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Research Article

Diagnostic Performance of AFP, Autotoxin and Collagen IV and their Combinations for Non-Invasive Assessment of Hepatic Fibrosis Staging in Liver Fibrosis Patients Associated with Chronic HCV

Entsar A Saad[1*], Salem A Habib[1,2], Mona S Eltabeey[1]

[1]*Chemistry Department, Faculty of Science, Damietta University, Damietta, Egypt*
[2]*Department of Biochemistry, Faculty of Science, University of Tabuk, Saudi Arabia*

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ABSTRACT

Background: Lately, several studies have utilized non-invasive serological markers to assess liver fibrosis and some are currently being validated as potential tools to determine liver damage. Purpose: Our aim was to investigate the diagnostic performance of AFP, autotoxin and collagen IV as non-invasive biomarkers of hepatic fibrosis. Patients and methods: 45 males and 15 females with chronic hepatitis C were enrolled in the current study. Laboratory assessment was done for all subjects in form of complete blood picture, liver function test, alpha fetoprotein (AFP), collagen IV and autotaxin. Patients were grouped according to the stage of fibrosis into F1, F2 and F3. Results: Mean serum values of AFP, autotaxin and collagen IV were elevated in all patients compared to healthy controls. Surprisingly, with increasing fibrosis stage AFP showed non-significant change while collagen IV and autotoxin showed significant increase ($P<0.01$ and $P<0.0001$, respectively). Autotaxin and collagen IV were significantly ($P<0.01$ and $P<0.05$, respectively) lower in F1 patients than those with F2-F3 but AFP level showed non-significant change. Autotaxin had the highest area under ROC curve and the highest accuracy for discrimination of F1 from F2-F3 patients and for discrimination of patients with F3 from F1-F2. Different combinations between AFP, collagen IV and autotoxin showed improvement in the accuracy. Conclusion: It was concluded that serum autotaxin may at least serve as a new clinical non-invasive alternative in patients who are not candidates for liver biopsy for diagnosis of liver damage. Autotaxin combination with collagen IV and AFP addition make them more useful.

Keywords: Hepatic fibrosis, Autotaxin, Collagen IV, Alpha fetoprotein, Hepatitis C virus.

INTRODUCTION

The liver is typically inflamed and shows signs of injury in hepatocellular diseases[1]. Hepatocellular injury may be related to variable pathologic conditions like exposure to different toxicants[2-4], obesity[5], diabetes[6,7] and even cancer[8-10]. Even though, viral infection still the most common cause[11] results in hepatocellular damage manifested by fatty infiltration (steatosis), inflammation (hepatitis) or cell death. In mild attack, the liver will recover and overall liver function will remain normal. Persistent injury will lead to fibrosis and cirrhosis and potentially severe liver dysfunction[12]. Liver damage and liver diseases are frequently monitored by liver function tests[6,13]. Lately, several studies have utilized non-invasive serological markers to assess liver fibrosis and some are currently being validated as potential tools to determine liver damage[11].

Liver fibrosis is scarring through excessive deposition of extracellular matrix (ECM) components as a result of liver's response to repair injury. One of the main causes of liver fibrosis is chronic viral hepatitis[11]. Hepatitis C virus (HCV) represents the chief cause of viral hepatitis[14] and consequently it is the chief cause for liver associated diseases[15]. It is more likely to end up in cirrhosis and hepatocellular carcinoma (HCC) that represents the third cause for tumor-related deaths globally[16].

Internationally, 171 million persons are HCV-infected and this chronic infection results in about 390,000 deaths per year due to linked cirrhosis and HCC[14]. Indirectly, detection of HCV specific antibodies is used to screen and diagnose infection. However, this assay does not distinguish active and resolved infections. So, detection of viral components (e.g., the core antigen or the viral genome) is greatly now used to directly diagnose HCV-infected patients[17].

Fibrosis staging is of great significance for patients with HCV related liver disease to indicate antiviral treatment, to monitor the response and to predict the prognosis. Liver biopsy (LB) is still considered the gold standard in assessment of the fibrosis stage[18]. However, LB is invasive, expensive and difficult to be repeated so it is non-acceptable by most patients. It also showed sampling errors, observer related variability of histo-pathological interpretation, and risk of rare but life-threatening complications[19].

Serum markers may present precious efficient alternative

**Author for Correspondence: entsarsaad@gmail.com*

Table 1: Demographic data of control and patients groups.

Variables		Healthy (n=20)	Fibrosis (n=60)			P value
			F1 (n=19)	F2 (n=23)	F3 (n=18)	
Gender	Male	15	45			-
	Female	5	15			-
Age (years)	Mean ± SD	42.5 ± 9.4	44.7 ± 8.1			> 0.05
HCV infection	anti-HCV	negative	positive			-
	PCR	-	positive			-
HBV infection	HBsAg	negative	negative			-

F1, moderate fibrosis stage; F2, intermediate fibrosis; and F3, extensive fibrosis stage. P>0.05 is considered non significant.

Table 2: Routine laboratory data of control and fibrosis patients groups.

Variables	Healthy (n=20)	Fibrosis (n=60)	P value	F1 (n=19)	F2 (n=23)	F3 (n=18)	P value
Liver Function Tests							
ALT (U/L)	12.2 ± 4.6	44.6 ± 37.6	<0.0001	30.6 ± 25.1	51.7 ± 50.3	50.3 ± 25.6	0.147
AST (U/L)	16.1 ± 4.9	41.2 ± 37.4	0.004	30.5 ± 28.3	47.0 ± 51.0	45.2 ± 20.9	0.317
T.bilirubin (mg/dL)	0.61 ± 0.14	0.79 ± 0.33	0.019	0.76 ± 0.38	0.85 ± 0.28	0.75 ± 0.32	0.557
D.bilirubin (mg/dL)	0.16 ± 0.04	0.22 ± 0.08	0.003	0.19 ± 0.06	0.23 ± 0.08	0.23 ± 0.10	0.202
ALP (U/L)	72.8 ± 16.3	89.2 ± 35.4	0.048	82.8 ± 22.7	86.9 ± 34.9	99.1 ± 45.4	0.355
Albumin (g/dL)	4.7 ± 0.38	4.1 ± 0.53	<0.0001	4.11 ± 0.41	4.07 ± 0.59	4.14 ± 0.58	0.940
P.T (Second)	12.9 ± 2.3	14.6 ± .9	<0.0001	14.1 ± 0.7	14.6 ± 0.7	15.2 ± 0.9	<0.0001
*Haematological parameter*s							
Hemoglobin (g/dL)	14.1 ± 0.63	12.0 ± 1.9	<0.0001	11.2 ± 1.2	12.4 ± 1.8	12.4 ± 2.4	0.062
RBCs ($\times10^9$/L)	4.2 ± 0.4	4.3 ± 1.0	0.957	4.1 ± 0.9	4.2 ± 0.7	4.4 ± 1.4	0.627
TLC ($\times10^9$/L)	6.7 ± 1.6	4.8 ± 1.8	<0.0001	4.3 ± 1.8	5.0 ± 1.5	4.9 ± 2.0	0.396
PLT ($\times10^9$/L)	252.8 ± 65.5	172.6 ± 66.3	<0.0001	194.4 ± 66.4	164.7 ± 73	159.6 ± 53.7	0.218

Continuous variables were expressed as mean ± SD. Reference values: Alanine aminotransferase (ALT) up to 45 U/L; aspartate aminotransferase (AST) up to 32 U/L; total bilirubin (T.bilirubin) up to 1.2 mg/dL; direct bilirubin (D.bilirubin) up to 0.25 mg/dL; alkaline phosphatase (ALP) 35 - 130 U/L; albumin 35-55 (g/L); hemoglobin (male 11–16 g/dL); red blood cells (RBCs) 3.5–5.5 ($\times10^9$/L); total leucocytic count (TLC) 6–11($\times10^9$/L); platelet count (PLT) 150-400 (10^9/L); prothrombin time (P.T) 10 - 14 Second. P>0.05 is considered non-significant; P<0.05 is considered significant. P<0.01 is considered highly significant, P<0.001 is considered very significant and P<0.0001 is considered extremely significant.

to LB for patients and clinicians allowing continual monitoring of fibrosis as they are non-invasive, repetitive, and mostly inexpensive with low risk of sampling errors and small observer-related variability[20]. Thus, the aim of this study was to investigate the diagnostic performance of AFP, autotoxin and collagen IV as non-invasive serum biomarkers of fibrosis.

MATERIALS AND METHODS

Subjects

This study was carried out on 80 subjects who signed informed consents. Of them, 60 chronic hepatitis C (CHC) patients with liver fibrosis, collected from Gastroenterology and Tropical Department and Outpatient Clinic at Al-Homiatte Hospital, Damietta, Egypt during the period from November 2014 to August 2016, of ages ranged between 25-59 years (44.7 ± 8.1 years) were used as the fibrosis group in this study. 75% of them were males (n=45) and 25% of them were females (n=15). All patients were positive for anti-HCV antibodies and HCV infection was confirmed via the presence of HCV-RNA using quantitative PCR assay. Regarding the distribution of liver fibrosis stages, CHC patients according to METAVIR scoring system were classified into 32% (n=19) portal fibrosis without septae (mild; F1), 38% (n=23) portal fibrosis with a few septae (moderate; F2) and 30% (n=18) septal fibrosis without cirrhosis (advanced; F3). On the other hand, 20 healthy individuals negative for anti-HCV antibodies (75%, n=15 males and 25%, n=5 females) were used as a control group. Their age ranged between 20-55 years (42.5 ± 9.4 years). All subjects (patients and controls) were free from HBV infection (Table 1).

All subjects were submitted to full history taking; including personal history (name, age, sex, residence, occupation, special habits of medical importance as smoking), and the present history and past history of HCV and HBV.

This study was performed in accordance with the Declaration of Helsinki of World Medical Association and

Table 3: Routine laboratory data of patients at fibrosis stages F1 versus F2-F3 and F3 versus F1-F2.

Variable	F1 (n=18)	F2-F3 (n=41)	P value	F3 (n=18)	F1-F2 (n=42)	P value
Liver Function Tests						
ALT (U/L)	30.6 ± 25.1	51.0 ± 40.9	0.050	50.3 ± 25.6	42.1 ± 41.8	0.447
AST(U/L)	30.5 ± 28.3	46.2 ± 40.2	0.130	45.2 ± 20.9	39.5 ± 42.6	0.593
T.bilirubin (mg/dL)	0.76 ± 0.38	0.81 ± 0.3	0.594	0.75 ± 0.32	0.81 ± 0.3	0.544
D.bilirubin (mg/dL)	0.19 ± 0.06	0.23 ± 0.1	0.074	0.23 ± 0.10	0.21 ± 0.1	0.526
ALP (U/L)	82.8 ± 22.7	92.3 ± 39.8	0.342	99.1 ± 45.4	85.1 ± 29.7	0.162
Albumin (g/dL)	4.11 ± 0.41	4.10 ± 0.58	0.973	4.14 ± 0.58	4.09 ± 0.51	0.765
P.T (Second)	14.1 ± 0.7	14.9 ± 0.9	0.001	15.2 ± 0.9	14.3 ± 0.8	<0.0001
*Haematological parameter*s						
Hemoglobin (g/dL)	11.2 ± 1.2	12.4 ± 2.1	0.018	12.4 ± 2.4	11.9 ± 1.7	0.331
RBCs ($\times 10^9$/L)	4.1 ± 0.9	4.3 ± 1.1	0.463	4.4 ± 1.4	4.2 ± 0.8	0.371
TLC ($\times 10^9$/L)	4.3 ± 1.8	4.96 ± 1.7	0.181	4.9 ± 2.0	4.7 ± 1.7	0.735
PLT ($\times 10^9$/L)	194.4 ± 66.4	162.5 ± 64.5	0.082	159.6 ± 3.7	178.2 ± 70.8	0.324

Continuous variables were expressed as mean ± SD. Reference values: Alanine aminotransferase (ALT) up to 45 U/L; aspartate aminotransferase (AST) up to 32 U/L; total bilirubin (T.bilirubin) up to 1.2 mg/dL; direct bilirubin (D.bilirubin) up to 0.25 mg/dL; alkaline phosphatase (ALP) 35 - 130 U/L; albumin 35-55 (g/L); hemoglobin (male 11–16 g/dL); red blood cells (RBCs) 3.5–5.5 ($\times 10^9$/L); total leucocytic count (TLC) 6–11($\times 10^9$/L); platelet count (PLT) 150-400 (10^9/L); prothrombin time (P.T) 10 - 14 Second. P>0.05 is considered non-significant; P<0.05 is considered significant. P<0.01 is considered highly significant, P<0.001 is considered very significant and P<0.0001 is considered extremely significant.

Table 4: Fibrosis biomarkers [alpha fetoprotien (AFP), autotoxin and collagen IV] in control and patients groups.

Group/Biomarker		Collagen IV (ng/mL)	Autotaxin (ng/mL)	AFP (U/L)
Healthy (n=20)		0.71 ± 0.59	0.63 ± 0.49	4.8 ± 1.6
Fibrosis (n=60)		1.9 ± 1.1	2.37 ± 1.8	8.0 ± 6.2
	P Value	<0.0001	<0.0001	0.023
F1 (n=19)		1.4 ± 0.82	1.3 ± 1.1	7.0 ± 7.6
F2 (n=23)		1.8 ± 0.9	2.13 ± 1.67	6.6 ± 4.2
F3 (n=18)		2.6 ± 1.3	3.8 ± 1.8	10.95 ± 6.0
	P Value	0.002	<0.0001	0.055
	P Value (F1 vs. F2)	0.143	0.075	0.828
	P Value (F1 vs. F3)	0.001	<0.0001	0.091
	P Value (F2 vs. F3)	0.018	0.004	0.01
F1-F2 (n=42)		1.63 ± 0.9	1.76 ± 1.5	6.8 ± 5.9
F2-F3 (n=41)		2.17 ± 1.1	2.87 ± 1.9	8.5 ± 5.5
	P Value (F1 vs. F2-F3)	0.001	<0.0001	0.016
	P Value (F3 vs. F1-F2)	0.011	0.002	0.388

Continuous variables were expressed as mean ± SD.
Reference values: Alpha fetoprotein (AFP) up to 8.2 (U/L); autotaxin 0.16 - 10 ng/mL and Collagen IV 0.78 - 50 ng/mL. P>0.05 is considered non-significant; P<0.05 is considered significant. P<0.01 is considered highly significant, P<0.001 is considered very significant and P<0.0001 is considered extremely significant.

it was approved by Al-Homiatte Hospital, Damietta, Egypt.

Subjects exclusion criteria

Cases not fulfilling the above-mentioned inclusion criteria were excluded in addition to patients with the following conditions were also excluded from this study: co-infection with hepatitis B virus, prior antiviral or immunosuppressive therapy and decompensated liver disease (ascites, jaundice, variceal bleeding or encephalopathy). Moreover, patients with reduced production of platelets other than hepatic infection with HCV such as typhoid, deficiency of vitamin B12 and leukemia were excluded as well.

Samples

Needle LB specimens were obtained with an 18-gauge or larger needle. They were processed, investigated and blindly interpreted according to METAVIR scoring system at Clinical Pathology Department Research Laboratory, Al-Homiate Hospital, Damietta, Egypt.

Directly after staging of liver fibrosis, venous blood samples were taken on citrated (1:9), EDTA and plain tubes. Samples in plain tubes were centrifuged and serum was separated and divided into 2 aliquots, one for liver function tests (alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), albumin, total & direct bilirubin) on the day of blood collection. The other aliquot serum was stored at –70 ºC for alpha fetoprotein, collagen IV and autotaxin assessment. Samples in EDTA tubes were used for

Table 5: Diagnostic performance of individual fibrosis biomarkers for discrimination between F1 versus F2-F3 and for discriminating between F3 versus F1-F2.

	F1 versus F2-F3			F3 versus F1-F2		
	AFP (U/L)	Collagen IV (ng/mL)	Autotaxin (ng/mL)	AFP (U/L)	Collagen IV (ng/mL)	Autotaxin (ng/mL)
AUC	0.63	0.67	0.77	0.72	0.75	0.84
Cut-off	≥ 6.5	≥ 1.7	≥ 1.4	≥ 7.9	≥ 1.8	≥ 1.9
Sensitivity (%)	63%	68%	83%	72%	72%	88.9%
Specificity (%)	74%	53%	60%	67%	67%	74%
PPV (%)	84%	76%	81%	48%	48%	59%
NPV (%)	48%	44%	61%	85%	85%	94%
Accuracy (%)	67%	63%	75%	68%	68%	78%

AUC; Area under ROC curve, PPV; Positive predictive value, NPV; Negative predictive value.

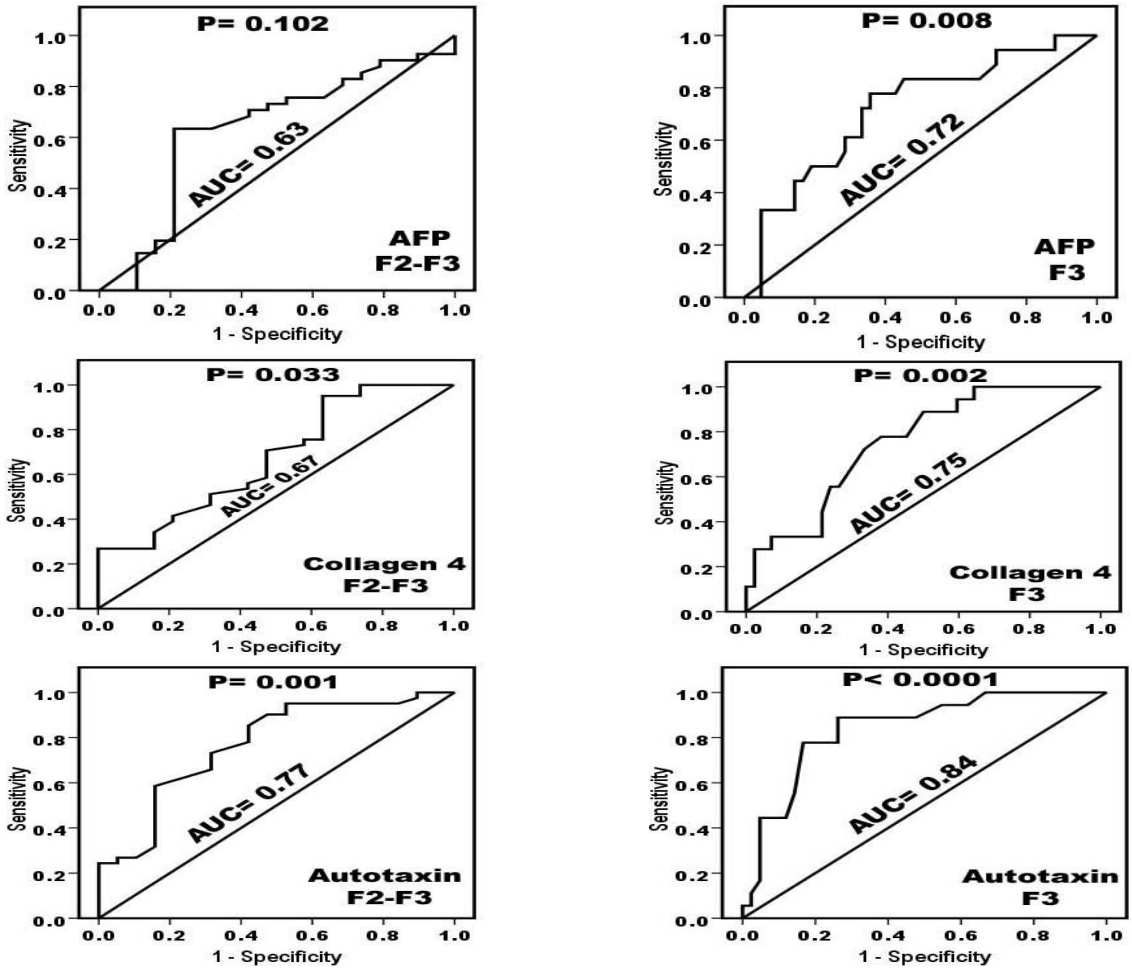

Figure 1: Receiver operator characteristic (ROC) curve of AFP, autotaxin, collagen IV (collagen 4) for discrimination of patients with F1 from F2-F3 (left) and for discrimination of patients with F3 from F1-F2 (right). The true positive rate (sensitivity) is plotted as a function of the false rate (1−specificity). Each point on the ROC plot represents a sensitivity/specificity pair corresponding to a particular decision threshold. Area under ROC curve (AUC) value represents the combined effects of both sensitivity and specificity of fibrosis markers in diagnosis of patients.

complete blood count (CBC) on the day of blood collection. Samples in citrated tubes were centrifuged and citrated plasma was separated and used for prothrombin time (P.T) measurement immediately.

Biochemical measurements

All subjects were screened for HBsAg (Dia. Pro, Milan, Italy) and for anti-HCV antibodies (Biomedica, Sorin, Italy). Patients were then confirmed for the presence of HCV-RNA using quantitative PCR assay (COBAS Ampliprep/COBAS TaqMan, Roche Diagnostics, Pleasanton, USA). Liver function tests (ALT, AST, ALP, albumin, total & direct bilirubin) were routinely measured in serum by the available commercial kits using the Automatic Analyzer (Hitachi 902). Complete blood count (CBC) was done using Cell-Dyn® 1800 Hematology Analyzer. P.T was measured using blood coagulometer

Table 6: Diagnostic performance of combined fibrosis biomarkers for discrimination of patients with F1 from F2-F3 and for discrimination of patients with F3 from F1-F2.

	F1 versus F2-F3			F3 versus F1-F2				
	AFP + Collagen IV	AFP + Autotaxin	Autotaxin + Collagen IV	AFP + Autotaxin + Collagen IV	AFP + Collagen IV	Autotaxin + Collagen IV	AFP + Autotaxin	AFP + Autotaxin + Collagen IV
AUC	0.68	0.76	0.78	0.79	0.797	0.88	0.895	0.93
Cut-off	≥ 0.24	≥ 0.17	≥ 0.19	≥ 0.2	≥ 0.32	≥ 31	≥ 0.35	≥ 0.35
Sensitivity (%)	66%	81%	73%	73%	72%	88.9%	94%	88.9%
Specificity (%)	63%	58%	63%	63%	76%	79%	83%	86%
PPV (%)	79%	80%	81%	81%	57%	54%	71%	73%
NPV (%)	46%	57%	52%	52%	87%	94%	97%	95%
Accuracy (%)	65%	73%	70%	70%	75%	82%	87%	87%

AUC; Area under ROC curve, PPV; Positive predictive value, NPV; Negative predictive value.

(SEAC S2) with the available kit (Biostec Liquiplstin, Egypt). Alpha fetoprotein (AFP) was estimated using a compact automated immunoassay system based on the Enzyme Linked Fluorescent Assay (ELFA) with mini-VIDAS® AFP kit (Biomerieux, Marcy-L'Etoile, France). Serum autotoxin, collagen IV levels were assayed using the human ENPP2 (Ectonucleotide Pyrophosphatase/Phosphodiesterase) and the human COL4 (Collagen Type IV) Enzyme Linked Immunosorbent Assay (ELISA) kits respectively (Guandong Science and Technology Industry Park, WuHan, China) according to the recommendations of the manufacturer. Measurements were performed on a Tecan SLT Rainbow Plate Reader (Tecan, Männedorf, Switzerland). Assays were done in duplicate and when results showed a difference with more than 10% the tests were repeated again.

Statistical analysis

All statistical analyses were done by the Statistical Package for Social Sciences (SPSS) version 15.0 for Microsoft Windows. Results were expressed as mean ± SD. Differences in continuous variables were assessed using Student's t-test or analysis of variance (ANOVA). All tests were two-tailed and statistical significance was assessed at the 0.05 level. Receiver operating characteristic (ROC) curves were created and areas under the ROC curves (AUC) were calculated. Diagnostic accuracy was also assessed using sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV).

RESULTS

As expected, patients with liver fibrosis were associated with significant (P<0.0001) lower platelets (PLT) count, total leucocytic count (TLC), hemoglobin and albumin levels, and with significant (P<0.05-P<0.0001) higher ALT, AST, total and direct bilirubin (T. and D.bilirubin), ALP, and P.T than those of healthy individuals. There was no significant difference in red blood cells (RBCs) (P>0.05, Table 2).

Comparison of the baseline characteristics of patients according to the stage of liver fibrosis is shown in Table 2. Statistically there were no significant difference between F1, F2 and F3 liver fibrosis stages in all studied haematological indices and liver function tests (P>0.05) except for P.T that increased significantly (P<0.0001) with increasing in fibrosis stage.

There was a significant difference between patients with F1stage and patients with F2-F3 in hemoglobin (P<0.05) and in P.T (P<0.01, Table 3). On the other hand, the laboratory data of patients with F3 stage were compared with those of patients with F1-F2 and the results show a highly significant difference in P.T (P<0.0001, Table 3).

There are significant elevations in AFP (P<0.05), in autotoxin (P<0.0001) and in collagen IV (P<0.0001) levels in patients with fibrosis were found when compared with their corresponding levels in healthy controls (Table 4). Regarding differences between the 3 fibrosis stages (F1, F2 and F3), AFP levels showed non-significant change (P>0.05) while collagen IV and autotoxin levels showed significant (P<0.01 and P<0.0001, respectively) increase with increasing fibrosis stage (Table 4).

Autotaxin and collagen IV levels were significantly (P<0.01 and P<0.05, respectively) lower in patients with F1 stage than those with higher stages (F2-F3) but AFP level showed non-significant change (P>0.05). On the other side, all of them showed significant (P<0.05-P<0.0001) elevations in patients with F3 stage as compared to patients with lower stages (F1-F2) (Table 4).

Diagnostic performance of individual biomarkers

Discriminating non-significant fibrosis (F1) from significant fibrosis (F2-F3)

AFP exhibited AUC of 0.63 at cut-off point of ≥6.5 U/L. The diagnostic performance of AFP at this cut-off was associated with sensitivity of 63%, specificity of 74%, positive predictive value (PPV) of 84%, negative predictive value (NPV) of 48% and 67% accuracy for discrimination of patients with F1 from F2-F3. Autotaxin showed AUC of 0.77 at cut-off point of ≥1.4 ng/mL. At this cut-off, autotaxin exhibited high accuracy (75%), sensitivity (83%), specificity (60%), PPV (81%) and NPV (61%) in discrimination of patients with F1 from F2-F3. Collagen IV showed AUC of 0.67 at cut-off point of ≥1.7 ng/mL. At this cut-off, sensitivity was 68%; specificity

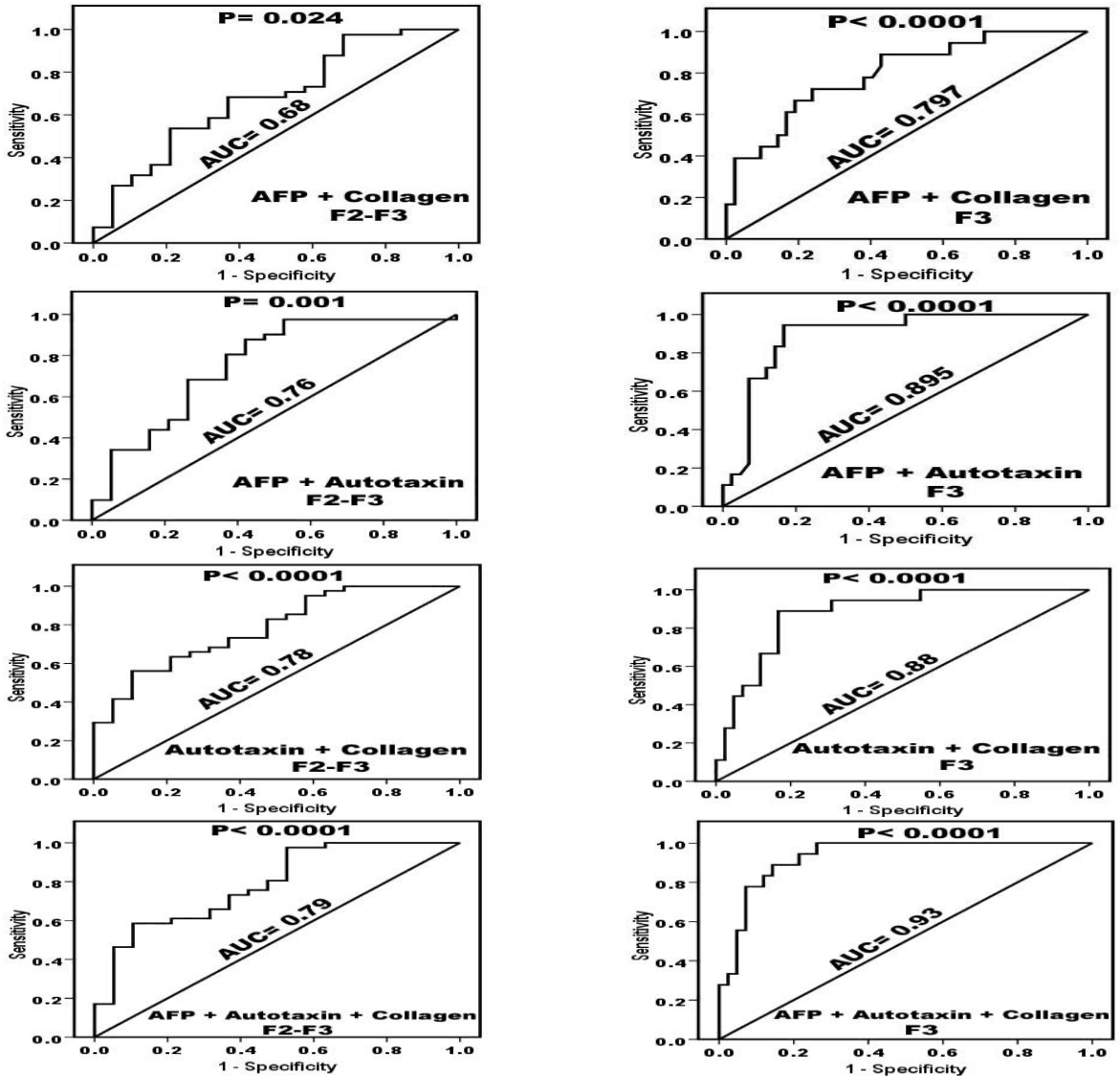

Figure 2: Receiver operator characteristic (ROC) curve of combined biomarkers (AFP, autotaxin, collagen IV (collagen 4)) for discrimination of patients with F1 from F2-F3 (left) and for discrimination of patients with F3 from F1-F2 (right).The true positive rate (sensitivity) is plotted as a function of the false rate (1−specificity). Each point on the ROC plot represents a sensitivity/specificity pair corresponding to a particular decision threshold. AUC value represents the combined effects of both sensitivity and specificity of combined biomarkers in diagnosis of patients.

was 53%, PPV was 76%, NPV was 44% and accuracy was 63% for discrimination of patients with F1 from F2-F3 (Table 5 and Figure 1). Based on the above diagnostic performances of the 3 biomarkers; autotaxin had the highest AUC and the highest accuracy for discrimination of patients with F1 from F2-F3.

Discriminating advanced fibrosis (F3) from non-advanced fibrosis (F1-F2)

AFP showed AUC of 0.72 at cut-off point of ≥7.9 U/L with sensitivity of 72%, specificity of 67%, PPV of 48%, NPV of 85% and accuracy of 68% for discrimination of patients with F3 from F1-F2. Autotaxin showed AUC of 0.84 at cut-off point of ≥1.9 ng/mL, high accuracy of 78%, sensitivity of 88.9%, and specificity of 74%, PPV of 59% and NPV of 94% for discrimination of patients with F3 from F1-F2. Collagen IV displayed AUC of 0.75 at cut-off point of ≥1.8 ng/mL. At this cut-off, sensitivity of 72%, specificity of 67%, PPV of 48%, NPV of 85% and accuracy of 68% were observed in discrimination of patients with F3 from F1-F2 (Table 5 and Fig. 1). Among these diagnostic performances; autotaxin had the highest AUC (0.84) and the highest accuracy (78%) for discrimination of patients with F3 from F1-F2.

Diagnostic performance of combined biomarkers

Discriminating non-significant fibrosis (F1) from significant fibrosis (F2-F3)

Aiming to improve the performance of the variables for discrimination of patients with F1 from F2-F3 diagnostic performance of different combinations of the 3 biomarkers (AFP, autotoxin and collagen IV) was studied (Table 6 and Fig. 2). ROC curve for combination of AFP and collagen IV showed AUC of 0.68 and low accuracy (65%) while combination of AFP and autotaxin resulted in increases in both AUC (0.76) and accuracy (73%). Combination of

autotaxin and collagen IV resulted in further increase in AUC (0.78) with accuracy of 70% whereas combination of the 3 blood markers (AFP, autotaxin and collagen IV) exhibited the highest AUC (0.79) with accuracy of 70%.

Discriminating advanced fibrosis (F3) from non-advanced fibrosis (F1-F2)

In a trial to improve the diagnostic performance of AFP, autotoxin and collagen IV in discrimination of patients with F3 from F1-F2 different combinations of the 3 biomarkers were used (Table 6 and Figure 2). ROC curve for combination between AFP and collagen IV showed AUC of 0.797 with accuracy of 75% in discrimination of patients with F3 from F1-F2. Combination of autotaxin and collagen IV resulted in higher AUC (0.88) with accuracy of 82%. Combination of AFP and autotaxin displayed further increase in AUC (0.895) with accuracy of 87% while combination of the three blood markers AFP, autotaxin and collagen IV exhibited the highest AUC (0.93) with the same accuracy (87%).

DISCUSSION

HCV is the main cause of chronic viral hepatitis and represents the main cause of hepatic fibrosis[11]. Our country, Egypt, has the highest HCV incidence (14.7%)[21] and it consequently has the highest CHC frequency. 10%-20% of CHC cases develop cirrhosis and about 7% of cirrhotic adults develop HCC[22]. Therefore, precise assessment of hepatic fibrosis become increasingly of great importance for diagnosis, prognosis, treatment decisions and following disease progression[23]. LB, the gold standard for fibrosis diagnosing and staging, is invasive method with associated morbidity; pain occurs in 20% and major complications (such as bleeding or hemobilia) in 0.5% of patients[24]. Less invasive, precise and reproducible methods with lower limitations than LB to assess the degree of liver fibrosis are therefore urgently needed[23]. Consequently, more research has been directed to search for non-invasive serum biomarkers of fibrosis[24]. Until now the developed non-invasive methods for liver fibrosis showed great variability among varied studies.

AFP is known as an important diagnostic tool of HCC; however increased AFP levels have also been noticed in CHC[25]. AFP was employed before in discriminating patients with significant fibrosis with AUR=0.61[26] and with AUR=0.77[25] and also in discriminating patients with advanced liver fibrosis with AUC= 0.75[25]. When AST/ALT and PLT count were added to AFP the AUC was lowered in patients with significant liver fibrosis whereas it was improved (AUC=0.82) in patients with advanced liver fibrosis[25].

Collagen IV is present in most connective tissue matrices. It forms an elastic filamentous net to link matrix macromolecules and cells[27]. When compared against LB, collagen IV showed 100% sensitivity and 68% specificity for advanced (F3) fibrosis in alcoholic liver disease[28]. In non-alcoholic steatohepatitis (NASH) collagen IV showed superiority to hyaluronan in detecting the presence of fibrosis[29]. In a study on patients with CHC, collagen IV was increased with progression of fibrosis. Used together with P3NP, they showed a sensitivity of 87% and specificity of 97% in detecting fibrosis[15].

Autotaxin is a secreted enzyme responsible for the hydrolysis of lysophospholipids producing lysophosphatidic acid (1- or 2-acyl-lysophosphatidic acid; LPA) in blood[30]. Serum autotaxin concentration has been assessed as a marker for liver fibrosis[31]. In this regard, Ikeda and Yatomi[30] stated that serum autotaxin should be evaluated as a possible liver fibrosis marker in not only patients with CHC, but also patients with liver fibrosis in general.

In the present study, serum concentrations of AFP, collagen IV and autotoxin were significantly elevated ($P<0.05$, $P<0.0001$ and $P<0.0001$, respectively) in CHC patients with fibrosis compared to their corresponding levels in healthy controls. High AFP levels indicate liver inflammation and regeneration. Elevated serum collagen IV may be related to portal hypertension degree. Increase in serum autotoxin may be attributed to decreased clearance occurs during hepatic fibrotic changes development and progression. Regarding differences between the 3 fibrosis stages (F1, F2 and F3), AFP levels showed non-significant change ($P>0.05$) while collagen IV and autotoxin levels showed significant ($P<0.01$ and $P<0.0001$, respectively) increase with increasing fibrosis stage. These results indicate autotoxin as the most efficient serum marker in detecting presence of hepatic fibrosis in this study. This was in consistence with Yamazaki et al.[32]. Moreover, when we studied the three biomarkers diagnostic performances autotaxin not only showed the highest AUC (0.77) and the highest accuracy (75%) for discrimination of patients with non-significant fibrosis (F1) from significant fibrosis (F2-F3) but also it showed the highest AUC (0.84) and the highest accuracy (78%) for discrimination of patients with advanced fibrosis (F3) from mild to moderate fibrosis (F1-F2). These results reflect autotoxin superiority to AFP and collagen IV in liver fibrosis stages differentiation. It is preferable for fibrosis staging markers to be capable of discrimination between non-significant (METAVIR scores F0-F1) and significant (score ≥ F2) fibrosis[33]. This is actually the case here with autotoxin as shown above. Our aforementioned findings were also in the same line with Manning and Afdhal[34] who reported that serum autotaxin was correlated to liver fibrotic stage in CHC patients and when compared to serum hyaluronate and aminotransferase/platelet ratio serum autotaxin concentration was the best parameter for predicting advanced fibrosis. Yamazaki et al.[32] reported almost comparable AUC (0.861) to our AUC (0.84) for autotaxin to diagnose advanced fibrosis (≥F2) in CHC male patients that was superior to those of FIB-4 and Forn's indices ($P < 0.001$) in their study.

In the present study, ROC curve for combination between autotaxin and AFP showed increments in both AUC (0.76) and accuracy (73%). Combination between autotaxin and collagen IV resulted in further increase in AUC (0.78) with accuracy of 70% in detecting significant fibrosis in CHC patients. While in discrimination of CHC patients with advanced fibrosis, the ROC curve for combination between autotaxin and collagen IV resulted in much higher

AUC (0.88) with accuracy of 82% compared to autotoxin alone. Combination between AFP and autotaxin displayed further increase in AUC (0.895) with accuracy of 87%. In addition, both AFP and collagen IV when added to autotoxin the AUC was improved (0.77 versus 0.79) for discrimination of CHC patients with non-significant (F1) from those with significant (F2-F3) fibrosis. It was also improved further from 0.84 with accuracy of 78% to 0.93 with accuracy of 87% for discrimination of CHC patients with advanced fibrosis (F3). These findings confirm that non-invasive methods accuracy can be improved when they are combined in diagnostic algorithms[25].

We conclude that serum concentrations of AFP, collagen IV and autotaxin changes in CHC disease and they were affected by the severity of liver fibrosis. Serum autotaxin may serve as a new clinical alternative to assess liver fibrosis in CHC patients who are not candidates for liver biopsy. Together when combined, serum AFP, collagen IV and autotaxin could be considered as a good marker for non-invasive diagnosis of liver damage. It is worthy to state that the combination of markers is more useful.

AUTHORS CONTRIBUTION

This work was done by all authors cooperatively.

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none declared

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