Can Fibrogenesis Markers Reflect Early Hepatic Histopathology in Chronic Hepatitis C?

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Background and study aim: Chronic HCV can progress to cirrhosis, and HCC. Liver biopsy is the best to assess and monitor progression. But it has limitations.The aim of this study was to evaluate noninvasive indicators of fibrogenesis, matrix metalloproteinase-2 (MMP-2), tissue inhibitor of metalloproteinase-1 (T1MP1) and AST/Platelet (APRI-score) for assessment of early hepatic histopathology in chronic-HCV, and cirrhosis.

Patients and methods: A cross section study included 344 participants from Tanta University Hospitals, (GI): 129 asymptomatic chronic-HCV, (GII): 135 with symptoms. (GIII): 80 patients with compensated HCV-related cirrhosis and 30 healthy controls. Investigations proved diagnosis, and excluded associated diseases. APRI-Score was evaluated. Quantitative immunoassay measured serum MMP-2 and TIMP-1, guided liver biopsy for histopathology staging and grading.

Results: Serum MMP-2& TIMP-1 showed significant difference between control& GI, GII, GIII, and GI, GII& GIII (P< 0.001) with significant correlation between GI& GIII, between GII & GIII, while insignificant between G1 & GII. There was significant positive correlation between APRI-score versus Metavir A, Metavir F, Ishak score of fibrosis, serum MMP-2 and serum TIMP-1 (P <0.001). Combined serum MMP-2, TIMP-1 and APRI-score showed high sensitivity, and specificity.

Conclusion: This combination of markers raised the sensitivity, specificity and correlations. It could reflect early hepatic histopathology, developing cirrhosis and potentially could replace liver biopsies in pre-treatment, follow up of chronic-HCV, and screening of asymptomatic patients.

INTRODUCTION

HCV infection has an estimated prevalence of 3% around the world [1], and Egypt is among the highest prevalence [2].

Asymptomatic HCV patients are underrepresented. Unfortunately, many persons with HCV infection are asymptomatic [3]. Many asymptomatic seropositive donors have clinically significant liver disease [4]. The progression to severe fibrosis and occurrence of HCC were reported [1, 2]. Patients with normal enzymes may have definite chances of chronic hepatitis on histological examination [6].

Percutaneous liver biopsy, is the gold standard for grading and staging liver diseases [7], but it is invasive, has limitations [8], and asymptomatic patients may not accept.

The matrix metalloproteinases (MMPs), and their inhibitors are groups of proteins involved in controlling matrix degradation. Therefore, it seems that imbalance between MMPs and TIMPs affects rate of fibrosis progression, and their estimation was correlated with the stage of fibrosis [9].

Aspartate aminotransferase to platelet ratio (APRI Score) was proved also useful to stage liver fibrosis [10]. It is an easy and validated predictor of hepatic fibrosis in chronic hepatitis C [11].

Non invasive diagnosis of liver fibrosis and cirrhosis in chronic hepatitis C, is required in pre-treatment and follow up [12]. So, we aimed to evaluate individual and combined non invasive indicators of fibrogenesis (MMP-2, T1MP1 and

APRI score) to assess early hepatic histopathology, and developing cirrhosis in chronic HCV patients with/without symptoms.

PATIENTS AND METHODS

A cross sectional study included 344 participants: (GI) 129 asymptomatic chronic HCV, (GII) 135 with HCV specific symptoms (abdominal pain, fatigue, tinge of jaundice etc…) and (GIII) 80 patients with compensated HCV-related cirrhosis in addition to 30 proved healthy subjects as control. They were collected between the years 2010 and 2013 from Department of Hepatolog, GIT & Infectious Diseases which is a pooling center for patients with viral hepatitis, and Tanta University blood bank with matched age and sex. Asymptomatic patients were selected from those accidentally discovered blood donors, pre-employment and during check up of cases contacts or pre-travel. They may have mild elevated liver functions or even average.

Exclusion criteria:

Patients proved, with associated diseases as other causes of hepatitis, decompensated liver cirrhosis, collagen diseases, blood diseases and active schistosomiasis were excluded.

Quantitative detection of serum matrix metalloproteinase (MMP-2) in patients with chronic HCV using Immunoassay kits R&D Systems Inc. McKinley Place N.E. Minneapolis, USA [13].

Quantitative detection of human TIMP-1 in the serum of patients, with chronic HCV using Flow Cytomix Human TIMP-1 Simplex Kit, Bender MedSystems GmbH, Campus Vienna Biocenter 2, A-1030 Vienna, Austria [14]. Sample collection and storage: a serum separator tube was used and allowed blood sample to clot 30 minutes. Once clotted, samples are centrifuged at 1000 x g for 10 min. Carefully remove serum and assay immediately or aliquot and store samples at <-20 c. Freeze/ thaw cycles were avoided.

APRI score (AST/Platelet ratio) was calculated after liver functions and blood picture

Guided liver biopsy: After completion of proper abdominal sonographic scanning, and checking the patient for any bleeding tendencies, and patient's consent, A18 G true- cut needle was set on a probe guide and a gun was used for biopsy. The biopsy was preserved in diluted formalin solution and sent for histopathological examination.

Histopathological examination of liver biopsy: The length of each histological specimen was 1.5 to 2.5 cm, and all specimens were placed in 40 g/L methanol for fixation immediately. After dehydration, they were embedded in paraffin, and sections were then stained with Hematoxylin-Eosin and Masson trichrome. The histological changes were examined under light microscopy for type, degree and activity of hepatic affection.

Statistical Analysis:

Was conducted, using the mean, standard deviation, by SPSS® V.16. Values were compared between groups by using the student's t-test. ANOVA test was used for comparison of quantitative data: has significant value: ($P < 0.05$*) and ($P < 0.001$**) is highly significant but insignificant: ($P > 0.05$).

RESULTS

There was insignificant difference between the studied groups as regard to age and sex ($P>0.05$).

Comparison between the studied groups in relation to serum MMP-2 and TIMP-1 (Table: 1 and Figure: 1a, b) showed a significant difference between control & GI, GII and GIII ($P < 0.001$) and G1, GII & GIII ($P < 0.001$) while insignificant between GI &GII ($P > 0.05$).

Correlation between AST/PLAT ratio (APRI score) and different scores of fibrosis, MMP2 and TIMP1 (Table: 2 and Figure 2a, b) showed significant positive correlation between APRI score and Ishak, Metavir A, Metavir F scores of fibrosis, serum MMP-2 and serum TIMP-1 ($P<0.001$*).

Histopathological staging and grading (Figure 3a) showed liver section from chronic-HCV patient showed ground glass, portal inflammation, fibrosis and piece meal inflammation (stage 2, grade 3) and (Figure 3b) showed porto-central fibrosis.

Inividual markers: MMP-2 (table: 3a), TIMP-1 (table: 3b) and APRI score (table: 3c) were compared as regard to AUC (Area under the curve), sensitivity and specificity. TIMP-1 was the highest.

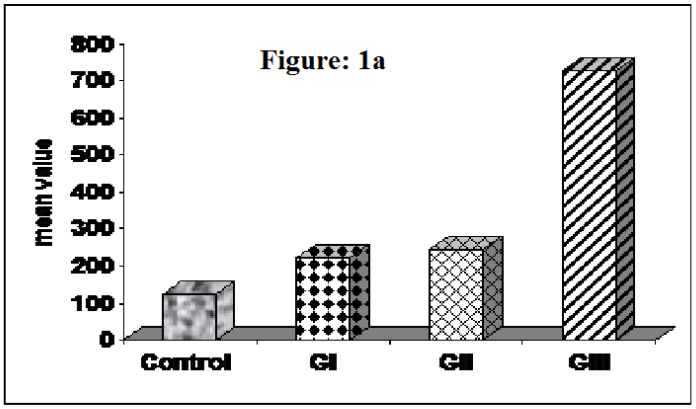
Combined markers: MMP-2 plus TIMP1 against APRI score in table 4a, combined APRI score plus MMP2, and TIMP1 (table: 4b) raised

sensitivity and specificity than any single marker, or combined MMP-2 and TIMP1. AUC indicated excellent test.

Table 1: Comparison between the studied groups in relation to serum MMP-2 and TIMP-1.

MMP-2:	Range	Mean + SD	p. value
Control	93-255	125.4±61.31	
GI	120-415	226.3±57.2	0.001*
GII	130-607	235.1±91.5	
GIII	520-936	727.5±121.5	
P1	0.314		
P2	0.001*		
P3	0.001*		
TIMP-1:			
Control	9-278	139.25±135.63	0.001*
GI	340-940	617.6±1569.04	
GII	200-966	691.3±196.1	
GIII	1400-2610	1878±317.2	
P1	0.517		
P2	0.001*		
P3	0.001*		

Figure 1: Comparison between the studied groups in relation to serum MMP-2 (1a) and TIMP-1 (1b).



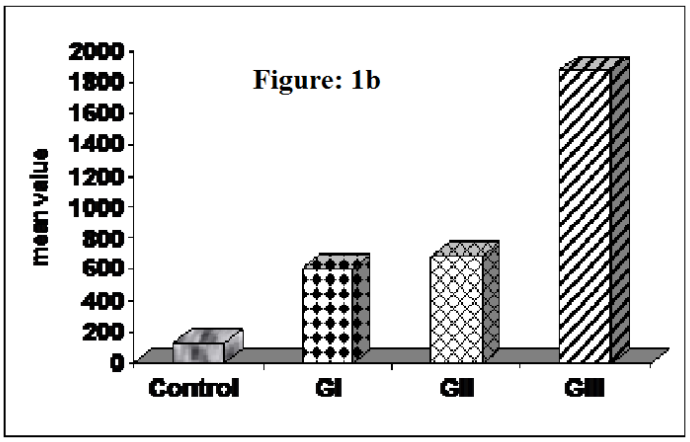
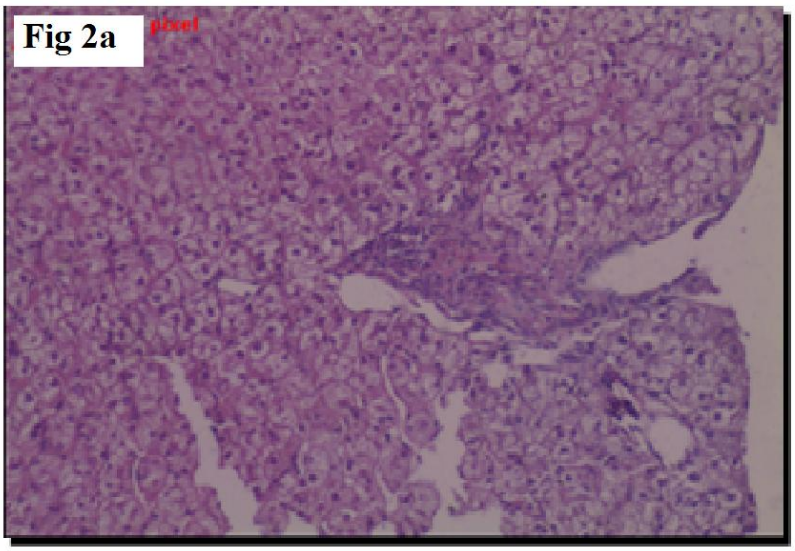
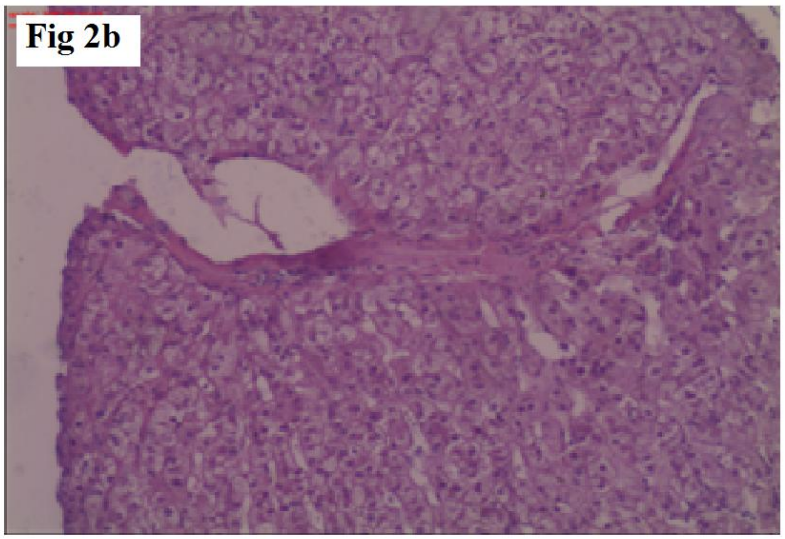


Table 2: Correlation between AST/PLAT ratio (APRI score) and different scores of fibrosis, MMP2 and TIMP1

	AST/Platelets	
	r.	**p. value**
ISHAK	0.514	0.001
METAVIR A	0.621	0.001
METAVIR F	0.471	0.001
MMP2	0.482	0.001
TIMP1	0.564	0.001

Figure 2a: liver section (HX &E) from chronic HCV patient showed ground glass, portal inflammation, fibrosis and piece meal inflammation (stage 2, grade 3) and (**Figure 2b**) showed porto-central fibrosis.





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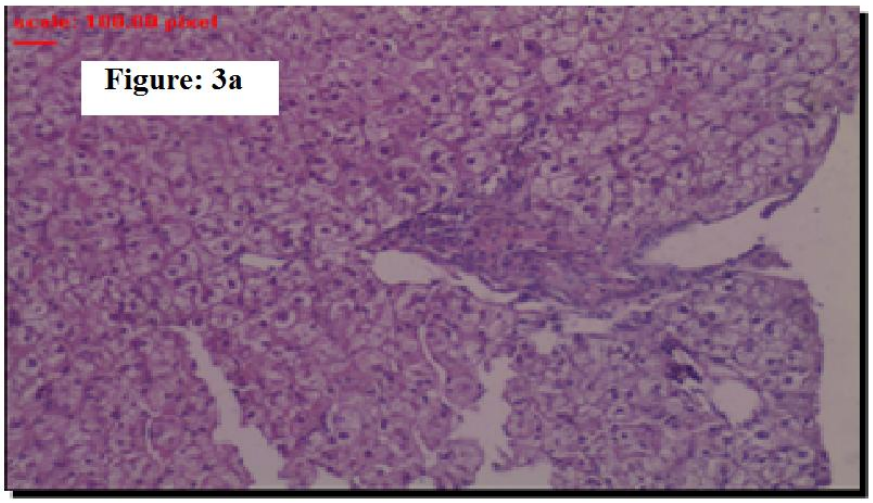

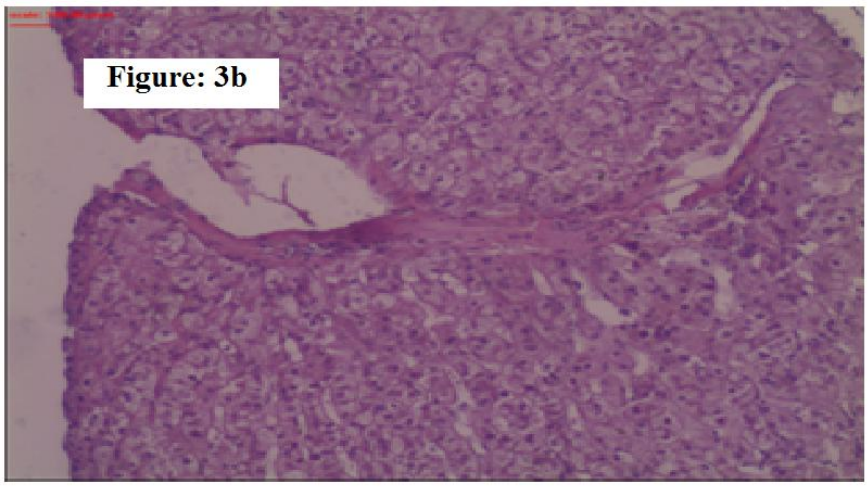

Table 3: AUC, Sensitivity and Specificity of individual markers: APRI score, MMP2 and TIMP-1 & Metavir A and Metavir F

Table 3a: APRI score & Metavir A and Metavir F

	AST/platelets (APRI score)		
	AUC	Sensitivity	Specificity
METAVIR A	0.752	90%	88%
METAVIR F	0.796	92%	86%

Table 3b: MMP-2 & Metavir A and Metavir F

	MMP2		
	AUC	Sensitivity	Specificity
METAVIR A	0.869	89%	85%
METAVIR F	0.874	87%	86%

Table: 3c: TIMP-1 & Metavir A and Metavir F

	TIMP1		
	AUC	Sensitivity	Specificity
METAVIR A	0.925	91%	92%
METAVIR F	0.936	95%	89%

AUC = Area Under the Curve AUC=1.0 indicate perfect test,
0.9 to 0.99 indicate excellent test 0.8 to 0.89 indicate good test
0.7 to 0.79 indicate fair test, 0.6 to 0.69 indicate poor test and 0.5 indicate worthless test

Table 4: AUC, Sensitivity and Specificity of Combined markers:

Table 4a: Showed APRI score & combined MMP2& TIMP1

	AST/platelet (APRI score)		
	AUC	Sensitivity	Specificity
MMP2& TIMP1	0.947	96%	94%

Table 4b: Showed Combined AST/platelets, MMP2 and TIMP1 & Metavir A and Metavir F

	Combined AST/platelets, MMP2 and TIMP1		
	AUC	Sensitivity	Specificity
METAVIR A	0.925	95%	94%
METAVIR F	0.917	96.1%	97%

AUC indicate excellent test with highest sensitivity and specificity

DISCUSSION

In the current study, all groups showed insignificant difference as regard to age, and sex as fibrosis progression was influenced by duration of infection and the age at time of infection [15].

The present results of AST and ALT showed significant difference between GI and both: GII and GIII, also between GII and GIII. This means that asymptomatic patients had lower levels. AST findings disagree to some extent with the results of Wendy et al, [16] who detected no correlation between severity of symptoms and AST levels. This could be related to the younger age of their patients and the possibility of short period of exposure. We detected, also significant correlation of AST, with the stage of fibrosis in the studied patients which may be attributed to reduced clearance of AST by liver as fibrosis progress [17], and/or mitochondrial injury of active necrosis [18]. This finding was in consistent with the results mentioned that liver fibrosis severity and subsequent cirrhosis were correlated with high AST levels [19].

The results of platelet count showed significant difference between GI & G III and between GII & GIII but not between GI & GII, so platelets decreased significantly in severe fibrosis or cirrhosis and these results were in agreement with the previous results [20]. Thrombocytopenia may be related to the bone marrow supprression due to HCV infection as direct viral replication and/or in association with immune complexes deposition in bone marrow [21], and the decreased production of diseased liver to thrombopiotin. Decreased platelet count was the earliest indicator of cirrhosis [22].

The current study showed a significant positive correlation between fibrosis stage and grade of inflammation. These findings were in accordance with previous reports [23]. This could be attributed to that the necroinflammatory process implicated in fibrogenesis process and activation of stellate cells around the necroinflammatory lesions. Thus, severe degrees of inflammatory activity can predict worsening of hepatic fibrosis [24].

As regard to direct serum fibrogenesis markers, which reflect extracellular matrix turnover: MMP-2 & TIMP-1were used to assess stage of fibrosis, instead of invasive liver biopsy. Our MMP-2 results showed significant difference between GI & GIII and between GII & GIII, but insignificant difference between GI & GII. This means that serum MMP-2 was significantly higher in cirrhotic patients, significant positive correlation between serum MMP-2 and the stage

of fibrosis, and Ishak score of fibrosis. This was in accordance with the results of Abdel-Samea et al., [20]. This could suggest that MMP-2 reliably to differentiate between cirrhotic and non cirrhotic particularly when liver biopsy has obstacles.

TIMP-1 showed significant difference between GI & GIII, and between GII & GIII but no significant difference between GI & GII. This means that serum TIMP-1 was significantly higher in cirrhotic patients than that of chronic hepatitis patients in accordance with Badra et al., [25]. A significant correlation was noticed between serum TIMP-1 and the stage of fibrosis and this finding was in consistent with their results. It was mentioned, also that serum TIMP-1 values can detect fibrosis with comparable efficiency, which was correlated with histological activity [9, 26].

The efficacy of TIMP-1 in detection of stage of fibrosis could be attributed to that TIMP-1 is produced mainly by activated hepatic stellate cells, and kupffer cells [27]. It is well established that activation of hepatic stellate cells, is a key event in the pathophysiology of hepatic fibrosis, and is accompanied by induction of TIMP-1[28]. TIMP-1 has been suggested as a profibrogenic factor to promote liver fibrosis.

APRI score was reported to have correlations with the stages of histological fibrosis [29], in agreement with the present results .While Khairy et al, showed that APRI score had moderate degree of accuracy [10]. Ma et al, considered it as a tool with limited expense, widespread availability, a promising noninvasive alternative to liver biopsy for detecting hepatic fibrosis and treatment response in patients with chronic hepatitis C [30].

Although the outcome of non-invasive markers in different studies is not the same but multiplicity of markers can give more accuracy. The combined indicators of fibrosis: TIMP-1, MMP-2 and APRI score in the current study showed a higher sensitivity, specificity, and strong correlations, with histopathology staging of fibrosis and grading of liver inflammation. AUC also indicated excellent test.

CONCLUSION

Combined serum level of TIMP-1 and MMP-2 with APRI score, are noninvasive tests, simple, inexpensive, and capable of accurate reflection of hepatic inflammation and early fibrosis in patients with HCV-related hepatitis, and developing cirrhosis. So, they could replace liver biopsy in the future or potentially decrease the number of liver biopsies especially in presence of obstacles.

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Conflicts of interest: The authors declare no conflict of interest.

Ethical approval: The study was approved by the Ethical Committee of Tanta Faculty of Medicine and a written informed consent was taken from each participant that follows principles in the Declaration of Helsinki.

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