ejpmr, 2016,3(11), 70-78

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

CAN YKL-40 IMPROVE THE DIAGNOSTIC POWER OF NON-INVASIVE FIBROGENIC STAGING IN CHRONIC HEPATITIS B VIRUS INFECTED PATIENTS?

EL-Shahat A. Toson*[1], Gamal E. Shiha[2,3], EL-Sherbiny H. EL-Saied[4], Waleed Samir[3], Mohamed ELbasiony[3], Doaa A. Shoieb[4]

[1]Chemistry Department, Faculty of Science, Damietta University, Damietta, Egypt.
[2]Internal Medicine Department, Faculty of Medicine, Mansoura University, Mansoura, Egypt.
[3]Egyptian Liver Research Institute and Hospital (ELRIAH), Sherbin, Eldakahlia, Egypt.
[4]Chemistry Department, Faculty of Science, Port Said University, Port Said, Egypt.

Corresponding Author: EL-Shahat A. Toson

Chemistry Department, Faculty of Science, Damietta University, Damietta, Egypt.

Article Received on 30/08/2016 **Article Revised on20/09/2016** **Article Accepted on 11/10/2016**

ABSTRACT

Background: Chronic hepatitis B virus run the risk of developing fibrosis and in later life hepatocellular carcinoma. **Aim**: The aim is to test the diagnostic power of YKL-40 in assessing liver fibrosis and its ability to improve those of APRI, AGE/AST, AAR, FI, CDS, LOK, API, FIB4, FIBQ, GUCI and KING scores in the previous patients. **Methods:** Blood samples for complete blood count and serum for YKL-40, liver function tests, HBV DNA and HBV markers evaluation were taken (n=71, F0 - F4); beside, transient elastography. The numerical values of these scores were quantized and correlated with that of YKL-40. **Results:** YKL-40 can efficiently differentiate patients with non-significant (F0, F1) from those with significant fibrosis (F2 –F4); sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of 86, 100, 100, 87 and AUC of 0.941, respectively and those with severe (F3 , F4) from non-severe fibrosis (F0 – F2, 95, 74, 60, 97 and 0.932, respectively). The additions of the results of YKL-40 to those of the previous scores greatly enhance their AUCs and their diagnostic power in the majority of cases. **Conclusions:** YKL-40, itself, cannot only help in assessing hepatic fibrosis but also dramatically enhance the diagnostic power of the tested scores in their non-invasive abilities to discriminate the stages of the disease in HBV chronically infected Egyptian patients.

KEYWORDS: YKL-40, Liver fibrosis, Hepatitis B Virus, transient elastography**,** non-invasive scores**.**

INTRODUCTION

Liver fibrosis in chronic HBV infected patients is a reversible wound-healing response resulting from liver injury which is characterized by the accumulation of extracellular matrix (ECM) accumulation and inflammation. The persistence of them lead to distortion of liver architecture thence cirrhosis and high mortality. This progression is variable and slowly developed.[1,2] Actually, not all the infected patients will develop cirrhosis.[3]

Liver biopsy has been considered the "gold standard" for diagnosing chronic liver disease, grading its necro-inflammatory activity, and finally, staging liver damage.[4] However, sampling error can lead to underestimation of the degree of liver fibrosis e.g. small or fragmented tissues. The latter's and others, therefore, can subtract from goodness or interpretation of the results of liver biopsy. Moreover, the biopsy is not suitable for repetition because of it invasiveness and tendency to cause major complications, including hemorrhage and death.[5] Generally, the prognosis and management of chronic liver diseases not only depend strongly on the degree of liver fibrosis but also on the accuracy of estimation of the HBV-related chronic hepatitis.

However and until recently, liver biopsy (LB) examination was the only way of evaluating liver fibrosis.[6] Also, several serum biomarkers, combined scores, and imaging techniques have been introduced for the assessment of liver fibrosis non-invasively.

For these reasons, many non-invasive markers for assessing liver fibrosis have been developed, validated in different studies, their results were compared with that of liver biopsies to assess their accuracy and their frequent use in clinical diagnosis. These include indirect and direct markers. The first can reflect the alternation in hepatic function, e.g. AAR, API, Fib-4 index, FibroQ index, GUCI, King's score, APRI, CDS, FI and LOK (Model 3). The direct serum markers basically depend on extracellular matrix proteins (ECMP) evaluations and reflect the activity of the fibrotic process, and are thought to indicate the extent of connective tissue deposition; including YKL-40.[7,8]

YKL-40 (chitinase-3-like-1 and human cartilage glyco-protein-39) is an emerging new inflammatory biomarker.[9] The YKL-40 gene encodes a protein of 383 amino acids (40 kDa) with an N-terminal sequence of Tyr-Lys-Leu (YKL), hence the name YKL-40 was used.[10,11] It belongs to the chitinase family and is strongly expressed in human liver. It is also thought to contribute to tissue remodeling and degradation of extracellular matrix (ECM) deposited in the liver.[12]

Therefore, the first aim of the present study is to evaluate the ability of serum YKL-40, itself, to assess hepatic fibrosis in Egyptian patients chronically infected with HBV or not. The second is to test whether the addition of YKL-40values, as an direct marker, to those of the well-known selected 11 non-invasive fibrogenic scores, as non-direct markers, could enhance their diagnostic power in differentiating the fibrotic stages in HBV chronically infected patients or not.

PATIENTS AND METHODS

Patients

The present study was conducted on 71 patients with HBV and on 20 healthy controls. The patients were subdivided according to the liver biopsy into two groups. Group I (non-significant versus significant fibrosis) and group II (non-severe versus severe liver fibrosis). These patients were randomly chosen (males and females) from the Egyptian Liver Research institute and Hospital (ELRIAH), Mansoura, Egypt out patients. All patients were positive for HBV-DNA and HBsAg and negative for hepatitis C (HCV) antibody and HCV-RNA. Besides, the healthy controls were free from any disease; especially HBV and they were sex and age matched with those of the patients' group.

Samples and blood markers

1- Blood samples: They were withdrawn from all cases and either freshly used or kept frozen at -80 ^{o}C until use.
2-Blood markers: The following were measured in the serum of each patient and control subject.

2-1- Human Chitinase 3-like 1(YKL-40)

Sandwich ELISA kit was used (Boster Biological Technology Co., Ltd., Catalog No; EK0974, USA), according to the enclosed pamphlet.

2-2-Routine liver function tests and blood picture

They include: albumin (Alb.), bilirubin (total and direct), serum glutamic pyruvic transaminase (SGPT) and serum glutamic oxalloacetic transaminase (SGOT) using automated Biochemistry analyzer. Prothrombin activity (INR) was also performed. Complete blood picture (Hemoglobin, red blood cells, platelets and white blood cells count) were done on a D-cell 60 automated Hematology analyzer (Diagon Ltd, Budapest, Hungary).

2-3-Serological markers

These include; HBV-DNA and HBV markers; namely, hepatitis B surface antigen (HBsAg) quantization, hepatitis B envelope Ag (HBeAg) and HBsAb, HBeAb and HBcAb (IgM - IgG) using the ARCHITECT HBeAg assay (CMIA). Also, HCV infection was excluded.

2-4- Hepatitis B virus DNA quantification

A manual extraction of DNA from 100 µl of plasma followed by co amplifying a region of the precore or core HBV gene with internal addition of QS at known concentrations in the extraction step[13]

2-5-Patholigical examination

Biopsy specimens were routinely stained with hematoxyline-eosin stain, blindly examined and METAVIR scored F0, no fibrosis, F1, portal fibrosis alone, F2, portal fibrosis with rare septae, F3, portal fibrosis with many septae, and F4, cirrhosis.

2-6- Clinical investigations, Fibroscan and ultrasonography

They were done by members of ELRIAH.

3- Formulas of the selected Scores

1-AST: platelets ratio index (APRI) was calculated using Wai's formula[14]

AST (upper limit of normal)/platelet count (expressed as platelets x 10^9/L) X 100

2-Fib-4 index was calculated using Sterling's formula[15]

Age (years) x AST (IU/L)/platelet count (109/L) x ALT (IU/L)$^{1/2}$

3-Fibro-quotient (FibroQ) index using this formula

10 × (age × AST × INR)/(ALT × platelet count)

4-Göteborg University Cirrhosis Index (GUCI) using this formula

(ASTxINRx100)/platelet count (109/L)

5- King's score using this formula

Age (years) x AST (IU/L) x INR/platelet count (109/L)

7-LOK (Model 3) using this formula

Log odds = -5.56 - 0.0089 × platelet (× 109/L) + 1.26 × AST/ALT ratio + 5.27 × INR.

8-Fibrosis index (FI) was calculated using this formula[16] as:

8.0-0.01 x platelet count (x 10^9/L) – serum albumin (g/dl)

9- Cirrhosis discriminant score (CDS) derived from routine LFTs:

Platelets, ALT/AST ratio and INR.

10-AAR: AST/alanine aminotransferase (ALT) ratio (AAR).

11- AGE/AST.

Statistical analyses

The data were analyzed using SPSS program (Statistical Package for Social Sciences) software version 20.

Analyses were done for parametric quantitative variables using one way ANOVA test and for non-Parametric quantitative variables using Kruskal Wallis test. ROC curve was done to determine the cutoff point, AUC, sensitivity, specificity, PPV and NPV of presences of fibrosis. The level of significance was taken at P value ≤0.05.

RESULTS

Discriminating power of YKL-40

According to Metavair fibrosis stages, 13 patients were with F0 (18.3%), 21 patients with F1 (29.6%), 17 patients with F2 (23.9%), 13 patients with F3 (18.3%) and 8 patients with F4 (9.8%) as in **table 1**.

The results of YKL-40 has a highly discriminating power between the fibrotic stage of liver disorder (Table 1); The mean value was 724.0±102.4 in F4, 497.3±122.0 in F3, 404.8±99.0 in F2, 252.4± 61.0 in F1 and 171.0±46.0 in F0 but was 110.0±20.3 in sera of control subjects. The P <0.001) were highly significant when YKL-40 levels were compared with each other or with that of the control value.

Correlations between YKL-40 and routine liver functions

Table 2 showed that,YKL-40, GOT and INR were significantly increased in significant as well as severe fibrosis compared with those of non-significant and non-severe fibrosis, respectively (P<0.044 or less). On the other hand, serum albumin and platelets count were decreased in significant as well as in severe fibrosis compared with those of non-significant and non-severe one (P<0.017 or less). Comparing the ability of these biochemical markers to discriminate between groups of both classifications, it was found that the extent of increase of YKL-40, GOT and INR in severe fibrosis (F3, F4, n=20) was higher than that of the significant fibrosis (F2, F3, F4, n=36), Also, the extent of decrease of albumin and platelet count was higher in the severe fibrotic group compared with those of patients with significant fibrosis.

Discriminating power of YKL-40 versus those of the selected 11 non-invasive scores in hepatic fibrosis

The ability of YKL-40 or the numerical values of the selected 11 non-invasive scores to stage liver fibrosis were listed in **table 3**. The level of YKL-40 at a cutoff value more than **336** can able to discriminate patients with significant from those with non-significant fibrosis with sensitivity of 86%, specificity 100%, PPV 100% and NPV 87% with an area under curve of 0.941 and p<0.001.The latter performance characteristics were much higher than those which were listed in the same table for the other selected scores; except for the sensitivity of APRI which was 91%. In addition, the level of YKL-40 at a cutoff value more than 336 can able to discriminate patients with severe from those with a non-severe fibrosis with sensitivity of 95%, specificity 74%, PPV 60% and NPV 97% with area under curve of 0.932 and a p<0.001 **(Figure 1 and 2)**.

Role of serum YKL-40 levels in the improvement of the diagnostic power of the non-invasive fibrogenic scores.

1-Performance characteristics and AUCs

The results of the ability of YKL-40 to enhancing the power of the other selected 11 non-invasive scores were tabulated **(Table 4)**. In fact, the performance characteristic of each score in discriminating patients with significant and non-significant fibrosis was highly elevated with AUCs values ranging between 0.955 and 0.986, sensitivity (86-94), specificity (94-100), PPV (94-100) and NPV (87-94) but with new cutoff values. Also; and as was expected, the same findings were observed during discrimination between severe and non-sever liver fibrosis; except for APRI and AGE/AST. In the two latter scores, the specificity were decreased **(Figure 1 and 2).**

2-Correlation coefficient

Table 5 list the correlation coefficient between serum YKL-40 levels and that of age, blood picture, liver function tests and the numerical values of the 11 selected scores in non-significant versus significant and in non-severe versus severe fibrosis. The r values which were higher than 0.5 were found to be with Age, albumin, FI, CDS, API, FIB4, FIBQ and KING score in both classifications.

DISCUSSION

Liver disease develops silently and many people have no idea that they have liver failure until it is too late. Also, one third of people admitted to hospital with end stage liver disease die within the first few months.[17] Therefore, early diagnosis is important for preventing complications and treatment of the disease.[18]

At present, pathological examination of liver puncture tissue is the way to diagnose liver fibrosis. On the other hand and in clinical practice, the use of liver biopsy is still limited due to its invasivement and sampling errors. However, it is the gold standard till now.

In addition, the usefulness of noninvasive evaluations for predicting liver fibrosis has been less extensively studied and validated for chronic HBV than for chronic HCV. Few scores based on combinations of serum biomarkers were originally proposed for use in patients with chronic HBV.[19] YKL-40 has emerged as a promising marker of fibrotic disease.[20]

In addition, the future researches; including the present study, around YKL-40 should concentrate further on establishing whether YKL-40 could gain the value of specific, early detector biomarker or differentiator between the fibrotic stages in clinical practice or not. YKL-40 seems to be useful for screening because it is detectable in early stage or in subclinical disease, and it

also seems to have the potential of becoming a prognosticator of fibrosis events in the liver; and thence, mortality. Therefore, the main aim of this study was to assess the diagnostic value of serum YKL-40 (direct marker) in Egyptian patient's chronically infected with HBV. Its usefulness as a non-invasive diagnostic test to detect early stages of fibrosis (Non-significant, non-severe) versus the late ones (significant and severe); either alone or in combination with some of the well-known non-invasive scores (indirect markers), will also be investigated.[21]

This is actually the case in the present study because its levels were significantly increased with the increase in the severity of the disease **(Table 1).** Also, YKL-40 itself discriminate non-significant from those with significant fibrosis with sensitivity, specificity, positive predictive value (PPV) and negative predictive values (NPV) of 86, 100, 100, 87 and AUC of 0.941, respectively and those with severe from non-severe fibrosis (95, 74, 60, 97 and 0.932, respectively).

Not only the latter findings concerning YKL-40 were observed in the present study but also the parameters of its diagnostic power was promissory to efficiently diagnose hepatic fibrosis when compared with those of the indirect non-invasive markers; which were re-evaluated in the present study. These markers include AAR, API, Fib-4 index, FibroQ index, GUCI, King's score, APRI, CDS, FI and LOK (Model 3). Moreover, the additions of the results of YKL-40 to these scores participate in their native diagnostic power exegravation and in their AUCs elevation; in the majority of cases. The latter findings could be explained by participation of YKL-40 in liver cells deterioration via acting as a growth-factor for fibroblasts activation.[22,23] Nojgaard, et al.[22] thought that YKL-40 contributes to tissue remodeling and to the deposited extracellular matrix (ECM) degradation. Since YKL-40 act as a growth-factor for fibroblasts and this type of cells is a well-known synthesizer of collagen-related and non-collagen-related materials; it is expected for this molecule to enhance ECM deposition with a resultant increase in the severity of hepatic fibrosis not only based on the latter findings but also on our experience in the development and assessment of hepatic fibrosis,[24,25,26,27,28,29] In addition, Kamal et al (2006) added that a strong correlation was found between serum YKL-40 and hepatic mRNA levels in patients with chronic hepatitis which suggests that YKL-40, in part, reflect ongoing hepatic fibrogenesis.[30] Since Fontana et al, (2008) showed thatYKL-40 act as a chemo-attractant for endothelial cells and thereby a modulator of angiogenesis during tissue damage[31], therefore, one can suggest that this molecule cannot only act as an angiogenic but also as a non-invasive marker for chronic inflammatory and fibrotic disorders in the liver. **Roslind and Johansen (2009**), showed thatYKL-40 is produced by local inflammatory cells; mainly macrophages, neutrophils, endothelial and vascular smooth muscle cells, and perhaps also hepatic stellate cells (Ito cells) at the site of inflammation.[32] Considering macrophages to be the main secretory cells of YKL-40; one can expect for that marker to be increased during viral attack by macrophages even at the early stages of liver disorders compared with that of the control. Since Ito cells play a vital role in the deposition of ECM[33], one cannot neglect the participation of these cells in the over expression of YKL-40 in sera of HBV infected patients, especially in the advanced stages of liver fibrosis; which is actually the case in the present study.

In the present study, AUC of YKL-40 was the highest among those of the selected 11 scores. Therefore one can expected that, the stepwise addition of YKL-40 values to those of the previous scores can improve their diagnostic power to stage liver diseases **(Tables 3, 4 and Figure1, 2)**.

In a recent study[34], the mean APRI values were significantly increased with fibrosis and its AUC distinguishing severe (F3-F4) from mild-to-moderate fibrosis (F0-F2) was 0.8. In the present study, the re-validation of APRI in HBV infected patients; for example, gave an AUROC of 0.787, 0.818 which are similar to the results of the previous studies. Surprisingly, the addition of the numerical values of YKL-40 (direct marker) to those of APRI (indirect) results in a significant increase in such area to reach to 0.967 and 0.942 for discriminating patients with significant from those with non- significant and those with non-severe from those with severe liver disorders, respectively. Based on the combined diagnostic power of YKL-40 and the native values of these indirect markers, the need to do liver biopsy can be avoided in the future which is actually one of the aims of the present study.

Table 1: Discriminating power of YKL-40 in various fibrotic stages in HBV infected patients and in controls

Marker Group	YKL-40
Control(n=20) Mean±SD Range	 110±20.32 73.0-142.0
F0 (n=13) (18.3%) Mean±SD Range P-value	 171±46.0 65.0-323.0 <0.001
F1 (n=21) (29.6%) Mean±SD	 252.4±61.0

Range P-value	116.0-336.0 <0.001
F2 (n=17) (23.9%) Mean±SD Range P-value	 404.8±99.0 205.0-546.0 <0.001
F3 (n=13) (18.3%) Mean±SD Range P-value	 497.3±122.0 321.0-686.0 <0.001
F4 (n=7) (9.8%) Mean±SD Range P-value	 724.0±102.4 520.0-792.0 <0.001

n= no of patients; SD: standard deviation.

Table 2: The mean level and standard deviations (SD) of YKL-40, alanine amino transeferase (ALT) and aspartate amino-transeferase (AST), albumin and the international normalization ratio (INR) as well as platelets count of hepatitis B virally-infected patients (n=71).

	1st Classification			2nd Classification		
	P-value	**Significant fibrosis (F2,3,4) n=36**	**Non- significant fibrosis (F0,1) n=35**	**P-value**	**Severe fibrosis(F3,4) n=20**	**Non severe fibrosis (F0,1,2) n=51**
YKL-40	<0.0001	497.0±159.14 [235%] †	211.4±73.0	<0.0001	576.9±159.1 [204%] ‡	282.5±119.9
Age	<0.0001	47.6±11.1	35.1±10.6	<0.0001	52.5±5.8	37.4±11.9
GPT	0.66	54.3±24 [139%] †	49.3±65.5	0.255	62.5±27.4 [131%] ‡	47.7±54.8
GOT	0.044	41.6±23.0 [141%] †	29.9±24.3	0.01	50.4±28.5 [124%] ‡	31.1±20.6
Albumin	<0.001	4.1±0.7 [112%] †	4.6±0.3	<0.0001	3.7±0.63 [193%] ‡	4.6±0.33
Platelets	<0.0001	175.3±58.5 [141%] †	247.6±84.9	0.002	164.4±63 [139%] ‡	229.1±80.0
INR	0.017	2.9±4.6 [207%] †	1.04±0.04	<0.0001	4.4±5.9 [433%] ‡	1.06±0.1

[†]: Percent of change compared to Non- significant fibrosis. [‡]: Percent of change compared to Non- sever fibrosis. P>005 is considered non-significant; P<0.05 is considered significant; P<0.001 is considered very significant and P<0.0001 is considered extremely significant.

Table 3: **Diagnostic values of YKL-40 versus those of the selected 11 non-invasive scores (APRI, AGE/AST, AAR, FI, CDS, Lok Mode, API, FIB-4, FIBQ, GUCI and KING Score) with optimal cut-off for discriminating between non-significant via significant non-severe via severe fibrosis.**

Group	Non-significant ¥ significant							Non-sever ¥ severe						
Variable	Cut-off	Sensitivity	Specificity	PPV	NPV	AUC	P value	Cut-off	Sensitivity	Specificity	PPV	NPV	AUC	P value
APRI	>0.25	91	50	66	85	0.787	<0.001	>0.53	65	88	68	86	0.818	<0.001
AGE/AST	1.4	61	59	54	50	0.497	0.975	≤0.9	35	88	53	77	0.565	0.41
AAR	>0.74	62	61	63	60	0.615	0.089	>0.74	60	72	46	82	0.673	0.0204
FI	>1.09	78	67	72	74	0.780	<0.001	>1.27	90	64	50	94	0.815	<0.001
CDS	>3.0	86	73	78	73	0.837	<0.001	>4.0	70	76	53	86	0.805	<0.001
LOK	>0,03	64	76	75	66	0.695	0.007	>0.04	70	68	46	85	0.662	0.178
API	>0.18	83	76	79	81	0.828	<0.001	>0.22	80	76	57	90	0.842	<0.001
FIB4	>1.0	70	85	83	72	0.838	<0.001	>1.1	80	78	59	90	0.875	<0.001
FIBQ	>1.64	73	85	84	74	0.823	<0.001	>1.84	85	76	58	92	0.846	<0.001
GUCI	>16.9	64	82	80	68	0.797	<0.001	>21.5	70	86	66	88	0.831	<0.001
KING	>7.13	70	88	86	73	0.841	<0.001	>7.13	90	76	60	95	0.887	<0.001
YKL-40	>336	86	100	100	87.2	0.941	<0.001	>336	95	74	60	97	0.932	<0.001

AUC:area under the receiver-operating characteristic curve; PPV: positive predictive value; NPV: negative predictive value. P>005 is considered non-significant; P<0.05 is considered significant and P<0.001 is considered very significant.

Table 4: **Combination between YKL-40 and the other 11 non-invasive scores (APRI, AGE/AST, AAR, FI, CDS, Lok Mode, API, FIB-4, FIBQ, GUCI and KING Score in non-significant versus significant one and non-severe versus severe fibrosis.**

Group	Non-significant ¥ significant							Non-sever ¥ severe						
Variable	Cut-off	Sensitivity	Specificity	PPV	NPV	AUC	P value	Cut-off	Sensitivity	Specificity	PPV	NPV	AUC	P value
APRI	>0.2037	86	100	100	87	0.967	<0.001	>-1.839	95	76	61	97	0.942	<0.001
AGE/AST	>0.5197	86	100	100	87	0.957	<0.001	>10.67	95	74	59	97	0.928	<0.001
AAR	>0.3522	86	100	100	87	0.955	<0.001	>-1.94	95	76	61	97	0.928	<0.001
FI	>0.3731	86	100	100	87	0.972	<0.001	>-1.547	90	86	72	96	0.948	<0.001
CDS	>-0.204	94	94	94	94	0.986	<0.001	>-2.144	100	76	62	100	0.941	<0.001
LOK	>0.212	94	97	97	94	0.979	<0.001	>-1.339	95	82	68	97	0.940	<0.001
API	>0.3923	92	97	97	91	0.972	<0.001	>-1.485	90	82	66	95	0.942	<0.001
FIB4	>0.1186	91	97	97	91	0.947	<0.001	>-0.201	80	94	84	92	0.948	<0.001
FIBQ	>0.556	94	94	94	94	0.973	<0.001	>-2.257	100	74	60	100	0.943	<0.001
GUCI	>-0.883	86	100	100	87	0.957	<0.001	>0.372	75	96	88	90	0.941	<0.001
KING	>0.6904	91	100	100	92	0.983	<0.001	>-1.339	95	82	78	97	0.940	<0.001

AUC:area under the receiver-operating characteristic curve; PPV: positive predictive value; NPV: negative predictive value. P>005 is considered non-significant; P<0.05 is considered significant and P<0.001 is considered very significant.

Table 5: Correlation between YKL-40 expression levels and the studied parameters in non-significant versus significant (Group 1) and in non-severe and severe (Group 2).

Variable	YKL-40			
	Group 2		Group 1	
	R	P value	R	P value
Age	0.531	<0.0001	0.531	<0.0001
Hb	- 0.05	0.65	- 0.05	0.65
RBCs	- 0.326	0.005	- 0.326	0.0054
WBCs	0.136	0.256	0.136	0.25
Platelets	- 0.38	0.001	-0.383	<0.001
T,Bil	0.006	0.958	0.006	0.9
D.Bil	0.461	< 0.0001	0.46	< 0.0001
SGOT	0.35	0.0024	0.354	0.002
SGPT	0.061	0.61	0.06	0.613
Albumin	-0.508	< 0.0001	-0.595	<0.001
INR	0.202	0.09	0.2	0.09
APRI	0.496	< 0.0001	0.496	<0.0001
AGE-AST	-0.091	0.4490	-0.0912	0.4493
AAR	0.384	0.0009	0.3844	0.0009
FI	0.551	<0.0001	0.552	<0.0001
CDS	0.5228	<0.0001	0.5228	<0.0001
LOK	0.112	0.3505	0.1123	0.351
APL	0.536	<0.0001	0.536	<0.0001
FIB-4	0.617	<0.0001	0.617	<0.0001
FIBQ	0.573	<0.0001	0.5731	<0.0001
GUCI	0.488	<0.0001	0.4880	<0.0001
KING	0.5462	<0.0001	0.5462	<0.0001

Grade of r: 0.00-0.24 = weak or no correlation; 0.25-0.49 = fair correlation; 0.50-0.74= moderate correlation; ≥0.75 = strong correlation. P>005 is considered non-significant; P<0.05 is considered significant; P<0.001 is considered very significant and P<0.0001 is considered extremely significant.

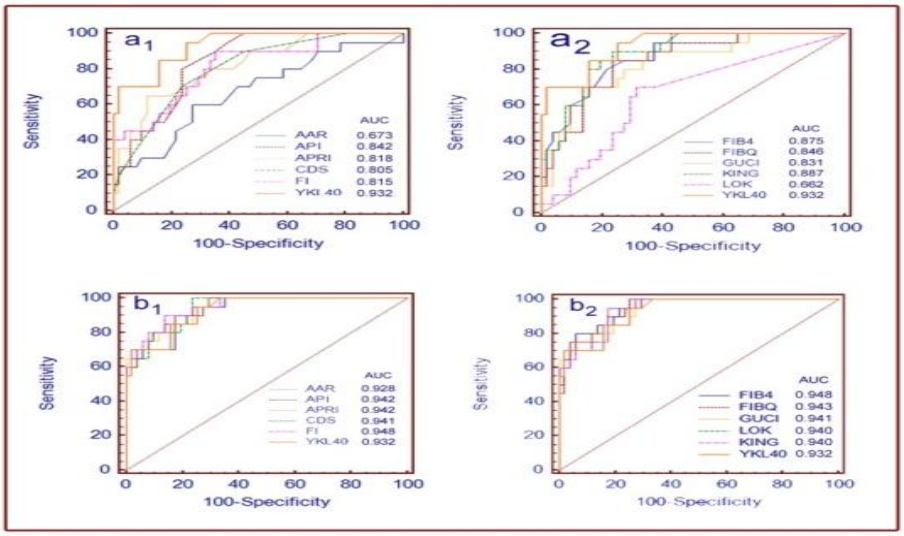

Figure1: **ROC curve of YKL-40 versus those of the selected 10 non-invasive scores (APRI, AAR, FI, CDS, Lok Mode, API, FIB-4, FIBQ, GUCI and KING Score) a_1, a_2 and ROC curve of YKL-40 in combination with those 10 scores for discriminating between non-significant via significant fibrosis b_1 and b_2.**

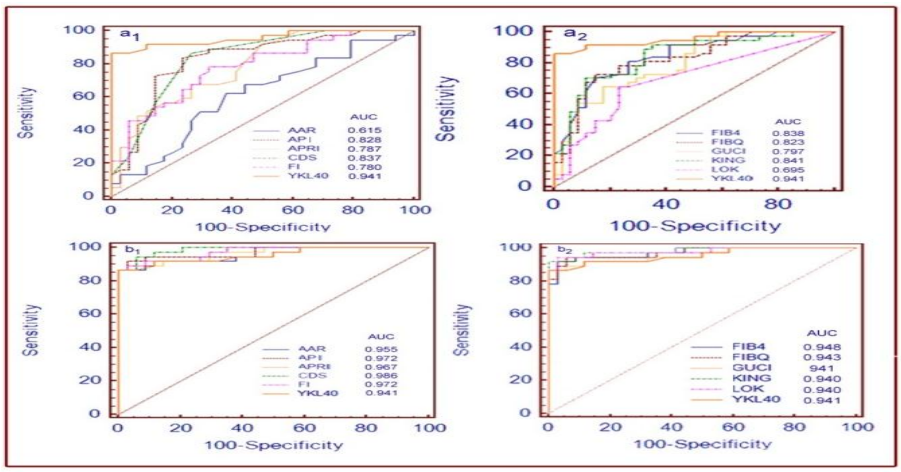

Figure 2: ROC curve of YKL-40 versus those of the selected 10 non-invasive scores (APRI, AAR, FI, CDS, Lok Mode, API, FIB-4, FIBQ, GUCI and KING Score) a_1, a_2 and ROC curve of YKL-40 in combination with those 10 scores for discriminating between severe and non-Severe fibrosis group's b_1 and b_2.

ACKNOWLEDGMENTS

We sincerely acknowledge authors for editing the paper and native English usage.

REFERENCES

1. Hernandez-Gea V and Friedman SL. Pathogenesis of liver fibrosis. *Annu Rev Pathol, 2011*; 6: 425–456
2. Toson EA., EL-Bendary M M, El-mezayen HA, and Sahyon H A. Longitudinal Evaluation of Hyaluronic Acid and Its Degrading Enzymes and Degradation Products in Egyptian Patients with Chronic Hepatitis C treated By Pegylated Interferon and Ribavirin". *International Conference on Advances in Agricultural, Biological &Environmental Sciences* (AABES-2014); Oct15-16, Dubai (UAE): 103-114.
3. Fattovich G, Bortolotti F and Donato F. Natural history of chronic hepatitis B: special emphasis on disease progression and prognostic factors. *J Hepatol*, 2008; 48:335–352.
4. Rockey DC, Caldwell SH, Goodman ZD, Nelson RC and Smith AD. Liver biopsy. *Hepatology*, 2009; 49: 1017-1044.
5. Myers RP, Fong A, Shaheen AA. Utilization rates, complications and costs of percutaneous liver biopsy: a population based study including 4275 biopsies. *Liver Int.*, 2008; 28: 705-712.
6. Friedman SL. Evolving challenges in hepatic fibrosis. *Nat Rev Gastroenterol Hepatol*, 2010; 7: 425-436.
7. Berres ML[1], Papen S, Pauels K, Schmitz P, Zaldivar MM, Hellerbrand C, Mueller T, Berg T, Weiskirchen R, Trautwein C and Wasmuth HE. A functional variation in CHI3L1 is associated with severity of liver fibrosis and YKL- 40 serum levels in chronic hepatitis C infection. *Journal of Hepatology*, *2009*; 50(2): 370-376.
8. Baranova A et al. Non-Invasive markers for hepatic fibrosis. BMC Gastroenterology, 2011; 11: 91-105.

9. Kamal SM, Turner B, He Q, Rasenack J, Bianchi L, Al Tawil A, et al. Progression of fibrosis in hepatitis C with and without schistosomiasis: correlation with serum markers of fibrosis.*Hepatology,* 2006; 43: 771–779.
10. Johansen JS. Studies on serum YKL-40 as a biomarker in diseases with inflammation, tissue remodeling, fibroses and cancer. *Dan Med Bull*, 2006; 53: 172-209.
11. Hu B, Trinh K, Figueira WF; Price PA. Isolation and sequence of a novel human chondrocyte protein related to mammalian members of the chitinase protein family. *J Biol Chem*, 1996; 271: 19415–19420.
12. Nojgaard C, Johansen JS, Christensen E, Skovgaard LT, Price PA; Becker U. Serum levels of YKL-40 and PIIINP as prognostic markers in patients with alcoholic liver disease. *J Hepatol*, 2003; 39: 179–186.
13. Tiziano Allice, Francesco Cerutti,1 Fabrizia Pittaluga, Silvia Varetto, Silvia Gabella, Alfredo Marzano et al. COBAS AmpliPrep-COBAS TaqMan Hepatitis B Virus (HBV) Test: a Novel Automated Real-Time PCR Assay for Quantification of HBV DNA in Plasma. *JOURNAL OF CLINICAL MICROBIOLOGY*, Mar, 2007; 828–834.
14. Wai CT, Greenson JK, Fontana RJ, Kalbfleisch JD, Marrero JA, Conjeevaram HS and Lok As. A simple noninvasive index can predict both significant fibrosis and cirrhosis in patients with chronic hepatitis C. *Hepatology,* 2003; 38: 518-26.
15. Sterling RK, Lissen E, Clumeek N, Sola R, Correa MC, Montaner J. et al. Development of a simple noninvasive index to predict significant fibrosis in patients with HIV/HCV co infection. *Hepatology, 2006*; 43(6): 1317-25.
16. Ohta T, Sakaguchi K, Fujiwara A, Fujioka S, Iwasaki Y, makino Y, Araki Y, Shiratori Y.S. Simple surrogate index of the fibrosis stage in chronic hepatitis C patients using platelet count and serum albumin level. *Acta Med Okayama, 2006*; 60: 77–84.
17. Dechêne A , Sowa JP, Gieseler RK, Jochum C, Bechmann LP, El Fouly A, Schlattjan M, Saner F, Baba HA, Paul A, Dries V, Odenthal M, Gerken G, Friedman SL, Canbay A.. Acute liver failure is associated with elevated liver stiffness and hepatic stellate cell activation. *Hepatology*, 2010; 52:, 1008–1016.
18. Seyed Mohsen Dehghani, Mohammad Hadi Imanieh, Mahmood Haghighat, Abdorrasoul Malekpour and Zeinab Falizkar. Etiology and Complications of Liver Cirrhosis in Children: Report of a Single Center from Southern Iran. *Middle East J Dig Dis*., Jan, 2013; 5(1): 41–46.
19. Masaru Enomoto, Hiroyasu Morikawa, Akihiro Tamori and Norifumi Kawada. Noninvasive assessment of liver fibrosis in patients with chronic hepatitis B. *World J Gastroenterol*, 2014; 20(34): 12031–12038.
20. Johansen JS, Jensen BV, Roslind A, Nielsen D and Price PA: Serum YKL-40, a new prognostic biomarker in cancer patients? *Cancer Epidemiology Biomarkers Prev*., 2006; 15: 194-202.
21. Julia S. Johansen, Per Christoffersen, Soren Moller, Paul A. Price, Jens H, Charly Garbarsch and Flemming Bendtsen. Serum YKL-40 is increased in patients with hepatic fibrosis. *Journal of Hepatology*, 2000; 32: 91 l-920.
22. Nojgaard C, Johansen JS, Christensen E, Skovgaard LT, Price PA; Becker U. Serum levels of YKL-40 and PIIINP as prognostic markers in patients with alcoholic liver disease. *J Hepatol*, 2003; 39: (179–186).
23. Saitou Y, Shiraki K, Yamanaka Y, Yamaguchi Y, Kawakita T, Yamamoto N, et al. Noninvasive estimation of liver fibrosis and response to interferon therapy by a serum fibrogenesis marker, YKL-40, in patients with HCV-associated liver disease. *World JGastroenterol*, 2005; 11(476–481).
24. Berres ML[1], Papen S, Pauels K, Schmitz P, Zaldivar MM, Hellerbrand C, Mueller T, Berg T, Weiskirchen R, Trautwein C and Wasmuth HE. A functional variation in CHI3L1 is associated with severity of liver fibrosis and YKL- 40 serum levels in chronic hepatitis C infection. *Journal of Hepatology, 2009*; 50(2): 370-376.
25. Attallah AM, Toson EA, El-Waseef A M, Abo-Seif MA , Omran MM and Shiha GE. 2006. "Discriminant function based on hyaluronic acid and its degrading enzymes and degradation products for differentiating cirrhotic from non-cirrhotic liver disease patients in chronic HCV infection". *Clin Chim Acta*, 2006; 369(1): 66-72.
26. Attallah AM, Toson EA, Shiha GE, Omran MM and Abdel-Aziz MM. Evaluation of serum procollagen amino terminal propeptide III, lamininand hydroxyproline as predictors of severe fibrosis in patients with chronic hepatitis C. *Journal of immunoassay &immunochemistry*, 2007; 28(3): 199-211.
27. Toson EA., EL-Bendary M M, El-mezayen HA, and Sahyon H A. Longitudinal Evaluation of Hyaluronic Acid and Its Degrading Enzymes and Degradation Products in Egyptian Patients with Chronic Hepatitis C treated By Pegylated Interferon and Ribavirin". *International Conference on Advances in Agricultural, Biological &Environmental Sciences* (AABES-2014); Oct15-16, Dubai (UAE): 103-114.
28. Julia S. Johansen', Per Christoffersen, Soren Moller, Paul A. Price, Jens H Charly Garbarsch and Flemming Bendtsen. Serum YKL-40 is increased in patients with hepatic fibrosis. *Journal of Hepatology*, 2000; 32: 91 l-920.
29. Toson, El-Shatat A.; Shiha, Gamal E.; El-Mezayen, Hatem A.; El-Sharkawy, Aml M. Diagnostic performance of T lymphocyte subpopulations in assessment of liver fibrosis stages in hepatitis C

virus patients: simple noninvasive score. *European Journal of Gastroenterology & Hepatology, 2016*; 28(8): 931–939 **.**

30. Kamal SM, Turner B, He Q, Rasenack J, Bianchi L, Al Tawil A, et al. Progression of fibrosis in hepatitis C with and without schistosomiasis: correlation with serum markers of fibrosis.*Hepatology*, 2006; 43(771–779).
31. Fontana RJ, Goodman ZD, Dienstag JL, Bonkovsky HL, Naishadham D, Sterling RK, et al. Relationship of serum fibrosis markers with liver fibrosis stage and collagen content in patients with advanced chronic hepatitis C. *Hepatology*, 2008; 47(789–798).
32. Roslind, A and Johansen, JS. 'YKL-40: a novel marker shared by chronic inflammation and oncogenic transformation. *Methods in Molecular Biology*, 2009; 511: 159-84.
33. H. L. Reeves and S. L. Friedman. Activation of hepatic stellate cells a key issue in liver fibrosis. *Frontiers in Bioscience, 2002;* 7: d808–d826.
34. Holmberg SD , Lu M, Rupp LB, Lamerato LE, Moorman AC, Vijayadeva V et al. Chronic Hepatitis Cohort Study (CHeCS) Investigators. Noninvasive serum fibrosis markers for screening and staging chronic hepatitis C virus patients in a larges US cohort. *Clinical Infectious Diseases*, 2013; 57(2): 240-246.