Efficiency of alternative markers to assess liver fibrosis levels in viral hepatitis B patients.

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Abstract

Background: Chronic hepatitis B is a global public health problem. Histological staging of liver fibrosis is crucial to identify patients who need antiviral treatment. Correlation between perspective markers and liver fibrosis has been studied by current research.

Methods: Samples of blood were collected from 75 persons non-infected and 75 infected cases with HBV were classified according to Metavir system. ELISA technique is used to determine alternative markers levels. RT-PCR assay is used to quantify HBV DNA. Scoring systems are compared to non-invasive markers.

Results: Our research has guided us that Hyaluronic acid, COMP and Coll-IV and scoring indexes of fibrosis discriminate fibrosis cases from non-fibrosis group. The studied biomarkers' results differentiate early stages from advanced levels of fibrosis.

Conclusions: We expect that if these alternative biomarkers are combined with scoring system, they would be beneficial predictors to determine fibrosis levels in liver in order to eliminate needing to liver biopsy.

Keywords: Non-invasive biomarkers, Hepatitis B, Liver fibrosis, META VIR, Fibrosis scoring systems.

Abbreviations:

experience-based suggestions of a number of pathologists who
boosted by sequent stepwise discriminated analysis [7]. 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 [8]. Fibrosis of liver is graded into several
levels from F0 (healthy) to F4 (progressive liver scare) [9]. The
improvement of inter and intra variability of METAVIR is
observable [10]. The Metavir characterises 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

COMP is an extracellular matrix (ECM) protein primarily
present in cartilage and encoded by the COMP gene. It is a
non-collagenous protein mostly identifiable in cartilage which
contains of five arms 435 kDa [12]. 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 is found in a highest concentration in fluid
such as joint and eyes [13]. Nowadays, it has been performed
as an effective biomarker of hepatic fibrogenesis in cases who
suffer from chronic viral disease [14]. Type IV collagen is a
component of ECM that was investigated as a surrogate marker
of liver fibrosis [15]. It has three different regions (an amino-
terminal domain, a central helix domain, and a carboxyterminal
domain) [16]. Type IV collagen has been studied extensively in
liver diseases of different etiologies. The rate of endorsement
of different indirect markers in evaluation of liver fibrosis is
diverse in several countries over the world and bordered [17].
We have aimed to evaluate an 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.

Material and Methods

Sampling

Required amounts of blood samples were drawn by specific
needle after an overnight fasting from totally 150 blood
samples from 75 healthy volunteers were obtained from hospital of Mansoura university in Mansoura,
Egypt and assessed by ultrasound techniques. 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 include Platelets count, AST, ALT, Albumin levels
and fasting blood glucose.

Serum HCV RNA detection

Serum HCV RNA was detected by Real Time PCR assay.

Serum markers

Hyaluronic Acid, Comp and Collagen-IV levels have been
determined by commercially ELISA kits.

Fibrosis Scoring Systems

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

FIB-4: It is a score which includes platelet count, ALT, AST
levels and age.

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

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

BMI Kg/M2: Calculated by using equation between height
and weight.

IFG: It is calculated by WHO consideration of FBS level from
110 to 125 mg/dL is IGF [18].

Statistical data

Particular software version of IBM SPSS is exploited in
insertion of data into computer to be analysed (Armonk, NY:
IBM Corp) [19]. Number and percentage were described by
qualitative data. The normality of distribution Quantitative data
which is verified by The Kolmogorov-Smirnov test and that
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 is implemented by using of Chi-square test. Normally
distributed quantitative variables are presented by Student t-
test which helps in comparison between two studied groups.
Normally distributed quantitative variables are performed by
using of F-test (ANOVA) which assists in distinguish between
more than two groups. Differentiation of abnormally variables
are elaborated by using of Mann Whitney test and compare
between studied groups. Abnormally variables are presented by
using of Kruskal Wallis test which helps in comparison
between studied groups.
Results

In Figure 1, the precision of particular studied markers tests is presented by ROC curve which helps to discriminate cases with fibrosis of liver from healthy. The areas under curve (AUROC) of HA (0.988), CO-IV (0.870), COMP 0.965 , APRI 0.1.000 , SHASTA 1.000 , NAFLD 1.000 and FIB-4 1.000 are helpful in detection of different stages of fibrosis. The results illustrate cut off for HA, CO-IV, COMP (>36, >98.1, >15) respectively and the accuracy is (94.33, 92.2, 92.91) 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 presented in Table 1. By understanding of highlights of these reports, the accuracies of these markers are observable.

Table 1. Presentation of sensitivity and specificity and accuracy.

<table>
<thead>
<tr>
<th></th>
<th>AUC</th>
<th>p-value</th>
<th>95% C.I</th>
<th>Cut off</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>Accuracy</th>
</tr>
</thead>
<tbody>
<tr>
<td>HA</td>
<td>0.988’</td>
<td>&lt;0.001’</td>
<td>0.976</td>
<td>0.999</td>
<td>&gt;36</td>
<td>100</td>
<td>89.33</td>
<td>89.2</td>
<td>100</td>
</tr>
<tr>
<td>CO IV (μg/L)</td>
<td>0.870’</td>
<td>&lt;0.001’</td>
<td>0.798</td>
<td>0.942</td>
<td>&gt;98.1</td>
<td>83.33</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>COMP (μg/mL)</td>
<td>0.965’</td>
<td>&lt;0.001’</td>
<td>0.937</td>
<td>0.993</td>
<td>&gt;15</td>
<td>84.85</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>APRI</td>
<td>1.000’</td>
<td>&lt;0.001’</td>
<td>1</td>
<td>1</td>
<td>&gt;0.3</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>SHASTA</td>
<td>1.000’</td>
<td>&lt;0.001’</td>
<td>1</td>
<td>1</td>
<td>&gt;-2.1</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>NAFLD</td>
<td>1.000’</td>
<td>&lt;0.001’</td>
<td>1</td>
<td>1</td>
<td>&gt;-1.645</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>FIB-4</td>
<td>1.000’</td>
<td>&lt;0.001’</td>
<td>1</td>
<td>1</td>
<td>&gt;0.9</td>
<td>100</td>
<td>100</td>
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<td>100</td>
</tr>
</tbody>
</table>

In Figure 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 (0.866), CO-IV (0.838), COMP (0.668), APRI (0.844), SHASTA (0.725), NAFLD (0.893) and FIB-4 (0.880) and these reports helps definitely to discriminate several levels of fibrosis. The results elaborate the cut off for HA, CO-IV, COMP (>123, >163, >21) respectively, and the accuracy is (78.79, 80.30, 68.18) 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.

![Figure 1. Discrimination liver fibrosis from control by using ROC curve.](image1)

![Figure 2. Differentiation F3+F4 from F1+F2 by using ROC curve.](image2)
Discussion

The plan of antiviral therapy includes specific patients who have fibrosis of cirrhosis, to eradicate or eliminate the consequence complications [20]. Our studied scoring systems are regarded as good non-invasive markers which are massively demanded to clinical works as well as they are considered as perfect alternative to biopsy. Also, these indexes can be used in screening of treatment efficiency [21]. The AUC of FIB-4 resulted 0.85, 0.81 in perfect evaluation of several levels of fibrosis in HBV cases [22]. The accuracy of FIB-4, NAFLD and APRI are performed as great detectors of levels of fibrosis [23,24]. Fortunately, these results of these researches are agreed with our research.

In another study, the precision of COMP in evaluating cirrhosis was as excellent as another scoring systems with (AUC 0.884), sensitivity 83.3% and specificity 83.7% (cut-off >15U/L). The results of COMP searches showed that it is a good non-invasive marker in comparison with specific fibrosis indexes such as APRI and FIB-4. Thereafter, to prove comp test as a valid detector to evaluate fibrosis, the more studied are required [25]. In our study, accuracy of discrimination of COMP in evaluating cirrhosis was (AUC 0.965) with sensitivity 84.85% and specificity 100.0%, (cut-off >21U/L) and accuracy (92.91) in ROC curve for diagnosis liver fibrosis from control. Moreover, in Figure 2 showed that the results of COMP was (AUC 0.668) with sensitivity 75.0%, specificity 61.76% with cut-off >21U/L and accuracy (68.18).

By using ROC curve in current study, APRI index performed the good results which are beneficial to discriminate early stages and advanced levels of fibrosis in parallel to classification of METAVIR score, whereas APRI showed AUC 0.844, COLL-IV and HA performed AUC 0.838 and AUC 0.866 respectively. By studying of NAFLD, the reports performed that Hyluronic acid was found a perfect marker in fibrosis detection whereas AUC 0.97 [26]. Otherwise, HA has effective ability to distinguish advanced levels from initial stages of fibrosis by its results of NPV (98-100%) which is higher than PPV (61%) [27].

Recently by researches, several indirect non-invasive markers are reviewed in detection of progressed levels of fibrosis of liver in infected cases with different liver diseases [28]. 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 [29]. The NAFLD fibrosis score depend totally on using 6 variables and was advanced in an early study including 75 patients for purposes. The test performed well with AUROC of 1.000 and 0.893 in studied groups. In the HBV group, progressive fibrosis could be discriminated and diagnosed by a NPV of 81.6% for patients with score >1.208 and a PPV of 89.3%. Using these cut-offs which approve that a biopsy could be avoided in 89% of patients tested with only a 10% false prediction rate. Various AUROC of APRI (0.844-1.000), FIB-4 (0.880-1.000) and SHASTA (0.725–1.000) have provided and effective indicators in detection of advanced fibrosis as mentioned. Overall, as a result of current study, a good accuracy of non-invasive fibrosis scoring systems tests such as APRI 84.85, SHASTA 71.21, NAFLD 84.85 and FIB-4 84.85 provides 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 75 HBV co-infected patients, this index performed that a sensitivity of 100.0% and a NPV of 100.0%, to discriminate liver fibrosis from control as well as showing a sensitivity of 93.75% and a PPV of 89.5% provide assistance to exclude liver fibrosis stages F1+F2 from F3+F4 of patients [30]. Hence, the results of studied biomarkers have presented potential evidence to consider these markers are pivotal predictors to distinguish cases without fibrosis from patients with several levels of fibrosis from early, mild and advanced stages.

In summary, Alternative biomarkers which are including H.A, CO-LIV and COMP are considered as prospective markers to differentiate healthy individuals from chronic liver disease by their significant results in fibrosis detection. Thence, if serum H.A, CO-IV, SHASTA, COMP, and comparative indexes of

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<td>H.A</td>
<td>0.866'</td>
<td>&lt;0.001'</td>
<td>0.783</td>
<td>0.949</td>
<td>&gt;123</td>
<td>90.62</td>
<td>67.65</td>
<td>72.5</td>
<td>88.5</td>
</tr>
<tr>
<td>CO IV (μg/L)</td>
<td>0.838'</td>
<td>&lt;0.001'</td>
<td>0.743</td>
<td>0.934</td>
<td>&gt;163</td>
<td>84.37</td>
<td>76.47</td>
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<td>0.668'</td>
<td>0.019'</td>
<td>0.535</td>
<td>0.801</td>
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LL: lower limit, UL: upper limit.

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fibrosis system are measured together, it would be precise criterion of liver fibrosis detection rather than liver biopsy.

Conflicts of Interest

The authors hereby declare that they have no competing interests. Authors and corresponding authors have reviewed this paper and approved it for publication.

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Authors’ Contributions

Authors completed the study protocol and were the main organizer of data collection drafting and revising the manuscript perfectly. Tamer A. Addissoouk has written the article and guarantees 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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