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RESEARCH ARTICLE

SERUM SYNDECAN-1 AND APOLIPOPROTEIN A1 IN PATIENTS WITH CHRONIC HCV INFECTION

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ABSTRACT

Hepatitis C virus (HCV) is endemic in Egypt. Over 15% of populations of the people in Egypt are infected, this is ten times greater than in any other country in the world. To validate a simple, inexpensive, non invasive markers (Fib-4, APRI, syndecan-1 and apolipoprotein A-1 (ApoA-1) for detection of liver fibrosis in patients with chronic HCV and thereby reduce the need for liver biopsy. Estimation of serum syndecan-1 and apoA-1 by ELISA were done on 20 normal healthy persons and 57 chronic hepatitis C patients, the patients were staged according to liver biopsies (Metavir fibrosis staging) (stage f1=15, f2=15, f3=14, f4= 13). The area under the receiver operating characteristic (ROC) curve (AUC) of syndecan-1=(0.72), the mean level of plasma syndecan-1 was significantly higher in chronic HCV patients when compared to control subjects (P=0.0008), but the mean level of ApoA-1 was not significantly different from that of controls (p=0.65), and it had significant negative correlation to the stage of fibrosis (AUC=0.57). Moreover the APRI and Fib-4 were proved significantly directly correlated with fibrosis stage of the studied patients. This study not reveals putative biomarkers of liver fibrosis (syndecan-1, ApoA-1 APRI, Fib-4) but also proves the differential expression of those markers in different stages of fibrosis. It is expected that combination of these novel biomarkers could be applied clinically to predict the stage of liver fibrosis without the need of liver biopsy.

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INTRODUCTION

Hepatitis C virus (HCV) infects 170 million people worldwide and is thus a major public health problem (Thomas *et al.*, 2000; world health organization 2012). The infection is often asymptomatic, but chronic infection can lead to scarring of the liver and ultimately to cirrhosis, which is generally apparent after many years. In some cases, those with cirrhosis will go on to develop liver failure, liver cancer or life-threatening esophageal and gastric varices (Ryan *et al.*, 2004).

Liver fibrosis results from chronic damage to the liver in conjunction with progressive accumulation of fibrillar matrix "extracellular matrix" proteins (ECM). Hepatic fibrosis (HF) is a pathological condition resulting in abnormal proliferation and accumulation of tough fibrous connective tissue (scar tissue) in the liver. Although the formation of scar tissue is a normal body response to injury, in fibrosis, this healing process goes erroneous. Normal process of wound healing involves collagen deposition; however, the chronic activation of this healing mechanism leads to liver pathology.

Among a variety of causes/factors or stimuli, which bring about this transformation are: chronic infection by hepatitis B, C viruses and parasites as *Schistosoma* spp, chronic alcoholism and/or exposure to certain drugs and toxins, infections, non-alcoholic steatohepatitis (NASH), inherited metabolic diseases like hemochromatosis, Wilson's disease, α -1 antitrypsin deficiency, autoimmune diseases such as primary biliary cirrhosis, and auto-immune hepatitis (Friedman, 2008). Monitoring of progression of liver fibrosis is an important tool for the clinician in assessing the aggressiveness of infection and the level of hepatic injury. For treatment decisions and prognosis, the accurate staging of hepatic fibrosis is essential. Although the current gold standard is liver biopsy, this procedure is limited by invasiveness, complications, sampling error, variability in interpretation, and the reluctance of patients to undergo repeated biopsies to monitor disease progression (Bedossa *et al.*, 2006; Friedman, 2004). Due to these limitations, numerous studies have examined noninvasive measures for the prediction of HCV-related fibrosis (Afdhal and Nunes 2004, Parkes *et al.*, 2006). The first approach used to assess the degree of liver fibrosis by noninvasive means consisted of routine biochemical and/or hematological tests (Martínez *et al.*, 2011). These so-called

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class II biomarkers are indirect serum markers and are based on the evaluation of common functional alterations in the liver, alterations that do not necessarily reflect extracellular matrix turnover and/or fibrogenic cell changes. APRI (Wai *et al.*, 2003), Forns index (Forns *et al.*, 2002) and Fib4 (Sterling *et al.*, 2006) are examples of this class. More recently, a better understanding of the pathophysiology of liver fibrosis has prompted many investigators to use more refined markers to identify different fibrosis stages. The latter, termed class I biomarkers, are those intended to detect extracellular matrix turnover and/or fibrogenic cell changes. These include Fibrometer (Cales *et al.*, 2005) and Enhanced Liver Fibrosis panel (ELF) score (Rosenberg *et al.*, 2004)

Thus, it is not very far that this invasive method will be completely replaced by other noninvasive methods such as serum based biomarkers (Parsian *et al.*, 2012). Therefore development of non-invasive methods is still needed and we should work harder for finding an appropriate method. Proteoglycans are a diverse group of macromolecules that covalently bind a variable number of glycosaminoglycan (GAG) side chains to a core protein (Heinegard and Oldberg, 1989). In the liver, proteoglycans are localized to the extracellular, pericellular spaces and on the cell surface, where they participate in cell-cell, cell-matrix and protein-protein interactions (Jarnagin, *et al.*, 1994). In the normal liver, the net amount of proteoglycans is low (Martinez-Hernandez and Amenta, 1993), whereas the level of several proteoglycans is increased during liver fibrosis (Gressner *et al.*, 1994). Decorin is the most extensively studied proteoglycan in liver fibrosis (Hogemann *et al.*, 1997; Dudas *et al.*, 2001) followed by biglycan, perlecan, aggrecan (in rodents), syndecan and lumican all showing elevated levels as a response to chronic liver injury. Syndecan-1 and -2 are increased on both mRNA and protein levels in the cirrhotic livers (Jarnagin *et al.*, 1994). Syndecan-1 (CD 138) is one of four members of a family of transmembrane heparan sulfate proteoglycans with distinct expression patterns and function. CD138 is a new simple non-invasive marker for predicting liver fibrosis in patients with chronic hepatitis C. Epithelial cells primarily express syndecan-1, and observations from various models indicate that it participates in wound healing and has been shown to be present in normal hepatocytes (Li *et al.*, 2002; Stepp *et al.*, 2007).

Apolipoprotein A-I (apoA-I) is a single polypeptide of 243 amino acids with a molecular mass of 28.1 kDa, mainly present in plasma as a component of high density lipoprotein (HDL) (Zannis *et al.*, 2004). ApoA-I is expressed primarily by the intestine and the liver, although other tissues also express it. Serum concentration of apolipoprotein A1 was incorporated with other serum markers in the assessment of liver fibrosis (Imbert-Bismut *et al.*, 2001; Gangadharan *et al.*, 2007) and it was reported to be negatively correlated with the stage of liver fibrosis.

Aim of the work

The aims of the present study are: 1) to assess the level of Syndecan-1 (CD138) and apolipoprotein A-1 in patients with chronic HCV infection in comparison to healthy controls. 2) to

test the diagnostic accuracy of these novel biomarkers in comparison to previously validated ones (as APRI and FIB-4) as non-invasive markers for differentiating mild from advanced stages of liver fibrosis.

Patients and Methods

Patients

Fifty-seven patients with positive anti-HCV antibody and HCV RNA by PCR for more than 6 months and referred for liver biopsy were recruited for the study from the Tropical Medicine & Gastroenterology Department, Sohag University Hospital. The study was approved by the local institutional ethical committee and patient's consent was obtained. In addition, twenty healthy (with no evidence of acute or chronic liver disease or other medical disorders), adult age and sex-matched individuals were recruited as a control group. Liver biopsy was performed for all patients to assess the severity of fibrosis (staging) and inflammation (grading) prior to treatment.

Exclusion criteria were as follows: any disorders associated with extra hepatic fibrosis, decompensated liver cirrhosis, hepatitis B co-infection, hepatomegaly due to autoimmune disease, fatty liver, liver failure, hepatocellular carcinoma, uncontrolled diabetes mellitus and nephrotic syndrome.

Methods

All patients were subjected to the following:

- 1- Complete medical history taking, clinical and radiological examination
- 2- The histological evaluation of paraffin-embedded liver specimens was carried out at the Pathology Department, Sohag University Hospital, according to METAVIR scoring system (Bedossa and Poynard, 2006). Liver biopsies were evaluated by two independent pathologists without prior information to patient's history. Histological staging based on the degree of fibrosis: as F0 (no fibrosis), F1 (mild fibrosis without septa), F2 (moderate fibrosis with few septa), F3 (severe fibrosis with numerous septa without cirrhosis) and F4 (cirrhosis).
- 3- Abdominal Imaging: ultrasound was done to evaluate liver size, echopattern (normal, bright or coarse), regularity of the surface, presence of splenomegaly, dilated portal vein or presence of portosystemic collaterals and ascites.
- 4- Blood sampling:

Four ml of blood were collected from all patients and controls, and left to clot for 20–30 min at room temperature and followed by centrifugation at 1500 r.p.m for 10 min. The serum was then transferred to a polypropylene tube and kept frozen at –80°C until assayed. Serum ALT, AST, alkaline phosphatase, albumin and total bilirubin concentrations were measured using standard methodologies. Hemoglobin concentration was determined by a colorimetric method. Red blood corpuscles, white blood cells and platelets were counted by hemocytometer apparatus. All biochemical tests were done without knowledge of liver biopsy results and all patients were

evaluated for, APRI and FIB-4, indexes. The following formulas were used to calculate the predicted scores.

APRI (Wai *et al.*, 2003) = $[\{AST (IU/l) / ALT_{ULN} (IU/l)\} \times 100] / \text{platelet count} (10^9/l)$.

FIB-4 (Sterling *et al.*, 2006) (= $[\text{Age (Years)} \times \text{AST (IU/l)}] / [\text{Platelet count} (\times 10^9/l) \times \text{ALT (IU/l)}]^{1/2}$).

Serum Apolipoprotein A1 (Apo - A1): from Assaypro and syndecan-1 from Wkea were used. They were a solid phase capture sandwich ELISA assay using a microwell format.

Statistical analysis

Data were analyzed using STATA intercooled version 9.2. Quantitative data were analyzed using student t-test to compare means of two groups and ANOVA for comparison of the means of three groups or more. When the data were not normally distributed Mann-Whitney test was used. Qualitative data were compared using either Chi square test or fisher exact test. univariate and multivariate logistic regression analysis was used to identify data that predict advanced fibrosis. Data were analyzed by sensitivity, specificity, positive, and negative predictive value derived from the receiver operating characteristic (ROC) curve by using Medcalc for Windows (version 11.0) and STATA (version 9.2). The diagnostic accuracy of syndecan-1, apolipoprotein- A-1, Fib-4, and APRI for detecting advanced fibrosis was expressed as the area under the ROC curve (AUC). P value was considered significant if it was less than 0.05.

RESULTS

The demographic and clinical data of all patients are illustrated in Table (1).

Table 1. Clinical data of the studied group

Clinical data	Patients (n=57)
Age	39.8±10.5
Sex	18(31.6%)
Female	39 (68.4%)
male	
BMI (Kg/m ²)	24.5±3.75
DM (%)	
No	47(82.5%)
Yes	10 (17.5%)
Hypertension	
No	49(86%)
Yes	8(14%)
Pallor	
No	54(94.7%)
Yes	3(5.3%)
Jaundice (No)	57(100%)
Liver size by U/S	
Normal	29(50.9%)
Hepatomegaly	15(26.3%)
Shrunken liver	13(22.8%)
Splenomegaly	
normal	41(71.9%)
enlarged	16(28.1%)
Ascites (No)	57(100%)
Hepatic focal lesion (No)	57(100%)

All data are mean ±SD or proportions [N (%)],

Table (2) shows a comparison of laboratory data between patients and controls. Serum syndecan-1 was significantly elevated in patients compared to the controls. While, Apo-1 was insignificantly higher in patients in comparison with the controls.

Table 2. Laboratory characteristics of the studied groups

	Patients	Control	P value
ALT (IU/L)	66.75±76.16	19.5±3.17	<0.001
AST(IU/L)	52.68±54.57	18.55±3.95	<0.001
TBil (mg/dl)	0.85±0.43	0.89±0.11	=0.045
Total Protein (g/dl)	7.66±0.71	7.42±0.33	=0.002
Albumin (g/dl)	3.93±0.54	3.95±0.34	=0.005
INR	1.15±0.25	0.95±0.1	<0.0003
Prothrombin Conc. %	84.89±15.76	98±1.78	<0.0001
FBG (mg/dl)	103.64±22.71	95.15±9.16	0.28
HB(gm/dl)	13.95±1.65	13.05±0.65	0.008
WBCs (x10 ³ /mm ³)	6.16±2.05	7.4±1.05	<0.0001
Platelets (x10 ⁹ /L)	220.55±65.25	255.9±33.6	0.03
Syndecan-1 (pg/ml)	5.60±2.426	4.155.6±3.98	0.008
ApoA-1 (mg/dl)	81.66±97.19	74.55±48.42	0.65

All data are mean ± SD. AST, aspartate amino- transferase; ALT, alanine amino - transferase; T.Bil, total billirubin; TP total protein., FBG, fasting blood glucose., HB, hemoglobin, WBCs., White blood corpuscles., (t -test for continuous variables), *P<0.05.

According to the results of Metavir histological staging and grading, patients were categorized into either F0=(0%), F1=(26.31%), F2=(26.31), F3=(24.56), F4=(22.80). This is illustrated in Table (3)

Table 3. Liver fibrosis

	N%
F1	15 (26.31%)
F2	15 (26.31%)
F3	14 (24.56%)
F4 (Cirrhosis)	13 (22.80%)

Table 4. Comparison between mild and advanced fibrosis as regard demographic and laboratory data

	F1+f2 (n=30)	F3+f4 (n=27)	P value
Age	38.43±10.50	41.37±10.49	0.30
BMI(Kg/m ²)	23.60±3.11	25.44±4.20	
Sex			
Females	7(23.33%)	11(40.74%)	0.16
Males	23(76.67%)	16(59.26%)	
ALT(IU/L)	58.70±57.67	75.59±91.7	0.24
AST(IU/L)	44.90±16.55	61.89±76.46	0.37
TBil (mg/dl)	0.66±0.249	1.04±0.49	0.005
Total Protein (g/dl)	7.94±0.612	7.35±0.66	0.001
HB(gm/dl)	14.00±1.80	13.68±1.85	0.52
WBCs (x10 ³ /mm ³)	5.70±1.63	6.65±2.33	0.07
Platelets (x10 ⁹ /L)	245.6±46.42	192.8±71.27	0.002
Albumin(g/dl)	4.160±0.453	3.65±0.499	0.0002
INR	1.023±0.077	1.27±0.29	0.001
Prothrombin Conc.%	93.10±7.16	75.81±17.49	0.0002
FBG (mg/dl)	100.9±18.29	106.8±26.98	0.41
Syndecan (pg/ml)	4.598±9.21	6.715±3.04	0.005
apoA-1 (mg/dl)	83.61± 126.4	79.91±62.52	0.36
FIB-4	1.06±0.60	1.56±0.73	0.004
APRI	0.48 ± 0.18	0.71± 0.36	0.009

Performance of serum fibrosis markers for prediction of advanced fibrosis (stage 3 and 4)

For evaluation of the performance of serum fibrosis markers in prediction of advanced fibrosis, their results were compared with that of liver histopathology taken as a gold standard. For each serum fibrosis marker, sensitivity, specificity, positive and negative predictive values were calculated at different cut-offs and ROC curve analysis was performed for them. Table (5) show that syndecan-1 at a cut off (>5.917) has an area under curve (AUC=0.72) similar to APRI and Fib4. However, it shows the best sensitivity (although unsatisfactory), best specificity and highest PPV and NPV. Fig (1) shows comparison of the AUC of the 4 tested serum fibrosis markers.

minority suffers from long term complications (Chen and Morgan, 2006).

At present the diagnosis of liver fibrosis still depends on pathological examination of liver biopsy, which is an invasive procedure, associated with patient discomfort and in rare cases with serious complications (Thampanitchawong and Piratvisuth, 1999). In addition, the accuracy of liver biopsy is limited due to sampling error and significant intra and inter observer variability in histological examination (Regev *et al.*, 2002). Non invasive assessment of liver histology has been the focus of research for many years, which is important to measure the severity of liver injury in Egypt where advanced liver disease from HCV is common.

Table 5. Sensitivity, specificity, PPV and NPV of syndican-1, apoA1, FIB-4 and APRI to diagnose correctly advanced fibrosis

	Cut off	Area under the curve (AUC)	Sensitivity	Specificity	PPV	NPV	P value
Syndecan -1(pg/ml)	>5.917	0.72 95%CI (0.58-0.83)	51.85	93.33	87.50	68.29	0.002
Apolipoprotein A1 (mg/dl)	<20.8	0.57 95%CI (0.43-0.70)	48.15	83.33	72.22	64.10	0.38
Fib4	>1.62	0.72 95%CI (0.59-0.83)	48.15	86.67	76.47	65.00	0.001
APRI	>0.66	0.72 95%CI (0.57-0.73)	48.67	86.67	76.47	65.00	0.005

Univariate analysis to the significant variables, * $P < 0.05$ PPV, positive predictive value; NPV, negative predictive value

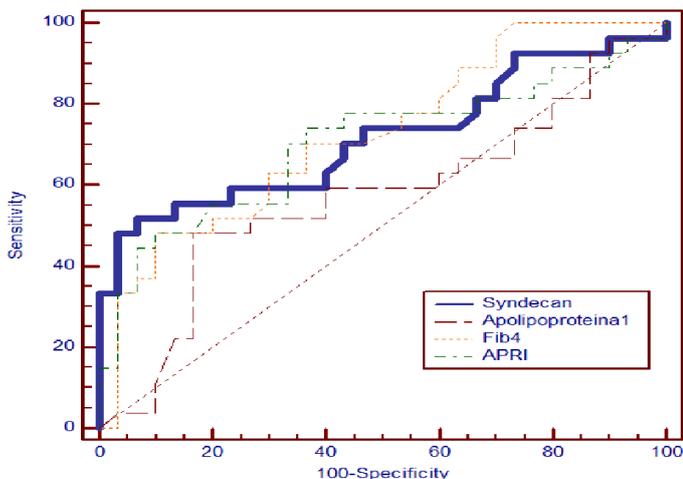


Fig. 1. Receiver operating characteristic (ROC) curves of different biomarkers to predict advanced fibrosis

DISCUSSION

The liver is of vital importance in intermediate metabolism, detoxification and elimination of toxic substance (Gressner., 1998). Damage of this organ affects liver function tests. Liver fibrosis, a common sequel of chronic inflammatory liver disease, is a complex dynamic process that includes an increase in ECM components, activation of cells producing matrix material, cytokine release and tissue remodeling (Vozar., 2005). The rate of fibrosis progression in HCV patients varies markedly from person to person, and only a

We have tested the possibility that the presence in the serum of cell surface proteoglycans shed by cells in several pathological conditions may correlate with liver fibrosis. The proteoglycans we investigated are the syndecan family, which are transmembrane glycoproteins with either heparan sulphate or chondroitin sulphate chains (Kim *et al.*, 1994). In the present study, the syndecan level was increased significantly from mild to advanced fibrosis at a cut off >5.91 with AUC (0.72), specificity (86.67) the PPV (76.47) and the NPV (65.00). These results are comparable to those of Zvibel *et al.* (2008) in chronic hepatitis C patients.

Syndecans can exert their effect by acting as co-receptors to various growth factors such as basic fibroblast growth factor and regulate their activity. It interacts with growth factors, matrix components and other extracellular proteins and is thought to be involved in processes such as cell growth, differentiation and adhesion. (CD138) is a marker of liver fibrosis, because it was shown to be present in high amounts in wound fluids (Subramanian *et al.*, 1997, Kainulainen *et al.*, 1998). Syndecan-1 is shed by many cell types during pathological processes, and it was also found to be a prognostic marker for myelomas and small cell lung cancer (Anttonen *et al.*, 2003, Seidel *et al.*, 2000).

In the current study, we noted an insignificant decrease in serum Apolipoprotein-A1 in patients with advanced fibrosis compared to those with mild fibrosis. Miller *et al.* (1980) reported that ApolipoproteinA-1 is synthesized by the liver, and it is included in extracellular matrix and its level is decreased at increasing stages of fibrosis. In alcohol-induced

liver disease, serum Apo A-I increases in patients with normal liver, steatosis, and decreases in liver fibrosis and cirrhosis (Poynard *et al.*, 1987). Recently, Kim *et al.*, 2010 reported that ApoA-1 is expressed in the lung epithelium, that lung ApoA1 levels were reduced in patients with idiopathic pulmonary fibrosis, ApoA-1 protects against ongoing fibrosis and promotes the resolution of established experimental lung silicosis., (Lee *et al.*, 2013) this is agree with our results, Imbert-Bismut *et al.* (2001) found that of ApoA-1 together with other five serum fibrosis markers can substantially reduce the numbers of liver biopsies in chronic HCV patients. Also Myers *et al.* (2003) reported that GGT, AST, and apolipoprotein A1 were independently predictive of significant fibrosis in chronic hepatitis B patients.

The mechanisms of serum Apo A-I decrease in liver fibrosis are unknown. During liver fibrosis, several hypotheses can be evoked: a decrease in Apo A-I transcription, an increase in Apo A-I catabolism, or storage of Apo A-I by the developing ECM. The latter hypothesis suggesting that Apo A-I interacts with the ECM is supported by several arguments. First, immunohistochemical studies have shown that Apo A-I colocalizes with ECM. Another argument is that lipoproteins can be extracted from vessels only in association with constituents of the ECM (Bedossa *et al.*, 1989). Apolipoprotein A-1 serum concentration is associated with fibrosis and used with prothrombin and GGT as a serum marker. (Teare *et al.*, 1993, Naveau *et al.*, 1994, Poynard *et al.*, 1991) It is trapped on extracellular matrices (Paradis *et al.*, 1996) Apolipoproteins, especially A₂, (Sabile *et al.*, 1999) also interact with HCV capsid proteins

Our results show that APRI and Fib -4 score were significantly increased from stage F1 to F4 according to Metavir classification. We found that at a cut off for APRI > 0.66 with AUC(0.72), the specificity (86.67) the PPV(76.47) and the NPV(65.00). APRI was initially described by Wai *et al.* (2003). This index is dependent on two routine tests, i.e. aspartate aminotransferase and platelet count. In a study by Snyder *et al.* (2006) for validation of APRI a value of <0.42 were accurately predicting mild fibrosis, whereas a value >1.2 were accurately predicting significant fibrosis, these conflicting results may be due to difference in numbers of the patients, histopathological scoring system used, and/or prevalence of the diseases. Le Calvez *et al.* (2004) reported that APRI and Forn's index were not able to identify individual stages of fibrosis, where APRI cannot be completely standardized due to variability in normality ranges of AST in different laboratories.

Other simple index that related to age (years), AST and ALT levels (U/L) and platelet count, is FIB-4. Yang *et al.* (2012) reported that FIB-4 index had a significant power for differentiation between patients with mild and significant fibrosis in nonalcoholic fatty liver disease (NAFLD), and they reported that FIB-4 might be useful as a non invasive hepatic fibrosis scores for predicting hepatic fibrosis in patients with NAFLD. Shah *et al.* (2009) in a study on 541 NAFLD adults found that among various serum based algorithms, FIB4 had better diagnostic accuracy for estimation of liver fibrosis. Our result is less at cut off >1.62 with AUC (0.72), the specificity (86.67) the PPV (76.47) and the NPV (65.00). The FIB-4

index may be of value in several respects. First, it is easy to use; the calculations are simple, quick, and do not require standardization. Second, results are available immediately, during the patient's visit. Third, it is inexpensive; there is no need to invest in a costly apparatus, and there are no additional costs, because the constitutive FIB-4 parameters are included in the standard investigation of any liver disease (age, AST, ALT, platelet count) (Pichard *et al.*, 2007).

In conclusion, the non-invasive methods will reduce but not completely abolish the need for liver biopsy. The most rational way of using these tools is to make a compromise: use non-invasive methods to classify those patients in whom they perform with high accuracy, sensitivity and the specificity for limiting liver biopsy to the subset of patients in whom precise noninvasive staging is not possible. Also syndecan-1 and ApoA-1 are non invasive markers for predicting liver fibrosis. Application of biomarkers would provide the clinicians with improved tools to personalize the treatment, to follow the progression of the disease and to monitor the response to treatment.

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