Evaluation Of Serum Hyaluronic Acid And Matrix Metaloproteinase-2 As Non Invasive Markers Of Hepatic Fibrosis

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Abstract: Liver biopsy is currently the gold standard for assessing liver fibrosis and non reliable non invasive approach is available, therefore a suitable serologic Biomarker is needed. Several biochemical markers have shown promise for the detection of advanced fibrosis and cirrhosis. The aim of the present work is to study the diagnostic value of serum hyaluronic acid (SHA) and matrix metalloproteinase-2 (MMP-2) as indicators for the stage of hepatic fibrosis, and to correlate the liver pathology and liver function tests with serum fibrosis markers. Eighty treatment naïve patients with chronic hepatitis C [CHC] with or without HBV and forty healthy subjects are used as a control group. The patients were divided according to Metavir classification of liver biopsy into 3 groups. Group 1 with normal biopsy (17 patients), group 2 was 35 patients with mild fibrotic changes (stage 1-2) and group 3 of 28 patients with severe fibrosis (stage 3-4). SHA level was significantly higher in patients with severe fibrosis than patients with mild or no hepatic fibrosis. (378.7±147.5, 226.2±123.7 and 85.3±52.2 pg/ml). (P<0.0001). MMP-2 was also significantly higher in severe fibrosis (group 3) than group 2 or group 1 (1196.2±119.5, 918.1±175.8 and 841.1±224.5 pg/ml) respectively (P<0.001). SHA and MMP-2 were not correlated to age, S bilirubin AST, ALT or spleen size. Group 3 was correlated significantly to the SHA and MMP, platelet count, S albumin and liver size but not correlated to AST, ALT, S. bilirubin or spleen size. The Specificity of fibrosis markers SHA and MMP-2 in prediction of severe fibrosis were 94.4% and 90.0% respectively and the sensitivity were higher to SHA 90.0% than MMP-2 80% but not a predictor of mild or normal biopsy. The cut of value of SHA, MMP-2, platelet count and prothrombin time (PT Activity), in diagnosis of severe fibrosis were 294.84 pg/ml, 1003 pg/ml., 115.084/cmmm, 72.116% respectively. Measurement of SHA and MMP-2 can be used to differentiate cirrhotic from non-cirrhotic patient and can be regarded as a useful non invasive test in the diagnosis of liver cirrhosis.

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Introduction

Even after a long and asymptomatic course of chronic hepatitis C (CHC), mild disease can progress to cirrhosis[(1)]. Serial liver biopsies are the best way to diagnose and assess the severity of CHC and to monitor its progression. Liver biopsy can determine the degree of both the inflammatory component (grade) and that of fibrosis (histological stage)[(2)]. Although the liver biopsy is the key examination for the diagnosis of cirrhosis, the use of liver biopsy has several limitations[(3).] There are 24% sample errors of false result. Complications with death rate of 0.015%, discomfort, and the cost of hospitalization. These limitations prevent the use of liver biopsy as a general screening procedure for cirrhosis[(4)]. Early diagnosis is essential because cirrhosis is often revealed by complication. On the other hand cirrhosis is a common disease that is frequently undiagnosed. Several indirect diagnostic tests have been evaluated[(5)]. These tests include clinical signs, biochemical parameters, echogenic signs and endoscopic signs[(6)]. In the liver, SHA is mostly synthesized by the hepatic stellate cells and degraded by the sinusoidal endothelial cells[(7)]. The increase in SHA level occur together with the development of liver fibrosis by 2 mechanisms, first, enhancement of hyaluronan production by the activated stellate cells may contribute to the increase in serum hyaluronan levels observed in patients with chronic liver disease without cirrhosis. Later, when cirrhosis is established, reduced degradation by sinusoidal endothelial cells may cause greater hyaluronan increases[(8)]. Hepatic stellate cells are believed to be the main source of fibrillar and nonfibrillar collagens in the liver and also of certain matrix-degrading proteases (MMPs) and their specific inhibitors (TIMPs). Although some researches, studied the MMP-2 in CHC they reported that level is higher than control, they stated that measured MMP-2 values were less liver specific[(9)]. This work was to determine the diagnostic accuracy of serum markers of fibrosis (SHA and MMP-2) for the diagnosis of hepatic cirrhosis. Study of this non invasive serologic assessment of hepatic fibrosis is

to enable diagnosis of fibrosis in early stages allowing therapeutic intervention to prevent progression to cirrhosis and HCC.

Patients and Methods

The present study was performed on 120 subject (40 control and 80 patients) attending the university hospital of Benha and Mansoura from November 2008 until October 2009.

Control group: Forty healthy subjects (25 males and 15 females) selected from subjects attending for routine medical check up, the mean age (42.5±8.4 years) range from 31-61 years. They were negative for HbsAg, HCVAb and HCVRNA by PCR. They had normal liver function test, no history of schistomiasis and abdominal ultrasonography was normal.

Patient group: A total of 80 patients (47 men and 33 females), 20 to 65 years old (42.7±11.81 years) were participating in the study. At entry all patients with chronic hepatitis had persistently elevated serum alanine aminotransferase (ALT) (more than twice the normal upper limit) for at least 6months on three occasions. They had anti –HCV antibodies, and liver histological findings compatible with chronic hepatitis C. Twenty patients had evidence of coenfection with hepatitis B virus (+ve HbsAg). None had clinical, ultrasonographic or histological evidence of other causes of chronic liver disease (wilson's disease, hemochromatosis, α1-antitrypsin deficiency, autoimmune hepatitis or hepatocellular carcinoma). All patients had given prior informed consent. The patients were subjected to thorough history taking, clinical examination and laboratory investigation including: CBC, Liver function tests (AST, ALT, S Bilirubin, S Albumin and PT); HCV Ab by ELISA I and HCV PCR II, HbsAg, and HBcAb; Alpha-fetoprotein; Serum fibrosis markers (SHA measured by ELISA kit supplied by Corginex inc. USA. MMP-2 measured by ELISA kit supplied by Amersham UK); Abdominal ultrasonography for measurement of liver and spleen size; Rectal snip for detection of schistosomiasis; Liver biopsy for histological grading and staging. Histological features of liver biopsy specimens were analyzed according to the Metavir scoring system (1994)[10]. Fibrosis was staged on a scale of F0-F4, as follows: F0=no fibrosis, F1=portal fibrosis without septa, F2=few septa, F3= numerous septa without cirrhosis, and F4=cirrhosis. The histological activity, a measure of the intensity of necroinflammation was graded on a scale of A0-A3: A0=no activity, A1= mild activity, A2=moderate activity and A3=severe activity.

According to the stage of liver biopsy the patient were divided into 3 groups:

Group 1: included 17 participant with chronic HCV and normal liver biopsy.

Group 2: include 35 patients with mild fibrosis (stage 1 and 2).

Group 3: included 28 patients with severe fibrosis (stage 3 and 4).

Results

Table 1: shows some demographic data of the patients, denoting the most common causes of liver fibrosis in Egypt, as schistosomiasis, HCV and HBV.

Table 2: shows the clinical, biochemical and ultrasonographic variables of the 3 studied groups. There was a significant change in SHA, MMP-2 (figure1), platelet counts, serum albumin and PT between the 3 groups ($P<0.05$). While age, AST, ALT, spleen and liver size, S bilirubin or PV diameter showed no changes between the different groups.

Table 3: showed the correlation between serum markers of fibrosis and clinical, biochemical and ultrasonographic variables. SHA was correlated significantly to platelet counts, PT and S. albumin, MMP-2, was correlated to liver size in addition to the above parameters. Both markers were not correlated either to age, S. bilirubin, PV diameter or spleen size.

Table 4 showed correlation between liver histology and serum markers of fibrosis. There was a significant correlation between serum SHA, MMP-2 platelet counts, PT, S. Albumin and liver size ($P<0.05$) and histological scores. There was a very strong correlation between SHA, MMP-2, PT and the stage of fibrosis ($P<0.001$). Whereas no correlation was found between histological stages, age, S. bilirubin, PV diameter or size of the spleen.

Table 5 and figure 2 showed the ROC curve of sensitivity curve of sensitivity and specificity of fibrosis markers and severity of fibrosis. The SHA and MMP-2 were specific in diagnosis of severe fibrosis in 94.4% and 90.0% respectively and sensitive in 90% for SHA and 80% for MMP-2. The cut off value of significant variable for prediction of severe fibrosis was 294 pg/ml, 1003 pg/ml, 115.084/cmm and 72% for SHA, MMP-2, platelet count and PT respectively (table 6).

Table (1) Demographic data of the individual in different groups

	Control (no.=40)	No fibrosis Gr1(No.=17)	Mild Fibrosis Gr2(no.=35)	Sever fibrosis Gr3(No.=28)
Bl. Transfusion	0	5	7	8
schistosomiasis	0	5	13	9
HCV	0	17	35	28
HBV	0	3	8	9

Table (2) Different variables among the studied groups

	Control(40) X±SD	Gr1(17) X±SD	Gr2(35) X±SD	Gr3(28) X±SD	F	P
MMP-2(pg/ml)	582.72±107.4	841.1±224.5	918.1±175.8	1196.2±119.5	23.231	**0.000***
SHA(pg/ml)	59.80±17.4	85.3±52.2	226.2±123.7	378.7±147.5	24.292	**0.000****
Age(yr)	42.5±8.4	46.0±14.8	42.1±9.6	46.6±9.1	1.156	0.322
Platelet/cmm	264.8±42.6	147.6±38.7	129.6±47.9	99.6±28.7	6.610	**.003***
PT(%)	93.60±6.1	89.0±7.6	80.0±10.1	60.0±13.7	32.147	**.000***
PV(mm)	10.5±0.9	13.0±1.7	13.0±1.4	13.5±0.9	1.329	0.273
Albumin(gm/dl)	3.91±0.3	3.5±0.5	3.5±0.6	3.0±0.2	6.589	**.003***
Bilirubin(mg/dl)	1.01±0.11	2.3±0.8	2.27±1.0	2.58±1.1	.543	0.584
ALT(u/ml)	28.70±5.3	78.92±26.1	84.72±29.2	70.04±27.0	1.637	0.204
AST(u/ml)	25.70±5.6	84.61±24.0	87.96±25.6	74.86±27.9	1.520	0.227
Spleen size(cm)	9.94±0.6	14.45±2.7	13.88±2.9	14.71±3.3	.451	0.639
Liver size(cm)	14.94±0.5	13.74±1.7	13.74±1.7	13.47±1.5	2.542	0.088

*Significant difference between controls and patient groups.
** significant difference between the 3 patient groups

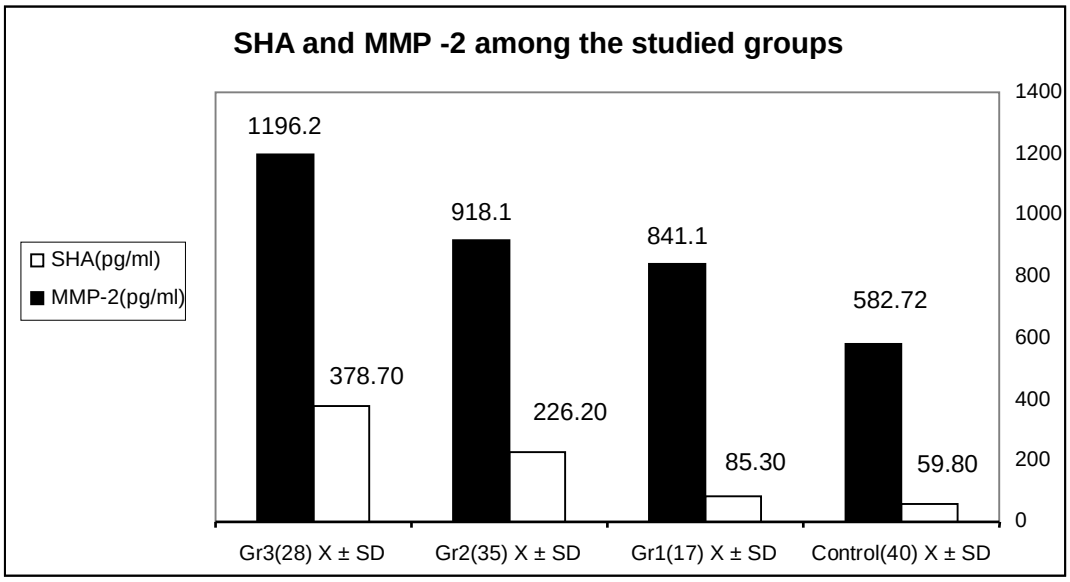

Figure (1): SHA and MMP-2 among the studied groups

Table (3) Correlation of MMP and SHA to other variables in 80 patients

	MMP		SHA	
	r	P value	r	P value
MMP	1		0.724	0.000
Age	0.178	0.174	0.165	0.198
Platelet	**-0.613**	**0.000**	-0.617	0.000
PT	**-0.563**	**0.000**	-0.503	0.000
S. Albumin	**-0.537**	**0.000**	-0.509	0.000
S. Bilirubin	0.033	0.803	0.144	0.27
ALT	-0.113	0.093	-0.229	0.053
AST	-0.231	0.075	-0.232	0.075
Spleen size	0.136	0.300	0.063	0.633
Liver size	-0.353	0.006	0.226	0.083

Table (4) Correlation of stage to other variables (80 patients)

P value	Chi-square	Variables
.000	22.109	MMP-2
.000	18.472	SHA
.081	10.475	Age
.001	11.077	Platelet
.002	9.710	PT
.106	2.610	PV
.005	7.772	S. albumin
.391	.736	S. bilirubin
.159	1.982	ALT
.102	2.668	AST
.411	.675	Spleen size
.024	5.118	Size liver
.076	3.140	GRADE

Table (5): Area under the curve

Test Result variable(s)	Area
SHA	.944
MMP-2	.900

Table (6) the cut off value of independent significant variable in prediction of severe fibrosis

SHA	294.8467
MMP-2	1003.4450
Platelet	115.084
PT	72.1167

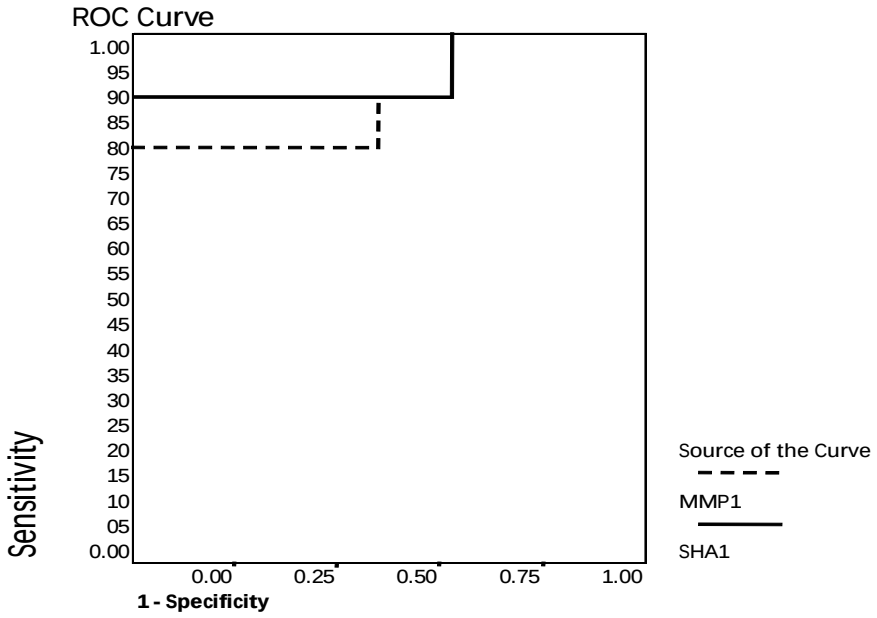

Figure (2) ROC curve of specificity of SHA and MMP in diagnosis of severe fibrosis

Discussion

Liver histology is the gold standard for establishing the severity of liver injury and fibrosis, although it is associated with complication and expense [(3)]. Practicing physicians are in need of simple, safe, inexpensive and non-invasively assess the severity in patients with liver disease. The serum fibrosis markers reflect the balance between fibrogenesis and fibrolysis have been proposed as a simple, non-invasive means of assessing hepatic fibrosis[(11,12)]. The aim of this study was to study the diagnostic accuracy of SHA and MMP-2 as indicators for the stages of hepatic fibrosis. The present study showed no significant difference of age in different studied groups. The value of age as a marker of fibrosis seems obvious, as fibrosis progression is time-dependent [(13)]. However the duration of HCV infection would be more precise indicator of fibrosis than age [(14)]. Platelet count were correlated significantly to severe fibrosis ($P<0.05$). This result comes in agreement with Bonacini et al[(15)]. Thrombocytopenia may be related to the development of portal hypertension and the decreased production of thrombopietin. Decreased platelet count is the earliest indicator or cirrhosis [(16)]. Fontana and Lok[(17)], found that the prothrombin index began to decrease when the Metavir fibrosis score was 2. Prothrombin index had a diagnostic accuracy that was nearly as high as that of the best serum marker of fibrosis [(18)]. In present study, the serum fibrosis markers (SHA and MMP-2) were significantly different between studied groups ($P<0.001$) and correlated significantly to the stage of fibrosis (Chi square 18.4 and 22.1). The level increased proportionally to the severity of cirrhosis. This result coincide with Kozlowska et al [(19)], they reported that the measurement of SHA and MMP-2 reliably differentiated cirrhotic from non-cirrhotic and can be regarded as a useful test in the diagnosis of liver cirrhosis[(12)(20)], particularly when a liver biopsy is contraindicated. However McHutchison et al[(21)], found no strong associations between SHA and MMP-2 and the components of the Knodell histological activity index score, they concluded that the clinical value of SHA measurement appears to be its ability to exclude cirrhosis[(8)], low level of SHA showed good correlation with low risk of fibrosis. Also SHA and MMP-2 can be helpful in discriminating patients of chronic hepatitis from the liver cirrhosis[(22)]. Gebo et al[(23)]., found that SHA and MMP-2 may have value in predicting fibrosis, and were poor at predicting intermediate levels of fibrosis[(24)]. In the present study the two fibrosis markers were not correlated to the grade of inflammatory activity ($P>0.05$). This was previously confirmed in many article as McHutchison et al[(21)], but disagree with Wang et al[(25)], who found a close

correlation between inflammation process and fibrosis, they suggest that the inflammatory process may play an important role in fibrogenesis. The serum fibrosis markers in the current study were highly correlated to PT, Platelet count, serum albumin and liver size ($P<0.05$) but not correlated to AST, ALT, S. Bilirubin or spleen size. This result consistent with most of previous published articles [18], found that the fibrosis markers correlated significantly with albumin, platelet count and PT but not to serum variables reflecting inflammatory activity. The receiver operating characteristic (ROC) curves in the present study shows specificity 94.4%, 90% and sensitivity 90%, 80% for SHA and MMP respectively in prediction of severe fibrosis. This result comes near the result of Xie et al[26], and Lee et al[8], who found ROC curve of 93% and 72% respectively. It is not surprising that the reported rate vary widely reflecting different patient etiologies. Pares et al[27], found the ROC curve of SHA is 91.4% in patients with fibrosis. Boeker et al[28], studied the MP-2 in cirrhosis and he mentioned that the diagnostic efficiency of 92%. The cut off value of independent significant variables in detecting the stage 3 and 4 (bridging and cirrhosis) in the current study were nearly similar to the figures in a number of other studies[26,29]. However , in the present study we did not correlated the effect of gender and ethenic origin with the fibrosis markers and staging because for establishing the actual changes rate of serum markers in male and female patients requires serial liver biopsies to correlate with Metavir fibrosis score, which is difficult and also need long time observations. Also it is known that, fibrosis grading and staging scores are higher in Egyptian patients infected with HCV due to concomitant schistosomiasis infection.

Conclusion

SHA and MMP reflect the severity of fibrosis in patient with chronic viral liver disease and useful as marker of precirrhotic and cirrhotic stage. Regular determination of both CHC in patients may be used as indicators of increasing fibrosis and development of cirrhosis. The addition of some laboratory parameters as PT and platelet count in addition to serum fibrosis markers SHA and MMP-2 may add a prognostic importance. Assessment of hepatic scarring may be performed with combination of novel fibrosis biomarkers, thus eliminating the need for liver biopsy. Further evaluation needs to be performed in large patient populations. Diagnosis of fibrosis during early stages will allow early treatment, thereby preventing fibrosis progression

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