatment cost [10]. Hence, looking for noninvasive alternatives to detect the levels of liver fibrosis is inevitable to be assessable in observation of treatment response and awareness of any harmful frequent of liver disease progression.

The scoring system of METAVIR was projected especially for patients who were co-infected hepatitis C virus using a sum of experience-based suggestions of a

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number of pathologists who boosted by sequent stepwise discriminated analysis [11]. The degree and stage of fibrosis include to couple of classified scores divided into one for necroinflammatory degree and other for fibrosis level (F). The activity of inflammation is defined by A1 to A4 which are described the severity of activity. An assessment of inflammation degree is a valuable in correlation with hepatic fibrosis. This assessment of activity degree is fully relied on integration of necrosis degree by using simple algorithm [12]. Fibrosis of liver is graded into several levels from F0 (healthy) to F4 (progressive liver scare) [13]. The improvement of inter and intra variability of METAVIT is observable [14]. The Metavir is characterised by feasibility, specificity on necroinflammatory lesion and fibrosis. The profitability, attraction and availability of serum markers which are used to determine fibrosis levels are pivotal for clinicians and patients as well. Above all, they are excellent alternatives due to their availability in repetition time which is practical in monitoring of fibrosis dynamically [15].

COMP is an extracellular matrix (ECM) protein primarily present in cartilage and encoded by the COMP gene [16]. It is a non-collagenous protein mostly identifiable in cartilage which includes five arms 435 kD [17]. It is considered a potential biomarker which interacts with collagen and is suggested to have a role in regulating fibril assembly as well as a structural role for maintaining the mature collagen network. HA is known as a glycosaminoglycan which is a vital substance of extracellular matrix found in a highest concentration in fluid such as joint and eyes [18]. Nowadays, it has been performed as an effective biomarker of hepatic fibrogenesis in cases who suffer from chronic viral disease [19]. Collagen type IV is a substance of extracellular matrix that was examined as substitute biomarker liver fibrosis patients [20]. It includes three different sections; a central helix domain, an amino-terminal domain and a carboxy-terminal domain [8]. It has been studied profoundly in several manners of chronic liver diseases [21].

There are specific markers that are extremely used in studies which include, SHASTA (ALB, HA and AST), PLT, fasting blood glucose, ALT and scoring systems such as APRI, FIB-4 and NAFLD. By far, the precision of these noninvasive biomarkers is argumentative [22]. Furthermore, cut-offs for fibrosis levels are ambiguous somewhat in these indirect noninvasive markers due to their main limitation [8]. Both direct and indirect tests have been integrated in patented commercial algorithms which verified the precision of tests diagnosis. Most importantly, noninvasive biomarkers should be evaluated to determine fibrosis stages in liver diseases particularly in era where the various HCV genotypes related to severe liver disease in contrast with other European regions [23]. The rate of endorsement of different indirect markers in evaluation of liver fibrosis is diverse in several countries over the world and bordered [24]. We have projected to appraise the efficiency of alternative non-invasive markers which include Coll-IV, COMP and HA as well as scoring systems: APRI, NAFLD, FIB-4 and SHASTA to detect fibrosis stages in HCV cases.

MATERIALS AND METHODS

Serum Sampling: required amounts of blood samples were drawn by specific needle after an overnight fasting from totally 136 blood samples from 68 infected HCV patients who had been excluded from 191 cases from the hospital of Mansoura University. Meanwhile, ten ml blood samples were taken from 75 healthy volunteers who assessed by ultrasound techniques at the same hospital. These samples were separated and used for their planned purposes. The retrospective analysis covered the period between July 2017 and January 2019 inside the hospital laboratory department.

Liver Biopsy: This operation has been implemented by Menghini's technique aspirating needle set. Expert pathologists examined the tissues of biopsy and then they have determined liver fibrosis stages according to METAVIR classification.

Hematology and Chemistry Tests: these tests included platelets count, AST, ALT, Albumin levels and fasting blood glucose.

Serum HCV RNA: Was detected by Real Time PCR assay.

Serum Markers: Hyaluronic Acid, Comp and Collagen-IV levels have been determined by commercial ELISA kits (HA, aviva, USA, OKEH02527), (COMP, biovendor, Czech, RD194080200), (COLL-IV, alpco, USA, 69-C4SHU-E01).

Fibrosis Scoring Systems

APRI: Is calculated by using platelets count and AST by measuring the ratio between them.

FIB-4: Score which includes platelet count, ALT, AST levels and age.

NAFLD: Is a score system depends on specific equation which includes age, FBS, BMI, PLT count, ALB and ratio between ALT and AST.

SHASTA Index: Is calculated by using panel of HA, AST and ALB.

BMI Kg/M^2: By using equation between height and weight.

IFG: The reports of Nichols *et al.* presented [25] Fasting Blood glucose level from 110 to 125 mg/dL initial impaired fasting glucose.

Statistical Data: Particular software version of IBM SPSS was exploited in insertion of data into computer to be analysed (Armonk, NY: IBM Corp) [26]. Number and percentage were described by qualitative data. The normality of distribution Quantitative data was verified by The Kolmogorov-Smirnov test and helps in description of using range (minimum and maximum), mean, standard deviation and median. Significant results were approved at the 5% level. Comparison between different groups was implemented by using of Chi-square test. Normally distributed quantitative variables were presented by Student t-test which helps in comparison between two studied groups. Normally distributed quantitative variables were performed by using of F-test (ANOVA) which assists in distinguish between more than two groups. Differentiations of abnormally variables were elaborated by using of Mann Whitney test and compared between studied groups. Abnormally variables were presented by using of Kruskal Wallis test which helps in comparison between studied groups.

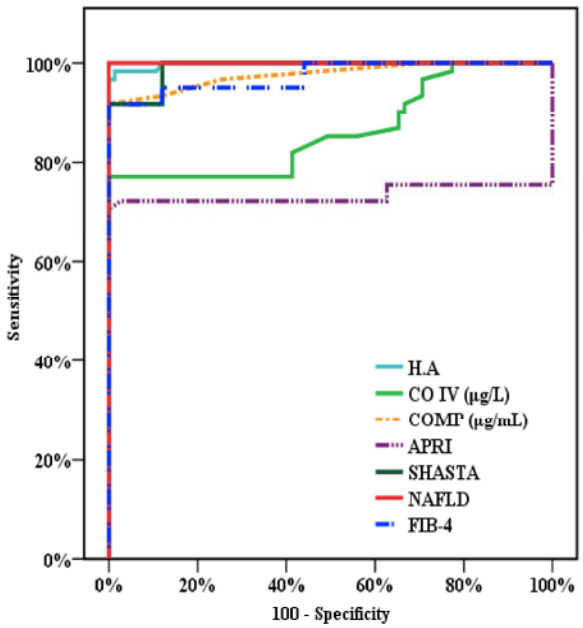

Fig. 1: Discrimination liver fibrosis from control by using ROC curve

RESULTS

The precision of particular studied markers tests is presented by ROC curve which helps to discriminate cases with fibrosis of liver from healthy (Fig. 1). The areas under curve (AUROC) of HA (0.988), CO-IV (0.863), COMP 0.977, APRI 0.733, SHASTA 0.990, NAFLD 1.000 and FIB-4 0.974 are helpful in detection of different stages of fibrosis. The results illustrated cut off for HA, CO-IV, COMP >46, >98.1, >15) respectively and the accuracy is (97.79, 89.71, 96.32) respectively too. The comparisons between the AUC of the calculated studied algorithm (APRI, NAFLD, FIB4 and SHASTA) and the AUC of the studied markers HA, CO-IV and COMP levels are presented and calculated in Table (1). By perceiving of highlights of these reports, the accuracies of these markers are observable.

Table 1: Presentation of sensitivity and specificity and accuracy of the studied biomarkers and scoring systems

			95% C.I							
	AUC	p	LL	UL	Cut off	Sensitivity	Specificity	PPV	NPV	Accuracy
H.A	0.998*	<0.001*	0.994	1.00	>46	96.72	98.67	98.3	97.4	97.79
CO IV (ig/L)	0.863*	<0.001*	0.795	0.932	>98.1	77.05	100.0	100.0	84.3	89.71
COMP (ig/mL)	0.977*	<0.001*	0.953	1.002	>15	91.80	100.0	100.0	93.7	96.32
APRI	0.733*	<0.001*	0.625	0.842	>0.25	72.13	97.33	95.7	81.1	86.03
SHASTA	0.990*	<0.001*	0.980	1.001	>-2.14	91.80	88.0	86.2	93.0	89.71
NAFLD	1.000*	<0.001*	1.000	1.000	>-1.645	100.0	100.0	100.0	100.0	100.0
FIB-4	0.974*	<0.001*	0.949	1.000	>0.9	91.80	100.0	100.0	93.7	96.32

Table 2: Presentation of sensitivity and specificity and accuracy of the studied biomarkers and scoring systems

			95% C.I							
	AUC	p	LL	UL	Cut off	Sensitivity	Specificity	PPV	NPV	Accuracy
H.A	0.957*	<0.001*	0.910	1.00	>161	96.15	85.71	83.3	96.8	90.16
CO IV (ig/L)	0.901*	<0.001*	0.824	0.978	>181	76.92	94.29	90.9	84.6	86.89
COMP (ig/mL)	0.676*	0.019*	0.537	0.816	>27	57.69	74.29	62.5	70.3	67.21
APRI	0.762*	0.001*	0.637	0.886	>1.41	61.54	82.86	72.7	74.4	73.77
SHASTA	0.757*	0.001*	0.637	0.877	>1.22	100.0	51.43	60.5	100.0	72.13
NAFLD	0.901*	<0.001*	0.816	0.986	>1.55	84.62	94.29	91.7	89.2	90.16
FIB-4	0.927*	<0.001*	0.858	0.996	>1.85	96.15	82.86	80.6	96.7	88.52

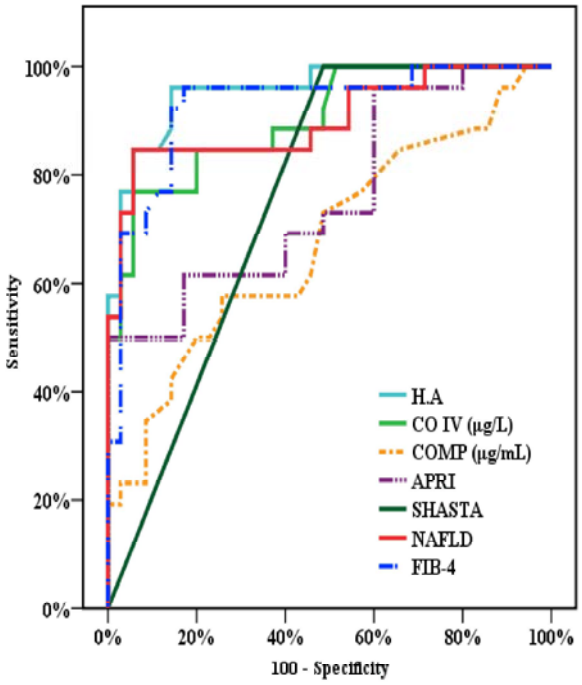

Fig. 2: Differentiation of F3+F4 from F1+F2 by using ROC curve

Fig. 2: The accuracy of specific studied biomarkers tests is performed by ROC curve which helps to exclude initial levels of liver fibrosis first and second stages from progressed liver fibrosis (F3 and F4). Thence, the areas under curve (AUROC) are presented by mentioned figure which describes that HA (0.957), CO-IV (0.901), COMP (0.676), APRI (0.762), SHASTA (0.757), NAFLD (0.901) and FIB-4 (0.927) and these reports helps definitely to discriminate several levels of fibrosis. The results elaborate the cut off for HA, CO-IV, COMP (>161, >181, >27) respectively and the accuracy is (90.16, 86.89, 67.21) respectively too. In comparison of the AUC of the studied calculated systems (APRI, NAFLD, FIB4 and SHASTA) to the AUC of the studied biomarker Hyaluronic Acid. On the other hand, the AUC of CO-IV and COMP were measured and the results are described profoundly in Table (2). By perceiving of highlights of these reports, the low accuracy of COMP test was noticeable in Figure 2.

DISCUSSION

By our study, we have detected that alternative noninvasive biomarkers of extra cellular matrix such as CO-IV, COMP and HA in addition to other indirect scoring systems such as APRI, FIB-4, SHASTA and NAFLD separately and in combine are effective parameters to determine liver fibrosis stages. On the other hand, biopsy of liver is an imperfect technique to assess the levels of hepatic fibrosis particularly severe status in patients who are infected by chronic HCV. As a result, a search for non-invasive assessment of liver fibrosis has emerged in the past few years. Ideally, these tests should be simple, cheap and easy to perform, safe, precise, validated externally and should be capable of differentiating patients in need of therapy and those with poorer prognosis bridging fibrosis and cirrhosis [27].

By observation of the results of our present study, the area under the ROC curve of APRI (AUC 0.733) results has shown the preferable precision which has perfect ability assistance to discriminate and exclude the HCV patients without fibrosis from those in the early stage of fibrosis. These results are relative to METAVIR score in comparison to our objected indirect serum markers of liver fibrosis detection as CO-IV, HA and COMP which their AUC were reported separately (0.863, 0.998 and 0.977) whilst the APRI cut off was 0.25 for HCV patients which showed moderate sensitivity 72.13 with higher specificity 97.33 and predictive values were 95.7 % for PPV and 81.1 % for NPV in HCV cases. These results indicate that the APRI has a moderate ability to prospect and change in hepatic fibrosis.

The sum of sensitivity and specificity of CO-IV is maximized by optimal cut off which is illustrated by the curves value to monitor HCV cases for fibrosis. The cut off was >98.1 ng/ml with a mild sensitivity, specificity,

NPV, PPV and Accuracy of 77.05, 100.0, 100.0, 84.3 and 89.71, respectively. The efficiency of diagnosis was performed by Coll-IV cutoff value of>181 ng/ml to exclude early liver fibrosis levels from advanced levels of fibrosis with sensitivity 76.92 and specificity of 94.29. These results are relatively close to those reported by numerous researchers in another era that presented several cut-off values and made studies on the CO-IV but their working was in other types of pathogenic diseases which enclose NAFLD and HCV patients [28]. Lower cut off value 0.770 ng/ml which was shown by Aida *et al*. [29] assisted to detect severe fibrosis stage F<2 in cases who are infected with HCV and increased to 0.827 ng/ml in case of NAFLD. Further particular researches which should exploit a large sample size are demanded to prove the cut off value and to guide the clinical utility which depends on the several types of pathogenesis and various genotypes of viral hepatitis which are distributed geographically.

In another study, the precision of COMP to diagnose cirrhosis was as good as APRI and FIB-4 index and the COMP results performed AUC 0.884, sensitivity 83.3%, specificity 83.7% and cut-off 11.5U/L.COMP serum levels presented as well as APRI and FIB4 score in evaluation of cirrhosis in CVH patients, prospecting COMP might be as an sensitive predictor and easy biomarker of liver fibrosis performance. Further studies are required in order to prove our results in cases who are HCV infected [30]. Current study presented the accuracy of assistance of COMP in diagnosis of fibrosis which has AUC 0.977, sensitivity 91.80%, specificity 100.00%, cut-off >15 U/L and accuracy 96.32 in ROC curve for diagnosis liver fibrosis from control. However, in ROC curve to diagnose F1+F2 from F3+F4 was AUC 0.676, sensitivity 57.69%, specificity 74.29%, cut-off >27 U/L and accuracy 67.21.

By our current study, we observed that the results of routine laboratory tests which assist to estimate infected patients with HCV and elaborate the relation of ALT level to the stage of fibrosis. Also, their reports affirmed that most patients with persistently normal ALT values have less inflammation and fibrosis. In concordance with our results, reported that AST > 60 IU/l. Seemingly, decreased platelet count is found out that it is the earliest indicator of cirrhosis. Low platelet count (less than 100) is predictive for significant hepatic fibrosis (METAVIR score F2–F4) and cirrhosis [31].

The reports of Guechot *et al*. [32] presented the significance of Hyaluronic acid serum concentrations to prove its correlation of histological degrees of liver fibrosis in untreated cases who are infected with chronic HCV (P <0.001). Furthermore, ROC curves performed the serum HA had greater performance than APRI in diagnosis which provides discrimination of patients with extensive liver fibrosis from those without or mild fibrosis AUC 0.864 vs. AUC 0.691 and P <0.001 or for exclusion of patients with cirrhosis from those without cirrhosis AUC: 0.924 vs. AUC 0.734 and P <0.001. The results of our current study illustrated that HA had sensitivity of 96.72% and specificity 98.67% differentiate patients with liver fibrosis from control whilst sensitivity 96.15% and specificity 85.71% help to discriminate cases who have severe liver fibrosis from mild liver fibrosis.

Recently by researches, several indirect noninvasive markers are reviewed in detection of progressed levels of fibrosis of liver in infected cases with different liver diseases [33]. The NAFLD fibrosis score is presented as the most well and a validated model in discrimination of patients who have advanced fibrosis stages from whom without fibrosis [34]. The NAFLD fibrosis score totally depends on using 6 variables which were used in an early study including 68 patients for implementation of purposes. The NAFLD scoring system has good performance with AUROC 1.000 and 0.901 in the evaluation and validation groups, respectively. In the HCV group, advanced fibrosis could be excluded and diagnosed by a NPV 89.2% for patients with score >1.55 and a PPV 91.7%. Using these cutoffs which approved that a biopsy could be avoided in 91% of patients tested with only a 9% false prediction rate. Various AUROC of APRI 0.733- 0.990, FIB-4 0.927 -0.974 and SHASTA 0.757-0.990 have provided effective indicators in detection of advanced fibrosis as mentioned. Overall, as a result of current study, a good accuracy of noninvasive fibrosis scoring system tests such as APRI 73.77, SHASTA 72.13, NAFLD 90.16 and FIB-4 88.52 provide a significant evidence to consider these algorithm parameters as excellent predictors for exclusion of advanced fibrosis and differentiate between milder forms of fibrosis.

SHASTA index is fully relied on several variables such as serum Hyaluronic acid, AST and albumin. By current study of 68 HCV co-infected patients, this index performed showed a sensitivity of 91.80% and a negative predictive value of 93.0% assist to discriminate liver fibrosis from control as well as showing a sensitivity of 100.0% and a negative predictive value of 100% provide assistance to exclude liver fibrosis stages F1+F2 from F3+F4 of patients.

CONCLUSIONS

Serum markers including H.A, CO-LIV and COMP are regarded as predictive markers to distinguish healthy individuals from chronic liver disease by their pivotal role in fibrosis detection. Therefore, if serum H.A, CO-IV, SHASTA, COMP, APRI and FIB-4 are measured together, it would be perfect gold standard of liver fibrosis detection rather than liver biopsy.

Availability of Data and Materials: All data are available and sharing is available as well as publication.

Competing Interests: The authors hereby that they have no competing interests.

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Consent for Publication: Authors and corresponding authors have reviewed this paper and approved it for publication.

Guarantor: Tamer Addissouky

Contributorship: Authors completed the study protocol and were the main organizer of data collection drafting and revising the manuscript perfectly. Tamer A. Addissouky has written the article and guaranteed the paper carefully. All authors contributed to the discussion and reviewed the manuscript as well as they helped in designing the study and protocol and engaged in a critical discussion of the draft manuscript. All authors have affirmed on the final copy of the manuscript.

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Abbreviations:

ECM (extracellular matrix),
COMP (cartilage oligomeric matrix protein),
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 (on-Alcoholic Fatty Liver Disease),
HCV (hepatitis C virus),
HSC (epatic stellate cells),
HA (Hyaluronic Acid),
CO-IV (Collagen type four).
IFG (Impaired fasting glucose).

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