Prediction of fibrosis in hepatitis C patients: assessment using hydroxyproline and oxidative stress biomarkers

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Abstract This study aims to evaluate the diagnostic accuracy of aspartate aminotransferase (AST), alanine aminotransferase (ALT), hydroxyproline (Hyp), malondialdehyde (MDA), superoxide dismutase (SOD), and total antioxidant status (TAS) biomarkers in comparison with Metavir scoring for assessing the severity of hepatic fibrosis in the HCV patients. The histological activity index (HAI) was evaluated in liver biopsy by Metavir scoring system in 150 patients with HCV. HCV initial screening, further genotyping and biochemical data analysis were performed in serum using ELISA and biochemical assays. Out of the 150 HCV patients in this study, the most prevalent HCV genotype was genotype 4 (97 %). The significant fibrosis was estimated in 83.3 % of patients using the Metavir scoring system. They classified into 40 % of patients with mild fibrosis (F0–F1); 60 % with significant fibrosis (F2–4) and 20 % had cirrhosis (F4). Patients with cirrhosis (F4) showed significant correlation ($P < 0.001$) with increase in ALT, AST, AST/ALT, Hyp, Hyp/platelet count ratio, APRI, MDA, older age, and decrease ($P < 0.001$) in SOD, TAS, and platelet count compared to other stages of liver fibrosis. In our population, using optimized cut-off values of AST/ALT, APRI, Hyp, MDA, SOD, and TAS, significant fibrosis could be predicted accurately with a range of (80–90 %), and cirrhosis with a

range of (67–97 %) of HCV patients. Our study showed that, oxidative stress and Hyp markers could be useful as noninvasive diagnostic markers in the assessment of hepatic fibrosis.

Keywords: Hydroxyproline · HCV · Liver fibrosis index · Oxidative stress · Metavir score

Introduction

The hepatitis C virus is a small (55–65 nm in size), enveloped, positive-sense single-stranded RNA virus belongs to the genus Hepacivirus, a member of the family Flaviviridae. Until recently it was considered to be the only member of this genus. Hepatitis C virus (HCV) is considered as a major basis of liver associated diseases throughout the world. More than 170 million people (3 % of the world's populations) [49]. Oxidative stress plays an important role in the formation of liver fibrosis via increasing the stellate cell activation and collagen synthesis [55]. HCV infection is characterized by increase of oxidative stress markers and depletion of antioxidant enzymes. Stimulation of lipid peroxidation and accumulation of malondialdehyde is an important event in hepatic fibrosis [25]. Oxidative stress is related to the liver fibrosis, anticipating the hepatic stellate cells' (HSC) activation. It was reported that MDA elevation offers the best prediction potential for liver fibrosis [12]. Direct measurements of oxidative stress markers in liver tissue and blood samples from CHC patients revealed an increase of ROS concentrations by two to five orders of magnitude [58, 59], suggesting a potential correlation between concentration of these compounds/enzymes and course of liver fibrosis. Also, Values of alanine aminotransferase

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(ALT) and aspartate aminotransferase (AST) positively correlate with various serum markers of oxidative stress [60]. Also, the alteration of hydroxyproline (Hyp) levels in liver, serum and urine is considered as an index of collagen metabolism and provides valuable information about the biochemical and pathologic events of hepatic fibrosis [64].

Progressive hepatic fibrosis with the development of cirrhosis is a feature in the majority of chronic liver disease cases. Therefore, liver fibrosis stage can be a significant predictive factor for mortality related to liver complications [39]. In HCV infected patients, histology is the gold standard for assessing the degree of hepatic fibrosis and for estimating prognosis [41]. Moreover, liver biopsy is not a perfect golden standard because it sometimes results in false positive and false negative diagnoses [6]. This hampers the primary determination of fibrosis and optimal management of therapy. Consequently, there is a need for non-invasive methods to accurately diagnose the presence of liver fibrosis and cirrhosis. Most previous studies on non-invasive diagnostics, including breath tests, serum biomarkers and transient elastography (TE), reliably determined the presence or absence of cirrhosis, but did not discriminate between the earlier stages of fibrosis very well [9, 15]. Non-invasive reliable biomarkers for diagnosing, grading hepatic fibrosis and monitor outcome of HCV infection treatment are active areas of clinical interest [11, 51].

The main objective of this study was to evaluate the diagnostic accuracy of AST, ALT, Hyp, malondialdehyde (MDA), superoxide dismutase (SOD), and total antioxidant status (TAS) biomarkers in comparison with Metavir scoring for assessing the severity of hepatic fibrosis in the CHC patients.

Materials and methods

Patients

This study included 250 adult subjects admitted to the outpatients of Gastroenterology Surgical Center, faculty of medicine, Mansoura University, Mansoura, Egypt. Out of these, 100 healthy individuals (70 men and 30 women, between 14 and 66 years of age) with a mean age of 38.6 ± 7.4 were selected as controls from a population undergoing standard annual physical examination and biological measurements for medical insurance and 150 treatment-naïve CHC patients who had undergone liver biopsy (100 men and 50 women, aged from 11 to 64 years of age) with a mean age of 39 ± 8.7 were included in this study. All gave their informed consent, which included undergoing a pretreatment of liver biopsy.

Patients selection

The inclusion criteria were: Patients who abstain from alcohol abuse for more than 6 months; with a proven HCV viremia, HCV RNA positivity and genotype determinations were selected. Liver biopsy was taken from patients prior to antiviral therapy or any other antifibrotic therapy. Serum marker levels (such as AST, ALT, SOD, MDA, TAS and Hyp) were performed on the day of biopsy or within 5 days after liver biopsy.

The exclusion criteria were: presence of HIV and/or HBV co-infection; other causes of chronic liver diseases; hepatocellular carcinoma and prior liver transplantation. Also, subjects with iron supplementation, overweight and obesity ($\text{BMI} \geq 25$ and $\geq 30 \text{ kg/m}^2$), previously received interferon therapy and insufficient liver biopsy were excluded from this study.

The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki, and was reviewed and approved by ethical committee of Gastroenterology Surgical center, Faculty of medicine, Mansoura University. All subjects completed a structured questionnaire with questions regarding demographic data, and daily medication use. The demographics and baseline characteristics of patients and controls are presented in (Table 1). Venous blood samples from each patient were collected either before the administration of preoperative drugs on the day of biopsy or within 5 days after biopsy. Samples were given a coded study identification number and were shipped frozen at -80°C for analysis.

HCV qualitative test

Diagnosis of chronic HCV was established by elevated alanine aminotransferase enzyme (ALT) levels in persons having HCV antibody (anti-HCV) by a third generation enzyme immunoassay (EIA). Samples were subjected firstly for the detection of HCV RNA qualitatively as previously describe by Abdel-Hamid et al. [1]. Reverse transcription PCR (RT-PCR) was done for the identification of HCV RNA. RNA was extracted from 100 μl patient's sera using Quigen RNA extraction kit according to the kit protocol. Nested PCR were performed using Taq DNA polymerase enzyme (Fermentas Technologies USA) in a volume of 20 μl reaction mix. The nested PCR products were visualized on 2 % agarose gel under "UV" light using "Uvitec" gel documentation system.

HCV quantitative test

HCV RNA quantification was done by using Smart Cycler II Real-time PCR (Cepheid, Sunnyvale, Calif., USA) with HCV RNA quantification kits (Sacace Biotechnologies,

Table 1 Demographic, laboratory and histological characteristics of 150 patients with chronic hepatitis C and control subjects

Characteristics	All CHC patients N, mean $\pm$ SD, (%)	Controls i, mean $\pm$ SD	<i>P</i> *
No.	150	100	
Age (year) ^a	39 $\pm$ 8.7	38.6 $\pm$ 7.4	0.001**
Sex (male/female) ^a	100/50 (76.9/23.1)	70/30	
BMI (kg/m ²) ^a	23.7 $\pm$ 3.4	24.7 $\pm$ 3.8	0.52
AST (IU/ml) ^b	63.56 $\pm$ 46.8	22.3 $\pm$ 6.3	0.001**
ALT (IU/ml) ^b	79.65 $\pm$ 58.51	28.3 $\pm$ 5.6	0.001**
Platelets (109/L) ^a	196 $\pm$ 56.5	250 $\pm$ 24.4	0.001**
HCV Genotypes			
4	145 (96.7)		
2,4	5 (3.3)		
Viral Load	21.6 $\pm$ 8.3		
Stage of Fibrosis, (METAVIR) <i>n</i> (%)	125/150 (83.3)		
F0	25 (16.7)		
F1	35 (23.3)		
F2	25 (16.7)		
F3	35 (23.3)		
F4	30 (20)		
Population, <i>n</i>	60 (40)		
F0–F1	90 (60)		
F2–F4	120 (80)		
F0–F3	30 (20)		
F4			
Mean length of liver biopsy core (LBC+SD)	16.8 $\pm$ 0.52 cm.		
Mean number of portal tracts (NoP + SD)	13 $\pm$ 4.5		
Necroinflammation			
A0–A1	80 (53.3)		
A2–A3	70 (46.7)		

SD standard deviation, BMI body mass index, ALT alanine amino-transferase, AST aspartate aminotransferase

^a Student *t* test

^b Mann–Whitney *U* test

P* for controls versus all HCV patients, **P* < 0.05, *P* < 0.01

Italy). The Smart Cycler II system is a PCR system by which amplification and diagnosis were accomplished at same time with Taq Man technology (Applied Biosystems, Foster City, Calif) using fluorescent probes to investigate amplification after each replicating cycle. The lower and upper detection limits of the used assay were 250 and 5.0×10^8 IU/ml, respectively. Specimens yielding values above the upper limit were diluted 100-fold, retested and the obtained values were multiplied by this dilution factor to get the actual HCV RNA concentration in international units (IU) per ml.

HCV genotyping

HCV genotypes were identified by reverse hybridization method using Line Probe assay (INNO-LiPA HCV II kit, Innogenetics, Swigdrecht, Belgium) according to the manufacturer's instructions.

Survey and laboratory data

A previously validated questionnaire was used to collect demographic and medical information. Laboratory test results used in this study, serum ALT, aspartate amino-transferase (AST) and blood platelet counts were all performed using standard methods. Serum malonaldehyde (MDA), superoxide dismutase (SOD) and total antioxidant status (TAS) were measured as described previously [22, 45, 53]. Serum hydroxyproline (Hyp; Cat. No. E0621Hu; Uscn Life Science Inc. Wuhan) was measured using commercially available bioassays. The ALT and AST indexes were calculated by dividing the patient's test results by the upper limit of normal (40 IU/l) for the test. The AST/platelet count ratio index (APRI) was calculated as AST index/platelet count divided by 10^3 times 100. The Hyp/platelet count ratio was calculated as the Hyp level/platelet count divided by 10^3 times 100 [15, 29, 47].

Liver biopsy

After submitting an informed consent document, all patients underwent a percutaneous liver biopsy to ascertain the diagnosis and their stage of liver injury. Biopsies were obtained using an automatic 16-gauge tru-cut needle (biopsy gun) which provides adequate specimens for evaluation and fewer cases with tissue fragmentations [50]. Liver biopsy specimens analyzed were at least 15–25 mm long with complete portal tracts (>10 CPTs). Formalin-fixed, paraffin-embedded sections were stained with hematoxylin and eosin and with Masson's Trichrome. Slides were labeled with patient identification numbers and then reviewed and graded blindly by a senior pathologist, the mean length of liver biopsy and the number of portal tracts were assessed (including only the complete, intact portal tracts). The degree of fibrosis was scored according to the METAVIR system, and no fibrosis was defined as F0, mild fibrosis as F1, moderate fibrosis as F2, severe fibrosis as F3, and cirrhosis as F4. Significant fibrosis was also defined as F2–F4. Hepatic inflammatory activity was also scored [37].

Statistical analysis

Statistical analysis was performed using the statistical package for social studies (SPSS) version 16 for windows. Patient baseline characteristics and results were descriptively

sum-marized and reported as mean $\pm$ standard deviation (SD) or number (percentage) of patients with a condition. Comparisons between groups were made using Student's *t* test or the Mann–Whitney *U* test for continuous variables. *P* values less than 0.05 were considered significant. The diagnostic performance of serum biomarkers for significant fibrosis and cirrhosis prediction was measured according to sensitivity, specificity, PPV and NPV parameters. They were expressed as percentage. The diagnostic value of the method was assessed by calculating the area under the curve ROC (AUROC) and their corresponding 95 % confidence intervals (CI).

Results

General characteristics and histological evaluation of the patients studied

One hundred and fifty patients with CHC were included in this study. The mean age of the patients was 39 ± 8.7 years and about 76.9 % were males. Based on genotype analysis, the most frequently detected genotype was 4 (97 %) along with 3 % of patients with mixed HCV genotype 2&4. The significant fibrosis was found in 83.3 % of CHC patients using the METAVIR system. Most patients had grade A0–A1 inflammation (53.3 %), while only a minority had grade A2–A3 with 46.7 %. On the other hand, biopsies from the majority of patients had stage F0–F1 (40 %) of mild fibrosis and stage (F2–4) (60 %) of significant fibrosis, while only about 20 % had cirrhosis (F4) (Table 1).

The mean length of liver biopsy core (LBC) and number of portal tracts were 16.8 ± 0.52 and 13 ± 4.5 cm respectively. There was statistical significance in the number of portal tracts towards grade of inflammation ($P = 0.001$) and fibrosis stages ($P = 0.001$). However, the size of liver biopsy core (LBC) only showed a significant correlation towards fibrosis stages ($P = 0.001$) in CHC patients (Table 2).

Laboratory variables and Age associated with the presence of significant fibrosis and cirrhosis showed increase in ALT ($P < 0.11$), AST ($P < 0.001$), AST/ALT ($P < 0.001$), hydroxyproline ($P < 0.001$), Hyp/platelet count ratio ($P < 0.001$), APRI ($P < 0.001$), MDA ($P < 0.001$), Age ($P < 0.001$), and decrease in SOD ($P < 0.001$), TAS ($P < 0.001$), and platelet count ($P < 0.001$) in patients with cirrhosis (F4) compared to other stages of liver fibrosis (Table 3).

Prediction of significant fibrosis

We analyzed the data comparing the different biomarkers to hepatic fibrosis using ROC curves. The results confirmed

Table 2 Distribution of length of liver biopsy core (LBC) and number of portal tracts (NoP) with grades of inflammation and stages of fibrosis

Grade of inflammation	Mean length of LBC $\pm$ SD, cm	<i>P</i>	Mean number of portal tracts NoP $\pm$ SD	<i>P</i>
A0–A1(<i>n</i> = 80) ^a	13.7 ± 0.3	0.12	9.7 ± 3.6	0.001
A2–A3(<i>n</i> = 70) ^a	15.7 ± 2.8	0.12	11.6 ± 3.9	0.001
Stage of fibrosis				
F0–F1 ^a	14.5 ± 1.7		9.8 ± 2.7	
F2 ^a	15.9 ± 1.5	0.01	12.7 ± 3.4	0.001
F3 ^a	16.8 ± 0.9	0.01	13.6 ± 1.8	0.001
F4 ^a	17.3 ± 1.2	0.01	16.7 ± 4.7	0.001

^a Mann–Whitney *U* test

* $P < 0.05$, ** $P < 0.01$

that APRI, AST/ALT, HypI, MDA, SOD and TAS were predictive of level of hepatic fibrosis. The AUROC curves of APRI, AST/ALT, HypI, MDA, SOD and TAS to predict significant fibrosis (F2–4) were 0.88, 0.73, 0.79, 0.94, 0.80 and 0.84, respectively. Together using APRI and AST/ALT cut-off values, liver fibrosis could be predicted in 80 % of patients. In the same manner, using hydroxyproline and MDA cut-off values liver, fibrosis could be predicted in 90 % of patients. However, SOD and TAS cut-off values could predict liver fibrosis in 96.7 and 86.7 % of HCV patients respectively (Tables 4, 5).

Prediction of cirrhosis

We analyzed the data comparing the different biomarkers to hepatic fibrosis using ROC curves. The results confirmed that APRI, AST/ALT, HypI, MDA, SOD and TAS were predictive of level of hepatic cirrhosis. The AUROC curves of APRI, AST/ALT, HypI, MDA, SOD and TAS to predict significant fibrosis (F2–4) were 0.912, 0.83, 0.92, 0.81, 0.89 and 0.92, respectively (Table 6). Together using APRI and AST/ALT cut-off values, liver fibrosis could be predicted in 73.3 and 80 % of patients respectively. In the same manner, using hydroxyproline and MDA cut-off values liver, fibrosis could be predicted in 93.3 and 67 % of patients respectively. However, SOD and TAS cut-off values could predict liver fibrosis in 83.3 and 81.3 % of HCV patients respectively (Table 6).

Discussion

Degree of Hepatic fibrosis is the important factor for initiation of treatment in patients of hepatitis C. HCV is the most serious form of infection which accounts for high proportion of liver diseases throughout the world. There are

Table 3 Comparison of variables associated with the presence of significant fibrosis and cirrhosis

Variables	Significant fibrosis		<i>P</i>	Cirrhosis		<i>P</i>
	F0–F1 Mean $\pm$ SD	F2–F4		F0–F3 Mean $\pm$ SD	F4	
Age (year) ^a	32 $\pm$ 2.5	46 $\pm$ 8.7	0.001**	37 $\pm$ 5.2	52 $\pm$ 1.3	0.001**
AST (IU/ml) ^b	69.7 $\pm$ 14.6	80.5 $\pm$ 22.0	0.001**	74.8 $\pm$ 16.4	110.5 $\pm$ 27.8	0.001**
ALT (IU/ml) ^b	96.3 $\pm$ 26.9	118.4 $\pm$ 17.6	0.11	105.3 $\pm$ 28.5	134.3 $\pm$ 27.14	0.11
Platelets (109/L) ^a	231.8 $\pm$ 29.2	212.3 $\pm$ 12.5	0.001**	200.3 $\pm$ 19.5	158.4 $\pm$ 84.5	0.001**
Hydroxyproline (μ g/ml) ^b	1.44 $\pm$ 0.7	7.9 $\pm$ 2.0	0.001**	11.24 $\pm$ 2.35	15.2 $\pm$ 2.2	0.001**
MDA (μ mol/l) ^b	6.47 $\pm$ 0.84	18.95 $\pm$ 2.12	0.001**	29.6 $\pm$ 6.3	42.3 $\pm$ 5.4	0.001**
SOD (Units/l) ^b	39.1 $\pm$ 7.1	26.4 $\pm$ 4.3	0.001**	20.3 $\pm$ 4.1	13.8 $\pm$ 3.25	0.001**
TAS (μ mol/l) ^a	615.8 $\pm$ 117	520 $\pm$ 74.1	0.001**	410 $\pm$ 77.3	336 $\pm$ 83.6	0.001**
AST/ALT ratio ^a	0.7 $\pm$ 0.49	0.98 $\pm$ 0.88	0.001**	1.65 $\pm$ 0.93	3.7 $\pm$ 2.58	0.001**
APRI ^a	0.6 $\pm$ 0.3	1.43 $\pm$ 0.22	0.001**	1.64 $\pm$ 0.18	2.5 $\pm$ 0.4	0.001**
Hyp index	1.69 $\pm$ 0.3	4.47 $\pm$ 0.95	0.001**	6.5 $\pm$ 1.4	11.7 $\pm$ 1.36	0.001**
Population, <i>n</i> (%)	60 (40)	90 (60)		120 (80)	30 (20)	

SD standard deviation, APRI AST to platelet ratio index, Hyp Index hydroxyproline to platelet ratio index, ALT alanine aminotransferase, AST aspartate aminotransferase

^a Student *t* test

^b Mann–Whitney *U* test

* *P* < 0.05, ***P* < 0.01

Table 4 Area under receiver operating characteristic (AUROC) curves of fibrosis tests in the prediction of significant fibrosis and cirrhosis

Serum markers	Significant fibrosis (F0–1 vs F2–4)		Cirrhosis (F0–3 vs F4)	
	Area	95 % CI	Area	95 % CI
AST/ALT ratio	0.73*	0.70–0.85	0.83**	0.77–0.91
APRI	0.88*	0.82–0.940	0.912*	0.82–0.932
Hyp Index	0.79**	0.70–0.85	0.92*	0.82–0.940
MDA	0.94**	0.89–0.99	0.81*	0.61–1.0
SOD	0.80*	0.67–0.93	0.89*	0.80–0.98
TAS	0.84*	0.72–0.96	0.92*	0.83–1.00

SD standard deviation, APRI AST to platelet ratio index, Hyp Index hydroxyproline to platelet ratio index, ALT alanine aminotransferase, AST aspartate aminotransferase

* *P* < 0.05, ***P* < 0.01

two major consequences of hepatitis C infection in liver, fibrosis and necroinflammatory activity [8].

Assessment of the degree of hepatic fibrosis is essential for estimating clinical outcome and deciding antiviral therapy for chronic HCV infection [46, 48]. Although liver biopsy remains the gold standard method for the assessment of hepatic fibrosis, it has some limitations [43, 52], hence, there is a need to develop accurate and reliable noninvasive means to assess the severity of hepatic fibrosis [42]. Realizing this need several non invasive biochemical markers for assessing the severity of hepatic fibrosis stage

and monitoring the degree of liver damage in patients of chronic hepatitis C have been developed [18].

Noninvasive methods to measure severity of liver injury are clinically important in Egypt where advanced liver disease from HCV is common and access to liver biopsy is limited [54]. Many of the reports evaluating biomarkers for detecting hepatic fibrosis have used scoring systems encompassing combinations of results from several blood tests and demographic data [19, 38]. Most of the indexes proposed in these studies would not be practical in Egypt and other developing countries because of cost and unavailability of some tests.

For this reason we evaluated a few blood tests routinely performed on patients with chronic HCV in addition to the levels of hydroxyproline (Hyp), malondialdehyde (MDA), superoxide dismutase (SOD), and Total antioxidant status (TAS) using commercially available tests for measuring hepatic fibrosis in CHC patients. The diagnostic performance of the AST-to platelet ratio index (APRI), AST-to ALT ratio index Hyp index, SOD and TAS to predict significant fibrosis and cirrhosis were also evaluated in our CHC patient.

In the present study, the significant fibrosis was found in 125 (83.3 %) of CHC patients using the METAVIR system. Ninety (60 %) patients had significant fibrosis (F2–F4), 30 (20 %) had cirrhosis (F4), and 60 (40 %) (F0–F1) had Mild or no fibrosis. Most patients had grade A0–A1 inflammation (53.3 %), while only a minority had grades A2–A3 with 46.7 %. It was found that HCV infection has a rapid course

Table 5 Diagnostic accuracy of tests in the prediction of significant fibrosis (F2–4)

Tests	Total (<i>n</i>)	Fibrosis		Sensitivity (95 % CI) ^a	Specificity. (95 % CI) ^a	Positive predictive value, (95 % CI) ^a	Negative predictive value, (95 % CI) ^a
		F0–F1 (<i>n</i> = 60) (40 %)	F2–F4 (<i>n</i> = 90) (60 %)				
AST/ALT							
≤0.65	60	45	15	85 (56–96)	56 (25–75)	68 (36–85)	81 (56–96)
>0.65	90	40	50				
≤0.72	90	60	25	46 (38–86)	95 (86–100)	89 (65–94)	57 (53–86)
>0.72	60	14	46				
APPRI							
≤0.5	65	40	25	96 (72–100)	92.1 (56–96)	78 (75–98)	72 (59–96)
>0.5	85	65	20				
≤1.5	95	45	25	92 (73–98)	94 (87–100)	86 (81–100)	90 (46–95)
>1.5	55	15	40				
Hyp index							
≤1.7	85	70	15	87 (81–100)	94 (87–100)	93 (73–98)	75 (54–96)
>1.7	65	50	15				
≤4.5	95	45	25	94 (87–100)	90 (73–97)	91(88–98)	85 (79–100)
>4.5	50	10	40				
MDA							
≤6.5	65	45	20	96 (81–100)	76 (72–100)	94 (73–98)	68.6 (59–96)
>6.5	85	60	25				
≤19.0	80	70	10	93 (86–100)	89 (87–100)	91 (86–98)	76 (72–96)
>19.0	70	25	45				
SOD							
≥45.0	80	50	30	78 (75–100)	89 (87–100)	86 (73–97.8)	69 (52–96)
<45.0	70	46	24				
≥18.5	85	60	25	87.5 (81–100)	95 (88–100)	82 (73–98)	78.6 (76–96)
<18.5	65	25	40				
TAS							
≥520	60	45	15	97 (81–100)	84 (76–100)	93 (84.6–98)	79 (73–96)
<520	90	60	30				
≥410	80	50	30	89 (83–100)	91 (68–100)	86 (78–98)	72 (62–96)
<410	70	20	50				

^a 95 % confidence interval

of disease progression in chronic hepatitis C patients [7, 30]. The data obtained supported with measuring the mean length of liver biopsy core (LBC) and number of portal tracts. There was statistical significance in the number of portal tracts towards grade of inflammation ($P = 0.001$) and fibrosis stages ($P = 0.001$). However, the size of liver biopsy core (LBC) only showed a significant correlation towards fibrosis stages ($P = 0.001$) in CHC patients. The results of this study suggested that the number of portal tracts is more important than the size of liver biopsy core (LBC) in reporting the grade of inflammation and stage of fibrosis in patients with chronic hepatitis C infection. The advancement in stage of fibrosis was directly proportional to the number of portal tracts and LBC in a liver biopsy core

which was statistically significant. Similarly, it has shown that degree of fibrosis increases with increase in length of specimen as in this study [14], this may also relate to the use of automated cutting needles which likely provide adequate specimens with fewer tissue fragmentations [50].

Although elevated levels of ALT and AST enzymes usually correlate with hepatic inflammation, they don't appear to give much information about the progression of fibrosis in chronic hepatitis C. So, the role of liver enzymes in the assessment of chronic hepatitis C remains important for the majority of clinical indexes estimating the degree of liver fibrosis [23].

In this study, all serum markers along with the older age associated with significant fibrosis and cirrhosis showed

Table 6 Diagnostic accuracy of tests in the prediction of cirrhosis (F4)

Tests	Total (<i>n</i>)	Fibrosis		Sensitivity (95 % CI) ^a	Specificity. (95 %CI) ^a	Positive predicative value, (95 % CI) ^a	Negative predictive value, (95 % CI) ^a
		F0–F3 (<i>n</i> = 120) (80 %)	F4 (<i>n</i> = 30) (20 %)				
AST/ALT							
≤0.85	50	45	5	84 (54.4–96)	48 (25–75.3)	65.6 (37–85)	69.8 (54.4–96)
>0. 85	100	70	30				
≤0.98	80	60	25	76.5 (35.4–85)	92 (86–100)	86 (64–93)	58 (53–84.1)
>0.98	70	45	25				
APPRI							
≤1.0	79	39	40	98 (72–100)	66 (56–96)	78.4 (73–98)	67 (59–96)
>1.0	71	55	16				
≤2.0	110	45	25	93 (73–98)	94 (87–100)	86 (81–100)	57 (46.2–95)
>2.0	40	10	30				
Hyp index							
≤6.5	60	56	4	87 (81–100)	94 (87–100)	92 (73–98)	59 (52–96)
>6.5	90	65	13				
≤8.5	70	45	15	94 (86.8–100)	75.3 (73–97)	64 (45–85)	84 (79.4–100)
>8.5	80	15	65				
MDA							
≤9.7	45	35	10	96 (81–100)	76 (72–100)	94 (73–97.8)	69 (59–96)
>9.7	105	60	45				
≤28.0	95	70	10	92 (86–100)	89 (87–100)	91 (86–98)	76 (73–96)
>28.0	55	15	30				
SOD							
≥32.0	70	60	10	77 (75–100)	90 (86–100)	86 (73–97.8)	69 (52–96)
<32.0	80	52	28				
≥12.5	95	63	32	89 (81–100)	92 (87–100)	82 (73–97.8)	79 (76–96)
<12.5	55	10	45				
TAS							
≥390	62	40	22	96 (89–100)	84 (76–100)	91 (85–98)	79 (72–96)
<390	88	70	18				
≥350	90	50	40	89.5 (83–100)	90 (68–100)	86 (78–98)	69 (62–96)
<350	60	25	35				

^a 95 % confidence interval

increase in ALT ($P < 0.11$), AST ($P < 0.001$), age ($P < 0.001$), and decrease in platelet count ($P < 0.001$) of patients with cirrhosis (F4) compared to other stages of liver fibrosis. It was reported a significant change in liver functions and reduction in platelet count due to the progression of liver fibrosis in CHC patients with older ages [35]. However, elevated ALT showed no correlation with significant fibrosis and cirrhosis, this may be due to older age and HCV viral load among HCV patients [2].

In our study, the area under the ROC curves of AST/ALT ratio for predicting significant fibrosis and cirrhosis were 0.73 (0.70–0.85) and 0.83 (0.77–0.91), respectively. The diagnostic accuracy of significant fibrosis and cirrhosis

in accordance to liver biopsy were reported in 80 % of CHC patients. This matched with other study which showed that platelet count, AST level, and AST/ALT ratio were important predictors of either significant fibrosis or cirrhosis [16]. Although another study confirmed the value of AST/ALT ratio in predicting cirrhosis [21], its accuracy varies widely among studies, with positive predictive values ranging from 0.64 to 1.00, and negative predictive values ranging from 0.72 to 0.88, respectively. However, another studies reported that, AST/ALT ratio alone has not been shown to be useful in predicting significant fibrosis [36]. Also, the diagnostic accuracy of significant cirrhosis in our patients infected with HCV shows a close correlation

to the average of Oberti et al. [33] and Nunes et al. [32], who reported a diagnostic accuracy of 86 % for detecting fibrosis and cirrhosis in HCV infected patients.

APRI is the simplest test with acceptable diagnostic accuracy. It employs routine lab parameters that are AST and platelet count to predict significant fibrosis as well as cirrhosis [62].

In our study, the AUROCs of the APRI for predicting significant fibrosis and cirrhosis were 0.88 (0.82–0.94) and 0.912 (0.82–0.93), respectively. Using APRI cut-off values, the diagnostic accuracy of significant fibrosis and cirrhosis in accordance to liver biopsy were reported in 80 and 73.2 % of CHC patients with a specificity of 92 and 93 % respectively. The data obtained were comparable to that of Wai et al. [62] and Silva et al. [51] who reported that APRI score of 0.88 and 0.92 (0.80–0.96) for predicting of significant fibrosis in 44 % of CHC patients. However latest studies comparing APRI with fibrotest and hepascor have shown lower accuracy rates than our data [10, 57].

Although the predictive value of both AST/ALT ratio and APRI have a prognostic values that were significantly higher than random but significantly lower than the prognostic value of other indices [28, 61], this may be due to low performance and that they include transaminases in their scores, which induces an interaction with necroinflammatory activity grades [40], and that, the variation in APRI or AST/ALT ratio results may be related to necrosis grade rather than fibrosis stage [26].

Therefore, the search for additional biomarkers was an aim of our study for achieving new non-invasive diagnostic parameters for hepatic fibrosis.

So, in the present study we focused on hydroxyproline, malondialdehyde (MDA), SOD, and TAS biomarkers as new targets for hepatic fibrosis.

Serum hydroxyproline showed significant ($P < 0.001$) increase in CHC patients. The data obtained were correlated with the stage of liver fibrosis. This reflects the extensive degradation of newly synthesized collagen in the liver by collagenolytic enzymes during the early stages of fibrosis in patients with chronic liver diseases [27], and elevation in the level of hydroxyproline argue its importance as good marker for staging of hepatic fibrosis [24].

In our study, the AUROCs of the hydroxyproline (Hyp) for predicting significant fibrosis and cirrhosis were 0.79 (0.70–0.85) and 0.92 (0.82–0.94), respectively. Together, using Hyp cut-off values, the diagnostic accuracy of significant fibrosis and cirrhosis in accordance to liver biopsy were reported in 90) and 93.3 % of CHC patients with a specificity of 94 % respectively. The data obtained were in accordance with Attallah et al. [3] who correctly classified 82 % of CHC patients with severe liver fibrosis using fibrosis discriminate score (FDS). Also, Lee et al. [24] and other previous reports [3], noted significant increment in

the level of hydroxyproline as the fibrosis score increased in CHC patients.

In the current study, a significant rise ($P < 0.0001$) in serum MDA and decrease ($P < 0.0001$) in the level of antioxidant enzyme (SOD) and total antioxidant status (TAS) were reported in CHC patients with cirrhosis (F4). It was found that HCV infection is characterized by remarkable levels of oxidative stress radical (MDA) and decrease in SOD activity [17, 56].

In our study, the AUROCs of MDA for predicting significant fibrosis and cirrhosis were 0.94 (0.89–0.99) and 0.81 (0.61–1.0), respectively. Together, using MDA cut-off values, the diagnostic accuracy of significant fibrosis and cirrhosis in accordance to liver biopsy were reported in 90 and 67 % of CHC patients respectively. The increase in oxidative stress commonly promotes liver fibrosis and excessively produces twofold increase of hepatic collagen via hepatic stellate cells (HSCs). The over production of collagen was accompanied with changes in liver markers and the corresponding histological stage [4, 44].

In our study, the AUROCs of SOD for predicting significant fibrosis and cirrhosis were 0.80 (0.67–0.93) and 0.89 (0.80–0.98), respectively. Together, using SOD cut-off values, the diagnostic accuracy of significant fibrosis and cirrhosis in accordance to liver biopsy were reported in 83.3 and 96.7 % of CHC patients respectively. The importance of oxidative stress markers as non-invasive parameters for the assessment of liver fibrosis were recently reported [31, 63], whereas glutathione (GSH) was used as non-invasive marker to predict liver fibrosis stages.

In our study, the AUROCs of TAS for predicting significant fibrosis and cirrhosis were 0.84 (0.72–0.96) and 0.92 (0.83–1.00), respectively. Together, using TAS cut-off values, the diagnostic accuracy of significant fibrosis and cirrhosis in accordance to liver biopsy were reported in 86.7 and 81.3 % of CHC patients respectively. The data obtained revealed like others, that oxidative stress markers, antioxidant enzymes and hydroxyproline have been documented to play a part in the pathogenesis of CHC [5, 13, 20, 34].

In our population, using optimized cut-off values of AST/ALT, APRI, Hyp, MDA, SOD, and TAS significant fibrosis could be predicted accurately in 80, 80, 90, 90, 83.3, and 86.7 % of CHC patients respectively. Also, cirrhosis was predicted correctly in 80, 73.2, 93, 67, 97, and 81.3 %, respectively of 150 CHC patients. Application of these Indexes may decrease the need for staging liver biopsy specimens among HCV patients. Oxidative stress and hydroxyproline markers could be useful as non-invasive diagnostic markers in the assessment of hepatic fibrosis. This may play a role in predicting severity of liver disease and possibility of treatment response in chronic HCV infection.

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