Serum angiopoietin-2 as a noninvasive diagnostic marker of stages of liver fibrosis in chronic hepatitis C patients

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Background
The process of fibrogenesis is associated with the development of disease-specific markers. The management of chronic hepatitis C (CHC) depends on the staging of liver fibrosis. Use of noninvasive methods is preferable in diagnosis and follow-up.
Objective
The aim of this study is to evaluate serum angiopoietin-2 (Ang-2) as a noninvasive marker in the diagnosis of different stages of liver fibrosis in CHC patients.
Materials and methods
A total of 90 individuals were included. They were divided into a patient group (75 patients) and a control group (15 normal individuals). Serum Ang-2 was measured using enzyme-linked immunosorbent assay. Pretreatment liver biopsy was performed for the patients. The METAVIR score was used in the staging of liver fibrosis. A comparison of Ang-2 was performed between patients and controls, and between different stages of liver fibrosis. A receiver operating characteristic curve analysis was carried out to determine the best cutoff values of Ang-2 in the differentiation of different stages of fibrosis.
Results
Ang-2 serum levels were significantly higher in advanced stages of liver fibrosis. The cutoff points 869.3, 2226, and 7205 pg/ml were the best for differentiating fibrosis stages >F1; >F2; and >F3, respectively. Ang-2, international normalized ratio, α-fetoprotein, and albumin were found to be independent predictors of liver fibrosis using univariate analysis.
Conclusion
Ang-2 correlated significantly with liver fibrosis stage. It can aid noninvasive differentiation between different stages of liver fibrosis in patients with CHC.

Keywords:
angiogenesis, angiopoietin, hepatitis C virus, liver fibrosis

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Introduction

Hepatitis C virus (HCV) infection is a major global health problem [1–3]. Patients with detectable levels of HCV-RNA have an increased risk of hepatic and extrahepatic disease [4]. The persistence of inflammatory responses and cellular damage of HCV promote disease progression toward fibrosis, cirrhosis, and hepatocellular carcinoma (HCC) [5]. Hepatic fibrosis is the deposition of excess extracellular matrix. The major effectors of fibrosis are the hepatic stellate cells and the portal fibroblasts, which are activated by soluble mediators produced by activated hepatic resident cells [6]. The lack of easy-to-use methods for the assessment of liver fibrosis has been a major limitation for clinical management and research [7]. Although liver biopsy (LB) is considered the standard for determination of the stage of fibrosis, it has disadvantages such as invasiveness, expensiveness, risk of complications, and potential for sampling errors. Noninvasive markers such as serum angiopoietin-2 (Ang-2) are being sought as an alternative [8]. Angiopoietins are a family of vascular growth factors that play a role in the physiological angiogenesis process [9]. Ang-2 is produced by the endothelial cells [10,11]. It has been shown that Ang-2 is overexpressed in fibrotic liver tissues, and therefore, play a pathophysiological role in chronic liver disease (CLD) [12]. Ang-2 is believed to be involved in both the angiogenesis and the inflammatory pathways in pathological situations. Ang-2 may result in leaky vasculatures that facilitate the extravasation of lymphocytes and promote the adhesion of rolling leukocytes to blood vessels [10].

The aim of the current study is to evaluate the role of serum Ang-2 as a noninvasive marker in the diagnosis of different stages of liver fibrosis in patients with chronic hepatitis C (CHC) infection.

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Materials and methods

This current study was carried out at the National Hepatology and Tropical Medicine Research Institute Cairo together with Ain Shams University Hospital, Internal Medicine, Gastrointestinal and Hepatology outpatient clinics. The study included 90 individuals. They were divided into two groups: group A, which included 75 patients in whom antiviral therapy was planned for CHC, and group B, which included 15 normal controls. Group A included 37 (49.3%) men and 38 (50.7%) women with BMI (mean±SD =31.07±8.55) and age (40.45±12.04). Group B included seven (46.7%) men and eight (53.3%) women with BMI (33±10.62) and age (39.40 ±15.18). Age, sex, and BMI were matched.

According to the METAVIR score for the classification of hepatic fibrosis [13], patients of group A were divided into five subgroups according to the stage of liver fibrosis: nine (10%) patients with stage F0, 16 (17%) patients with stage F1, 26 (28.8%) patients with stage F2, 18 (20%) patients with stage F3, and six (6.7%) patients with stage F4. The individuals in the control group (group B) were found to have no fibrosis by Fibroscan.

Inclusion criteria included patients with CHC diagnosed by countable HCV-RNA in their serum by PCR, and for whom oral antiviral therapy was planned. Exclusion criteria included patients with HIV coinfection, hepatitis B virus coinfection, patients with autoimmune liver disease, HCC, decompensated liver cirrhosis, and patients with hepatic or extrahepatic malignancies.

Informed written consent was obtained from patients and controls before inclusion. The study protocol was approved by the Research Ethical Committee of Faculty of Medicine, Ain Shams University, according to the ethical guidelines of the 1975 Declaration of Helsinki.

All the following were performed for recruited patients and controls.

(1) Complete assessment of history together with a full clinical examination.
(2) Laboratory investigations including complete blood count; liver function tests: alanine aminotransferase (ALT), aspartate aminotransferase (AST), total and direct bilirubin, international normalized ratio (INR), prothrombin time, serum albumin, and kidney function tests by standard laboratory tests.
(3) Radiological examination including pelviabdominal ultrasound with examination of liver size, echogenicity, splenomegaly, presence of ascites, portal vein diameter and patency, presence of any hepatic focal lesions, or any abdominal malignancy, and a detailed examination of the kidneys (equipment used: Hitachi, EUB-5500; Hitachi Medical Systems America, Inc.; Twinsburg, OH 44087, USA).
(4) LB was performed only in the patient group for histopathological examination, which was the standard for determining the stage of fibrosis according to the METAVIR score [13]. Exclusion of liver fibrosis in the control group was performed using Fibroscan.

Transient elastography (Fibroscan) was performed for the patients in group B to confirm the absence of any fibrosis using Fibroscan 502 equipment (ECHOSENS Company, Paris, France). The patients lay on their back with their arm raised behind their head. The physician applied a water-based gel to the skin, placed the probe with a slight pressure, and examines the right lobe of the liver through the intercostal space. The examination included 10 consecutive measurements performed on the same location. The result was obtained at the end of the examination in the form of a number in kilopascals (kPa).

Liver histology

Liver biopsies from patients were obtained by percutaneous needle extraction under ultrasound guidance and were embedded in paraffin for routine histopathological examination. The median length of liver biopsies was 1.9 mm. The METAVIR scoring system was used for staging liver fibrosis [13]. Liver fibrosis was divided into five stages from F0 to F4. F0 indicated the absence of fibrosis, F1 indicated the presence of portal fibrosis without septa, F2 indicated the presence of portal fibrosis with rare septa, F3 indicated the presence of numerous septa without cirrhosis, and F4 indicated is the presence of cirrhosis.

Angiopoietin-2 concentrations in the serum of chronic hepatitis C patients

Circulating Ang-2 levels were measured in the serum samples from CHC patients on the same day that they underwent LB, before initiation of antiviral combination therapy, using commercially available Human Angiopoieyin-2 ELISA kits following the manufacturer's instructions (Boster Immunoleader; Biological Technology Company Ltd, Pleasanton, California, USA). Blood samples (5 ml) were obtained from both groups A and B by a trained, professional nurse using sterile, disposable equipment. After centrifuging the blood samples, serum samples were separated in serum separator tubes and were stored horizontally at −20°C. Serum samples were mixed with diluents buffer samples

in a ratio of 1 : 2. Serum levels of human Ang-2 were estimated by enzyme-linked immunosorbent assay and measured in pg/ml with levels ranging from 156 to 10 000 pg/ml.

Serological indices of fibrosis were calculated for the patients and presented in the results. Comparisons of the diagnostic values of Ang-2, aspartate aminotransferase-platelet ratio index (APRI), and Fibrosis-4 (FIB4) were performed.

APRI: It was calculated as: (AST/upper limit of normal range)/[platelet count (10^9/l)]×100 [14].

The FIB4 score: this score was as calculated as: age (years)×AST (IU/l)/platelet count (10^9/l)×ALT (IU/l)$^{1/2}$ [15].

AAR (ALT/AST ratio): this score was calculated as: AST (IU/l)/ALT (IU/l) [16].

Statistical analysis

The statistical package for the social science (SPSS Inc., IBM Company, Chicago, USA), version 17 for Microsoft Windows, was used for data analysis. Quantitative variables were expressed as mean±SD, whereas qualitative variables were expressed in percentage or number. The correlation between Ang-2 serum levels and other variables was analyzed by the Pearson correlation coefficient. A univariate regression analysis was carried out. Significant factors were then subjected to a multivariate analysis. Comparison of quantitative variables was carried out using the Student t-test, whereas comparison between more than two groups was carried out using the analysis of variance test (ANOVA). The area under the receiver operating characteristic curve (AUROC=AUC) was used to assess the discriminatory ability of the test under study to predict the stages of liver fibrosis among CHC patients. Significance level (P) value: $P \leq 0.05$ is significant (S). $P < 0.01$ is highly significant (HS). $P > 0.05$ is insignificant (NS).

Results

There was a statistically significant higher mean of Ang-2 in higher stages of liver fibrosis (P<0.001) according to the ANOVA test (Table 1 and Fig. 1).

On comparing Ang-2 serum levels between different subgroups (Table 2).

(1) There was a statistically significantly higher mean of Ang-2 in stages F2, F3, and F4 of liver fibrosis in comparison with stage F0 (P<0.05).
(2) There was a statistically significantly higher mean of Ang-2 in stages F3 and F4 of liver fibrosis in comparison with stage F1 (P<0.05).
(3) There was a statistically significantly higher mean of Ang-2 in stages F3 and F4 of liver fibrosis in comparison with stage F2 (P<0.05).
(4) There was a statistically significantly higher mean of Ang-2 in stage F4 of liver fibrosis in comparison with stage F3 (P<0.05).
(5) There was a statistically significantly lower mean of Ang-2 in normal cases in comparison with stages F2, F3, and F4 of liver fibrosis (P<0.05).

On comparing between patients with liver fibrosis (n=66, F1–F4) and those without (n=9, F0) using the ANOVA test, there was a statistically significantly higher mean Ang-2 serum levels (Table 3 and Fig. 2), α-fetoprotein (AFP), and INR in cases with liver fibrosis than in those without fibrosis (P<0.05). However, there was a statistically significantly lower mean albumin in cases with liver fibrosis in comparison with cases without fibrosis (P<0.05).

Analysis of the relationship between liver fibrosis and different variables by univariate regression analysis (Table 4) indicated that:

(1) Ang-2 serum levels showed an independent significant positive relation with liver fibrosis (coefficient: 0.000023, P<0.05).
(2) AFP showed an independent significant positive relation with liver fibrosis (coefficient: 0.05848, P<0.05).
(3) Albumin showed an independent significant negative relation with liver fibrosis (coefficient: −0.09636, P<0.05).
(4) INR showed an independent significant positive relation with liver fibrosis (coefficient: 0.2451, P<0.05).

An analysis of therelationship between different variables and liver fibrosis was carried out using

Table 1 Comparison of angiopoietin-2 between normal controls and patients with different stages of liver fibrosis by the analysis of variance test

	Stages of fibrosis	Normal (control)	F0	F1	F2	F3	F4	*P* value
Angiopoietin-2 (pg/ml)	Mean	202.44	367.40	739.99	1578.16	3831.66	13095	<0.001
	SD	21.58	83.10	105.047	453.455	1386.09	2622.29	

multivariate regression analysis (Table 4). Among the total effects of all variables, only BMI showed a significant positive relation with liver fibrosis (coefficient: 0.01100, P=0.0463).

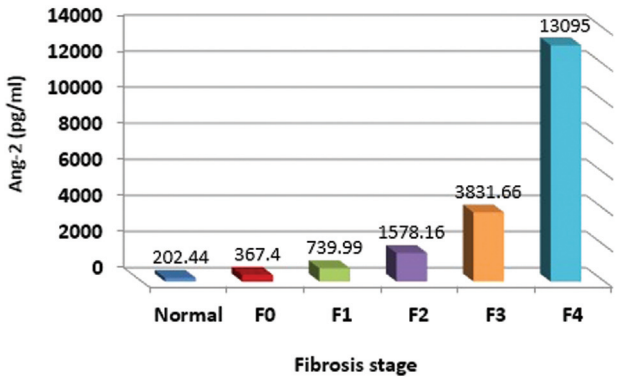
Pearson correlation analysis was used to find the correlation between Ang-2 and other variables in cases with CHC (n=75) (Table 5). There was a statistically significantly positive correlation between Ang-2 serum levels and AFP, FIB4, AST, APRI score, HCV-PCR, ALT, and total bilirubin (P<0.05). There was a statistically significantly negative correlation between Ang-2 and serum albumin (P<0.05).

Receiver operating characteristic curve (ROC) showed that the FIB4 score (Table 6) had a significant diagnostic value for liver fibrosis stages (F>2) at a cutoff more than 2.5 pg/ml (P=0.032) and F>3 at a cutoff more than 2.5 pg/ml (P=0.048). FIB4 showed 32% sensitivity and 93.7% specificity in the diagnosis of significant fibrosis (F>1) (Fig. 3).

The ROC curve showed that the APRI score (Table 6) had significant diagnostic value for liver fibrosis stages (F>2) at a cutoff more than 0.96 (P=0.046) and F>3 at a cutoff more than 1.06 (P=0.011). It showed 52% sensitivity and 75% specificity in differentiating patients with significant fibrosis (F>1) at a cutoff value more than 0.67 (Fig. 4).

AUC-ROC analysis of the diagnostic value of Ang-2 showed 100% sensitivity and 100% specificity in differentiating fibrosis stages(F>1, >2,>3) at cutoff values more than 869.3, 2226, and 7205 pg/ml, respectively, as shown in Table 6 and Fig. 5.

Figure 1



Comparison of angiopoietin-2 (Ang-2) between normal controls and patients with different stages of liver fibrosis.

Discussion

CHC viral infection represents a serious health problem for nearly 200 million infected individuals worldwide. Morbidity and mortality rates of chronic HCV infection have been increasing since 2007 [17]. Egypt has the highest prevalence of HCV worldwide (15%) [18] and the highest prevalence of HCV genotype 4, which is responsible for almost 90% of HCV infections [19]. Patients have different clinical outcomes, ranging from acute resolving hepatitis to CLDs including liver cirrhosis or HCC [20]. Accurate evaluation of hepatic

Table 2 Pairwise comparisons of the mean angiopoietin-2 between different stages of liver fibrosis by the analysis of variance test

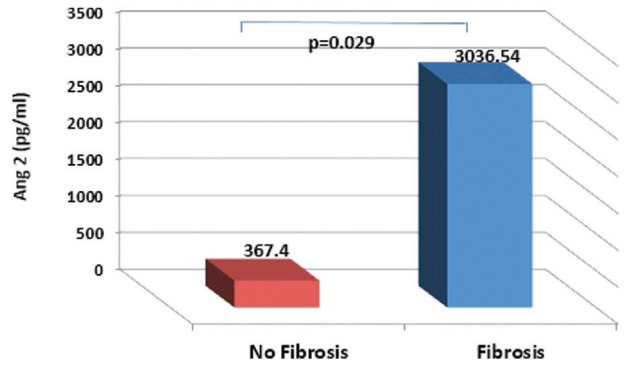
Fibrosis stages	Mean difference	*P* value	95% confidence interval
F0			
F1	−372.59	1.0000	−1541.4734–796.2859
F2	−1210.76	**0.0170**	−2295.7089 to −125.8219
F3	−3464.26	<**0.0001**	−4609.5301 to −2319.0032
F4	−12727.6	<**0.0001**	−14206.128–11249.0712
Normal	164.95	1.0000	−1017.8697–1347.7763
F1			
F2	−838.17	0.0846	−1729.5437–53.2004
F3	−3091.67	<**0.0001**	−4055.5557 to −2127.7901
F4	−12355	<**0.0001**	−13697.946–11012.0658
Normal	537.54	1.0000	−470.6751–1545.7693
F2			
F3	−2253.5	<**0.0001**	−3113.6716 to −1393.3310
F4	−11516.8	<**0.0001**	−12787.390–10246.2789
Normal	1375.7	**0.0002**	466.1392–2285.2982
F3			
F4	−9263.3	<**0.0001**	−10585.7697–7940.8970
Normal	3629.2	<**0.0001**	2648.4750–4609.9650
F4			
Normal	12892.5	<**0.0001**	11537.4593–14247.6473

Table 3 Comparison between patients with no fibrosis and patients with fibrosis in terms of angiopoietin-2 and the clinical variables using the analysis of variance test (*n*=75)

Variables	CHC (mean±SD)		*P* value
	No fibrosis (*n*=9)	Fibrosis (*n*=66)	
Angiopoietin-2 (pg/ml)	367.4±83.10	3036.54±3574.37	**0.029**
Age (years)	37.9±13.10	40.8±11.96	0.500
Albumin (g/dl)	3.9±0.49	3.13±0.95	**0.015**
ALT (U/l)	32.2±13.79	49.04±49.31	0.315
AFP (ng/ml)	0.39±0.15	1.28±1.32	**0.049**
APRI	0.47±0.19	0.88±0.97	0.162
AST (U/l)	35.4±12.83	61.48±58.27	0.188
AAR	1.2±0.57	1.40±0.66	0.467
BMI (kg/m^2)	28.6±8.29	31.41±8.59	0.360
FIB4	1.28±0.65	2.07±1.64	0.178
HCV-PCR (copies/ml)	306049±66164.34	298783.22±97574.66	0.830
INR	1.17±0.13	1.40±0.32	**0.039**
Platelets (10^9/l)	197.7±55.51	200.28±65.26	0.913
PT (s)	13.15±1.72	13.08±2.22	0.921
TB(mg/dl)	0.52±0.17	0.65±0.23	0.125

AAR, aspartate aminotransferase/alanine aminotransferase ratio; AFP, α-fetoprotein; ALT, alanine aminotransferase; APRI, the AST-to-platelet ratio index; AST, aspartate aminotransferase; FIB4, Fibrosis-4; HCV, hepatitis C virus; INR, international normalized ratio; PT, prothrombin time; TB, total bilirubin. The Bold values $P \leq 0.05$ are significant (S).

Figure 2



Comparison between angiopoietin-2 (Ang-2) mean levels in patients with fibrosis and patients without fibrosis using the analysis of variance test.

fibrosis has become the primary goal in managing the progression of CHC because its morbidity and mortality are linked to cirrhosis and its complications. In addition, the decision of physicians to administer antiviral therapy depends on the stage of fibrosis [21]. LB is considered the standard method for the diagnosis and staging of fibrosis on the basis of its value in assessing the stage of fibrosis and necroinflammatory grade [22]. However, LB can overestimate or underestimate the degree of fibrosis [23]. LB is not reasonable for the repetitive assessment of liver fibrosis during the long-term follow-up of patients, necessitating noninvasive markers that accurately diagnose the progression of CLDs as CHC before, during, and after treatment [24].

In the current study, using univariate regression analysis, there was a significant relation between liver fibrosis on the one hand and Ang-2, AFP, INR, and albumin on the other. INR and AFP were found to have an independent significant positive relation, whereas serum albumin was found to have a significant negative relation with liver fibrosis. Also, statistically significantly higher means of INR and AFP, and lower mean of serum albumin were found in cases with liver fibrosis in comparison with cases without fibrosis. This is in agreement with Hernández-Bartolomé *et al.* [25]. In a study carried out by Sebastiani and Alberti [26], age, platelet count, INR, AST, and GGT were found to be independent variables linked to liver fibrosis.

There was no statistically significant relation between liver fibrosis and the main demographic features of CHC patients in the present study. Hernández-Bartolomé *et al.* [25], found a statistically significant relation by univariate regression analysis between liver fibrosis and age. Poynard *et al.* [27] found a correlation between a higher grades of fibrosis and male patients. In a study carried out by Wong *et al.* [28], age at the time of infection was found to be one of the most important host-related factors in the progression of liver fibrosis. Costa *et al.* [29] reported that patients who acquired HCV after the age of 40 years had a higher rate of fibrosis progression and reached the stage of liver cirrhosis within a period of time that was four to five times shorter than that observed for patients who were infected at a younger age.

In the current study, univariate regression analysis showed no significant relation between liver fibrosis and liver enzymes. These parameters were found to

Table 4 Relation between liver fibrosis and angiopoietin-2 and other variables using univariate and multivariate regression analyses among chronic hepatitis C cases (*n*=75)

Variables	Univariate regression analysis		Multivariate regression analysis	
	Coefficient	*P* value	Coefficient	*P* value
Ang-2 (pg/ml)	0.000023	0.029	0.0000678	0.245
Age (years)	0.002148	0.499	–0.004439	0.506
Sex	0.22314	0.754	0.04288	0.621
BMI (kg/m^2)	0.004101	0.360	0.01100	**0.046**
AFP (ng/ml)	0.05848	0.049	–0.1233	0.407
Albumin (g/dl)	–0.09636	0.015	–0.06096	0.236
ALT (U/l)	0.0008232	0.314	0.001273	0.712
AST (U/l)	0.0009070	0.188	–0.002137	0.619
AAR	0.04272	0.466	0.03844	0.767
INR	0.2451	0.038	0.2519	0.070
Platelets (10^9/l)	0.000065	0.912	0.0008064	0.467
PT (s)	–0.001768	0.920	–0.007385	0.707
TB (mg/dl)	0.2486	0.125	–0.04796	0.811
TLC (×10^3/mm^3)	0.01781	0.546	0.04284	0.187
HCV-PCR (copies/ml)	–0.00000008	0.829	–0.00000033	0.481
APRI	1.72090	0.162	0.04742	0.875
FIB4	0.54212	0.178	0.06034	0.595

AAR, aspartate aminotransferase/alanine aminotransferase ratio; AFP, α-fetoprotein; ALT, alanine aminotransferase; Ang-2, angiopoietin-2; APRI, AST-to-platelet ratio index; AST, aspartate aminotransferase; FIB4, Fibrosis-4; HCV, hepatitis C virus; INR, international normalized ratio; PT, prothrombin time; *R*, correlation coefficient; TB, total bilirubin; TLC, total leukocyte count. The Bold value $P \leq 0.05$ is significant.

Table 5 Pearson correlation between angiopoietin-2 and other variables in patients with chronic hepatitis C (*n*=75)

Variables	Angiopoietin-2 (*R*)	*P* value
AFP (ng/ml)	0.9724	<**0.0001**
FIB4	0.3508	**0.0020**
AST (U/l)	0.4677	<**0.0001**
APRI	0.4020	**0.0004**
HCV-PCR (copies/ml)	0.2957	**0.0100**
ALT (U/l)	0.484	<**0.0001**
Albumin (g/dl)	–0.489	<**0.0001**
ALT/AST	–0.106	0.3648
INR	0.011	0.9287
Platelets (10^9/l)	–0.053	0.6489
PT (s)	–0.042	0.7175
TB (mg/dl)	0.456	<**0.0001**
TLC (×10^3/mm^3)	0.039	0.7395

AFP, α-fetoprotein; ALT, alanine aminotransferase; Ang-2, angiopoietin-2; APRI, AST-to-platelet ratio index; AST, aspartate aminotransferase; FIB4, Fibrosis-4; HCV, hepatitis C virus; INR, international normalized ratio; PT, prothrombin time; *R*, correlation coefficient; TB, total bilirubin; TLC, total leukocyte count. The Bold values $P \leq 0.05$ are significant.

have positive significant relations with liver fibrosis in the study by Hernández-Bartolomé *et al.* [25]. In studies carried out by Adinolfi *et al.* [30] and Zechini *et al.* [31], there was a correlation between liver fibrosis and serum ALT levels. It is well known that ALT is released by direct virus-related cytopathic activity and/or by an immune-mediated process [32]. In a study carried out by Liu *et al.* [33], serum ALT levels were not found to be a useful parameter to assess liver damage in the patients with CHC.

In the present study, the univariate regression analysis found no statistically significant relation between liver fibrosis and total bilirubin, which is different from the results of Hernández-Bartolomé *et al.* [25].

The ROC curve analysis in this study showed that the FIB4 score had significant diagnostic value in differentiating patients with fibrosis stages (F>2) at a cutoff more than 2.5 and patients with F>3 at a cutoff more than 2.5. It showed low sensitivity and high specificity in differentiating patients with significant fibrosis (those with >F1). In previous studies, the use of this index correctly classified 87% of patients with FIB4 values beyond 1.45–3.25 and biopsy could be avoided in 71% of the patients in the validation set with an AUC of 0.765, a sensitivity of 70%, and a specificity of 97% for differentiating fibrosis stages of Ishak score 0–3 from 4 to 6 [15].

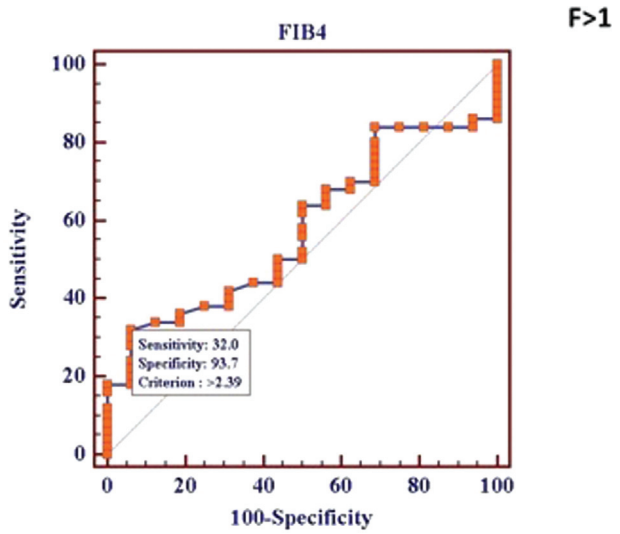
In the present study, the APRI score had significant diagnostic value in the stages F>2 at a cutoff more than 0.96 and F>3 at a cutoff more than 1.06 using ROC curve analysis. It showed 52% sensitivity and 75% specificity in the diagnosis of significant hepatic fibrosis (stages>F1). This is in agreement with a recent meta-analysis study carried out by Lin *et al.* [34], who suggested that the APRI score can identify hepatitis C-related fibrosis with only a moderate degree of accuracy. In the study by Giannini *et al.*

Table 6 Diagnostic value of each of the Fibrosis-4 score, the aspartate aminotransferase–platelet ratio index score, and angiopoietin-2 in discriminating different stages of liver fibrosis using receiver operating characteristic curve analysis (*n*=66)

Parameters	FIB4			APRI			Angiopoietin-2		
	F>1	F>2	F>3	F>1	F>2	F>3	F>1	F>2	F>3
AUROC	0.57	0.65	0.77	0.58	0.65	0.81	1	1	1
SE	0.07	0.07	0.14	0.07	0.07	0.12	0.00	0.00	0.00
95% CI	0.44–0.6	0.52–0.7	0.66–0.8	0.45–0.70	0.52–0.76	0.69–0.89	0.94–1	0.94–1	0.94–1
P value	0.31	**0.03**	**0.04**	0.28	**0.046**	**0.011**	**<0.0001**	**<0.0001**	**<0.0001**
Cutoff	>2.39	>2.5	>2.5	>0.67	>0.96	>1.06	>869.3	>2226	>7205
Sensitivity (%)	32	45.8	83.3	52	50	83.3	100	100	100
Specificity (%)	93.7	88.1	81.6	75	85.7	83.3	100	100	100
PPV (%)	94.1	68.7	31.2	86.7	66.7	33.3	100	100	100
NPV (%)	30.6	74	98	33.3	75	98	100	100	100

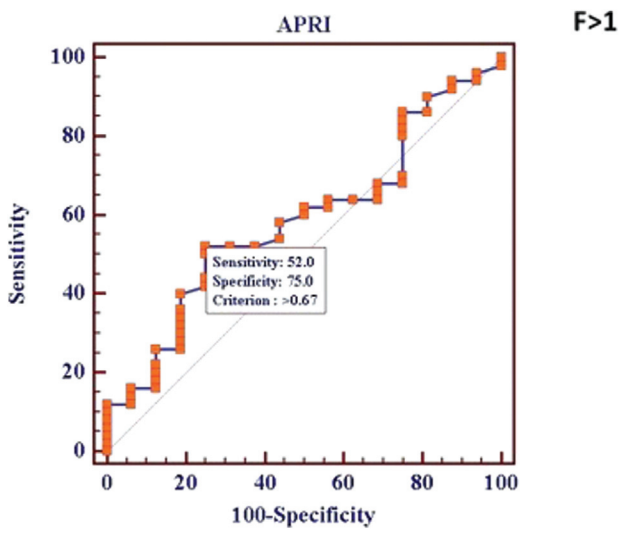
APRI, aspartate aminotransferase-to-platelet ratio index; AUROC, area under the receiver operating characteristic curve; CI, confidence interval; FIB4, Fibrosis-4; NPV, negative predictive value; PPV, positive predictive value. The Bold values $P \leq 0.05$ are significant.

Figure 3



Diagnostic value of the Fibrosis-4 (FIB4) score in differentiating patients with significant fibrosis (F>1), at cutoff more than 2.39, by receiver operating characteristic curve.

Figure 4



Diagnostic value of the APRI score in differentiating patients with significant fibrosis (F>1) at a cutoff more than 0.67 by receiver operating characteristic curve. APRI, aspartate aminotransferase-platelet ratio index.

[32], it was found that APRI scores in patients with CHC showed a rather good diagnostic performance and reproducibility, particularly for cirrhosis.

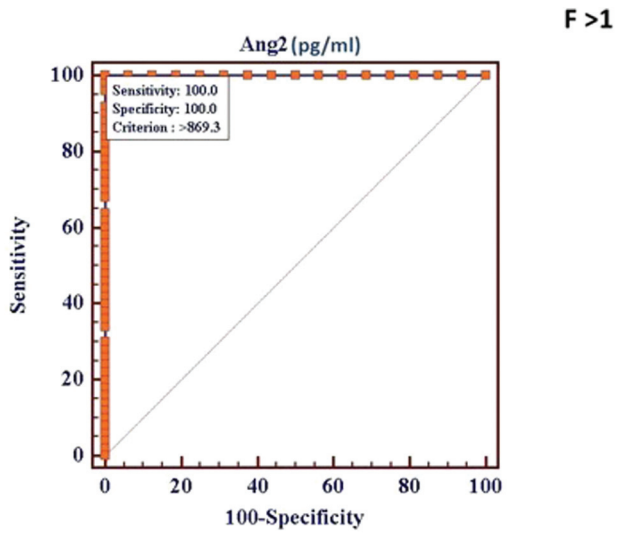
In this study, statistically significantly higher mean Ang-2 levels were found in CHC patients compared with normal individuals. Also, higher levels of Ang-2 were present in higher stages of liver fibrosis. A significant positive correlation was found between Ang-2 and AFP, FIB4, AST, APRI score, HCV-PCR, ALT, and total bilirubin, whereas there was a statistically significant negative correlation between Ang-2 serum levels and serum albumin.

Ang-2 is one of the regulators of neovascularization, vascular remodeling, and maturation in CHC patients through agonistic and antagonistic autophosphorylation of its common tyrosine kinase receptor, Tie2 [35]. Also, it is one of the most significant signaling pathways in pathological angiogenesis and HCC [36].

Serum vascular endothelial growth factor (VEGF), Ang-2, and Tie2 are increased in viral hepatitis and their concentrations could be valuable markers of hepatic inflammation, disease progression, and response to therapy. Treatment of CHC patients with pegylated interferon and ribavirin was found to reduce the inflammatory process and therefore could decrease VEGF and Ang-2 concentration [37].

In the current study, ROC curve analysis showed that the Ang-2 cutoff value more than 869.3 pg/ml was the best in differentiating patients with significant fibrosis (>F1) [sensitivity=100%, specificity=100%, 95% confidence interval (CI)=0.94–1, and AUC=1].

Figure 5



Diagnostic value of angiopoietin-2 (Ang-2) in differentiating patients with significant fibrosis (F>1) by receiver operating characteristic curve.

The Ang-2 cutoff value more than 2226 pg/ml was the best in differentiating patients with advanced fibrosis (F>2) [P<0.0001, positive predictive value=100%, negative predictive value=100%, sensitivity=100%, specificity=100%, 95% CI=0.94–1, SE=0, and AUC=1].

The cutoff value more than 7205 pg/ml was the best in differentiating patients with cirrhosis (F>3) [P<0.0001, positive predictive value=100%, negative predictive value=100%, sensitivity=100%, specificity=100%, 95% CI=0.94–1, SE=0, and AUC=1].

These findings are in agreement with Hernández-Bartolomé *et al.* [25], who found that Ang-2 serum levels increased progressively with the stages of fibrosis. Hernández-Bartolomé *et al.* [25] found that Ang-2 was accurate in differentiating between different fibrosis stages (F1, F2, and F3) in 107 CHC patients. In their study, AUC values were 0.886 for F1, 0.920 for F2, and 0.923 for F3. Their cutoffs had a sensitivity and specificity of ~80% or higher and accuracy above 80% for all stages (F1, F2, and F3) [25].

Conclusion

Ang-2 correlated significantly with liver fibrosis stage. It can aid noninvasive differentiation between different stages of liver fibrosis in patients with CHC.

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Conflicts of interest

There are no conflicts of interest.

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