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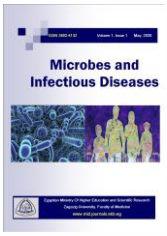

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Original article

Value of autotaxin as a serum marker for liver fibrosis in chronic HCV infected patients receiving direct-acting antiviral therapy

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ABSTRACT

Background: Non-invasive biomarkers have largely replaced liver biopsy in assessment of liver fibrosis in chronic HCV. Autotaxin (ATX) is a novel serum marker that may be related to liver fibrosis. **Aim:** to clarify the role of ATX as a biomarker for the estimation of hepatic fibrosis and to compare its sensitivity and specificity to the well-known fibrosis-4 score (FIB-4) and the AST-to-Platelet Ratio Index (APRI) before treatment with direct-acting antiviral drugs (DAAs) for chronic HCV as well as six months after the end of treatment. **Methods:** Plasma samples were obtained from 86 chronic HCV patients with different degrees of liver fibrosis. Routine laboratory, transient elastography (TE) and ultrasonographic assessments were done. Enzyme-linked immunosorbent assay (ELISA) technique was used to detect ATX. **Results:** ATX, FIB-4, and APRI had AUC of 0.57, 0.95, and 0.92, respectively for the detection of cirrhosis (F4). Baseline ATX was higher in cirrhotic group vs. non-cirrhotic group (250 vs. 210) pg/ml, although the difference was not significant (p= 0.3). Significant improvement of all the laboratory parameters, APRI, FIB-4 and liver stiffness occurred at sustained virological response after 24 weeks (SVR24). Non-significant increase of ATX level was noted six months after the end of treatment. **Conclusion:** ATX should be considered cautiously as a diagnostic marker for liver fibrosis in patients with chronic HCV.

Introduction

The emergence of effective direct acting antiviral drugs (DAAs) in recent years has offered opportunities to mitigate the burden of hepatitis C virus (HCV) disease and prevent its further transmission, potentially leading to the eradication of this blood-borne virus as an issue of public health [1].

It has been demonstrated that chronic HCV patients have different rates of histologic progression. Accelerated fibrosis progression has been linked to multiple variables, such as advanced age at infection, male gender, high alcohol use, co-infection with HIV, higher alanine aminotransferase (ALT) levels, and steatosis [2]. Assessment of hepatic fibrosis stage in chronic HCV patients is essential in the decision of surveillance interval and therapeutic intervention [3]. The most reliable and conventional examination technique for determining the stage of liver fibrosis is liver biopsy; nevertheless, it is invasive, prone to sample error,

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and has a significant risk of consequences. [4,5]. Hence, non-invasive biomarkers of liver fibrosis progression such as FIB-4 index and APRI have largely replaced liver biopsy in assessment of liver fibrosis in chronic HCV patients and are helpful in the clinical setting [6,7].

However, these markers are associated with "indeterminate" range scores in 30–50% of patients, and this is considered a remarkable limitation and opens the field for secondary diagnostic tests [8]. It was also found by de Oliveira et al, 2016 that by using the recommended cut off values of these markers, approximately 30-40 % of the patients could not be classified. In the rest of patients, either FIB-4 or APRI alone correctly diagnosed 60-70 % of cases. Concomitant or consecutive use of both scores increased the number of correctly diagnosed cases only slightly, but also increased the number of patients not classified within the cutoff values. Based on these results the investigators concluded that the use of FIB-4 or APRI scores for detection of hepatic fibrosis may be a viable alternative at referral centers to guide treatment of chronic HCV in low- and middle-income countries because despite relatively good accuracy, a significant number of patients could not be assessed by these methods [9].

Non-invasive assessment and follow up with the use of TE has been widely investigated in the setting of liver fibrosis as well as identification of the presence of portal hypertension and has also the potential for the dynamic assessment of cirrhosis regression after successful antiviral therapy [10]. Unfortunately, the main drawback of Fibroscan testing is that it cannot be performed in all patients. Due to technical restrictions, the test cannot be used on patients with ascites, people who are extremely obese, or people who have a lot of fat on their chest wall. Either the test cannot be administered to these groups, or the results are not trustworthy [11]. The study by Castera et al, 2010 confirmed this fact and revealed that approximately 15% of Fibroscan results may be unreliable and fail to obtain any liver stiffness measurements in about 3% of patients, mainly due to obesity or operator inexperience [12].

Autotaxin is an enzyme secreted in the conditioned media of human melanoma cell cultures A2058. It is also recognized as a member of the phosphodiesterase/pyronucleotide pyrophosphatase (ENPP 2) family [13]. It plays a crucial role in the conversion of lysophosphatidyl choline to lysophosphatidic acid (1- or 2-acyl-lysophosphatidic acid; LPA) [14] which is implicated in numerous physiological mechanisms as wound healing, platelet aggregation, smooth muscle contraction, neurogenesis, and angiogenesis [15,16]. Additionally, hepatic stellate cell proliferation and contractility are stimulated by LPA. ATX acts as an autocrine motility factor, so it has been linked to the invasion and metastasis of cancer [17]. Based on physiological considerations, the enzyme found in the serum is broken down by the liver's sinusoidal endothelial cells. Thus, it is believed that liver fibrosis reduces the breakdown of ATX, resulting in an increase in its serum levels.

These results suggest a possible direct link between ATX and liver fibrosis. [18,19]. **Yamazaki et al.** observed that ATX levels in patients exhibiting a sustained virological response (SVR) decreased significantly from the start of treatment to 4 weeks of treatment and remained low (p<0.001). Therefore, interferon-free DAA agents were linked to a considerable reduction in serum ATX levels in patients who achieved SVR, indicating an early reversal of liver fibrosis and inflammation [20].

The current study aims at evaluating the changes of serum levels of ATX prior to DAA treatment for patients with chronic HCV as well as six months after treatment and to clarify the role of ATX as a biochemical indicator for assessing fibrosis of liver and to compare its sensitivity and specificity to the well-known FIB4 and APRI.

Materials and procedures

The study participants

Eighty- six chronic HCV patients with varying degrees of liver fibrosis who met the criteria for antiviral treatment as per the National Committee for Control of Viral Hepatitis (NCCVH) in Egypt were recruited for this prospective analysis. All patients were enrolled from the virology outpatient clinic between April 2019 and February 2020 at Thabet Thabet Hospital, Cairo University, Egypt. The analysis was performed to only 74 patients at SVR12 and 67 patients at SVR24 due to incomplete follow-up. The study was approved by the Institutional Review Board (IRB) of the Faculty of Medicine, Cairo University (MD-52-2019) and informed written consent was taken from all subjects prior to enrollment, with explanation of all the study procedures.

Criteria for inclusion and exclusion

Patients recruited in the current study were adults (aged 18-80 years old), of both sexes, with chronic HCV infection diagnosed by HCV antibody testing with confirmation by HCV RNA PCR testing. Patients younger than 18 years of age, with Child-Pugh C score liver cirrhosis, platelets count <50000/mm^3, hepatocellular carcinoma (HCC), pregnancy, HBV or HIV co-infection, non-viral etiology of chronic liver disease (CLD) (e.g. autoimmune hepatitis), any psychiatric, neurological or any other disorder that prevented informed consent, any extra-hepatic malignancy (except two years of disease free after chemotherapy or radiotherapy), were excluded from our study.

Patients who were enrolled in the current study underwent the following baseline evaluation:

a. Informed consent that was signed by all patients.

b. Thorough clinical assessment.

c. Basic laboratory investigations including CBC, liver biochemical profile, kidney function tests and alpha fetoprotein (AFP).

d. Estimation of Child-Pugh score in case of cirrhotic patients [21].

e. Calculation of FIB-4 index using platelets, AST, ALT and age to assess the level of fibrosis. With a sensitivity of 74.3%, a FIB-4 index <1.45 showed a negative predictive value of 94.7% to rule out severe fibrosis. A FIB-4 index ≥3.25 exhibited a 98.2% specificity and an 82.1% positive predictive value to identify the presence of a severe fibrosis (F3-F4) [6].

f. Calculation of APRI score using platelets and AST; score higher than 0.7 was associated with a 77% sensitivity and a 72% specificity in predicting the presence of severe hepatic fibrosis [22].

g. Assessment of blood levels of ATX by ELISA.

h. Abdominal ultrasonography using real time scanning Ultrasound System device with convex transducer, 1.5 – 6.0 MHz. with details on the liver echopattern, presence of ascites or hepatic focal lesions.

i. Liver stiffness measurements (LSM) using TE: Fibroscan (Echosens Version 13 - 05/2009; software version 1.40) (23) (24); patients were categorized based on their fibrosis stages into non-significant fibrosis (<F2): 0-8.7 kPa, significant fibrosis to advanced fibrosis (≥F2-<F4): 8.8-14.4 kPa, and cirrhosis (F4): ≥14.5 kPa.

Post DAAs treatment follow-up was carried out twice; three and six months after cessation of therapy, by performing the same basic laboratory investigations for follow up. Additionally, a quantitative HCV RNA PCR was performed in the first follow up setting to detect SVR. Also, assessment of the degree of liver fibrosis was performed by calculation of APRI and FIB-4. In the second follow up setting, additional assessment of LSM by TE, FIB-4, APRI and ATX levels were performed as well as abdominal ultrasonography with details on the liver condition.

Statistical analysis

Descriptive statistics were done, and numerical data were displayed as mean (SD) / median (IQR) and categorical data as frequency (%). The change in different variables following treatment was compared to baseline using paired samples t- test or paired samples Wilcoxon rank sign test. Receiver operator characteristics curves (ROC) were performed to assess the diagnostic potential of the fibrosis markers and to determine the appropriate cutoff values with the highest sensitivity and specificity. *P-values* <0.05 were deemed significant. The analysis was conducted using STATA 15.1.

Results

This prospective study was conducted on 86 adult patients of both sexes with HCV-related CLD. The study included 46 non-cirrhotic HCV patients who received sofosbuvir (SOF) and daclatasvir (DAC) for 12 weeks and 40 patients with post HCV liver cirrhosis who received SOF, DAC and weight-based dose of ribavirin (RBV) for 12 weeks according to the NCCVH guidelines. The demographic features, baseline laboratory data and fibrosis parameters of the studied participants are shown in **table (1)**.

Baseline median serum ATX level was 220 pg/ml (150-350), with a detection range of (100-2700) pg/ml. All cirrhotic patients in our study were Child A according to Child-Pugh classification. All the parameters of cirrhotic patients are shown in **table (2)**. It was found that serum ATX was higher in cirrhotic group; 250 (150- 365) pg/ml while in non-cirrhotic group was 210 (150-280) pg/ml, although the difference between the two groups was non-significant (p= 0.3).

The laboratory parameters were analyzed at SVR12 and SVR24 as shown in **table (3)**. There was marked improvement in all parameters with

significantly lower level of ALT, AST, bilirubin, and AFP as well as significant lower values of fibrosis parameters including FIB-4 and APRI. Compared to the baseline Fibroscan readings, fibrosis improvement by at least 1 Metavir stage occurred in 6 of the 67 patients at SVR24. There was a significant lower value at SVR24 as the median value was 10.5 kPa (6.1-22) (p<0.0001).

Subgroup analysis for the changes of the laboratory and fibrosis parameters among patients according to the presence of baseline cirrhosis was shown in **table (4)**. There was significant improvement of FIB-4 and APRI from baseline to SVR12 in both non-cirrhotic and cirrhotic patients. Moreover, we noticed that there was also significant improvement in TE values from baseline to SVR24 in both non-cirrhotic and cirrhotic patients. However, serum ATX showed a significant increase in non- cirrhotic patients at SVR24 compared to baseline while cirrhotic patients showed non-significant changes of serum ATX from baseline to SVR24.

Serum ATX at SVR24 showed relative increase in median value of 287 (135-493) pg/ml, which is not statistically significant (p= 0.2) (Table 3). This is also shown in **figure (1)** which reflects the expression of serum ATX at the start of treatment and SVR24.

Receiver operator characteristics (ROC) curves for the discriminatory power of ATX, FIB-4 and APRI for advanced fibrosis (F3) showed that FIB-4 yielded (AUC=0.93 and p-value <0.001 with 95% CI; 0.877 – 0.98), while APRI yielded (AUC= 0.91, p-value <0.001 with 95% CI; 0.85 – 0.97), on the other hand ATX had (AUC= 0.526 with 95% CI; 0.40 – 0.64) as shown in **figure (2)**.

ROC curves for the diagnostic accuracy of ATX, FIB-4 and APRI for cirrhosis showed that FIB-4 yielded (AUC= 0.95, p-value <0.0001 with 95% CI; 0.89 – 1), while APRI showed (AUC= 0.92, p-value < 0.0001 with 95% CI; 0.864 – 0.98), on the other hand, ATX had (AUC= 0.57 with 95% CI; 0.45 – 0.69) as shown in **figure (3)**.

Table 1. Demographic features, baseline laboratory data and fibrosis parameters of all the participants .

		Range
Age /years *	57 (48-64)	22-77
Gender Male/Female†	44 (51.16%) /42(48.84%)	
Diabetes mellitus, n (%)	23 (26.74%)	
Hypertension, n (%)	18 (20.93%)	
Laboratory Parameters		
ALT (U/L)	47.5 (31-69)	8-386
AST (U/L)	46.5 (34-63)	17-166
Albumin (g/dL)	3.99 (0.56)	2.8-5
Bilirubin (mg/dL)	0.6 (0.5-0.8)	0.2-1.9
INR	1 (1-1.13)	0.86-1.59
White blood cell ($x10^3/mm^3$)	5.8 (4.6-7.7)	1.7-18
Hemoglobin (g/dL) Mean (SD)	13.85 (1.53)	9.7-16.9
Platelets ($x10^3/mm^3$)	158 (116-246)	55-390
Creatinine (mg/dL)	0.80 (0.7-1)	0.5-2.36
AFP (ng/dL)	5.7 (3-11.7)	0.9-81
Liver stiffness by TE	11.3 (6.3-22.3)	3.5-75
Distribution of Fibrosis stages F0-F1(<7.1 kPa) F2 (≥F7.1 kPa) F3 (≥9.5 kPa) F4 (≥12.5 kPa)	 27 (31.40%) 9 (10.47%) 10 (11.63%) 40 (46.51%)	
FIB-4	2.26 (1.06-4.83)	0.35-11.18
APRI	0.7 (0.4-1.5)	0.2-4.9
ATX (pg/ml)	220 (150-350)	100-2700
Liver U/S, n (%)		
Normal	18 (20.93%)	
Bright	15 (17.44%)	
Cirrhotic	37 (43.02%)	
Splenomegaly	10 (11.62%)	

Data are expressed as mean ± standard deviation (SD), median (range), or n (%). Abbreviations: AFP: alpha-fetoprotein; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; APRI: Aspartate aminotransferase-to-platelet ratio index; ATX: autotaxin; FIB-4: Fibrosis 4 score; INR: International normalized ratio; kPa: kilopascals; TE: Transient elastography.

Table 2. Comparison between non-cirrhotic and cirrhotic patients including demographics, baseline laboratory and fibrosis parameters

	Non-cirrhotic (n=46)	**Liver cirrhosis (n=40)**	***p-value***
Age (years), median (IQR)	51 (37-58)	63 (57-67)	**<0.0001**
Gender Male/Female	23/23	21/19	0.8
Diabetes mellitus, n (%)	7 (15.2%)	16 (40%)	**0.01**
Hypertension, n (%)	6 (13%)	12 (30%)	0.05
Baseline fibrosis parameters			
Liver stiffness by TE (kPa) Median (IQR)	6.4 (5.6-8.9)	23.9 (17.2-33.6)	**<0.0001**
FIB-4, median (IQR)	1.21 (0.8- 1.83)	4.97 (3.88 - 6.05)	**<0.0001**
APRI, median (IQR)	0.4 (0.3-0.6)	1.45 (1-2.1)	**<0.0001**
ATX (pg/ml), median (IQR)	210 (150-280)	250 (150-365)	0.3

Unless otherwise stated numerical data are expressed as median (IQR). ATX: autotaxin; APRI: aspartate aminotransferase to platelet ratio index; FIB-4: Fibrosis 4 score. TE: Transient elastography. Statistically significant values are in bold.

Table 3. Analysis of laboratory and fibrosis parameters 12 weeks and 24 weeks post DAAs of all participants

Laboratory & Fibrosis parameters	**Baseline**	**SVR12**	**SVR24**	***p-value***
ALT (U/L)	47.5 (31-69)	25 (22-32)	23 (18-28)	**<0.0001* 0.003****
AST (U/L)	46.5 (34-63)	22 (20-33)	23 (17-30)	**<0.0001*** 0.05**
Albumin (g/dL), mean (SD)	3.99 (0.56)	3.86 (0.40)	3.83 (0.40)	**0.04*** 0.9**
Bilirubin (mg/dL)	0.6 (0.5-0.8)	0.53 (0.4-0.8)	0.5 (0.4-0.7)	**0.02*** 0.7**
INR	1 (1-1.13)	1 (1-1.12)	1.04 (1-1.15)	0.9* 0.08**
White blood cell ($x10^3/mm^3$)	5.8 (4.6-7.7)	6.43 (4.5-8)	5.92 (4.5-7)	0.2* **0.005****
Hemoglobin (g/dL), mean (SD)	13.85 (1.57)	13.38 (1.55)	13.53 (1.39)	**0.0005*** 0.2**
Platelets ($x10^3/mm^3$)	158 (116-246)	186.5 (114-246)	186 (115-250)	0.8* 0.2**
Creatinine (mg/dL)	0.80 (0.7-1)	0.8 (0.67-1)	0.83 (0.7-1)	0.2* 0.7**
AFP (ng/dL)	5.7 (3-11.7)	5.55 (2.8-8.2)	4.5 (2-7)	**<0.0001*** **0.0001****
FIB-4	2.26 (1.06-4.83)	1.42 (0.95-2.94)	1.3 (0.93-2.88)	**<0.0001*** 0.2**
APRI	0.7 (0.4-1.5)	0.3 (0.2-0.7)	0.3 (0.2-0.6)	**<0.0001*** 0.05**
TE values (kPa)	11.3 (6.3-22.3)	-	10.5 (6.1-22)	**<0.0001**#
ATX **(**pg/ml)	220 (150-350)	-	287 (135-493)	0.2#

Data are expressed either as mean (SD) or median (IQR). Abbreviations: AFP: alpha-fetoprotein; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; APRI: Aspartate aminotransferase-to-platelet ratio index; ATX: autotaxin; FIB-4: Fibrosis 4 score; INR: International normalized ratio; kPa: kilopascals; TE: Transient elastography. SVR12: sustained virological response at week 12 after end of therapy. SVR24: sustained virological response at week 24 after end of therapy. Statistically significant values are in bold.
*p-value for baseline vs SVR12.
**p-value for SVR12 vs SVR24.
#p-value for baseline value vs SVR24.

Table 4. Analysis of laboratory and fibrosis parameters 12 weeks and 24 weeks post DAAs according to the presence or absence of baseline cirrhosis.

Laboratory & Fibrosis parameters		Non-cirrhotic (n=46)	Liver cirrhosis (n=40)
ALT (U/L)	Baseline	41 (30-58)	55.5 (38-92.5)
	SVR12	23 (20-28)	28.5 (23-35.5)
	SVR24	18 (16-22)	27.5 (23.5-31.5)
	P-value	**<0.0001*** **0.0003****	**<0.0001*** 0.5**
AST (U/L)	Baseline	39.5 (28-48)	63 (47-86)
	SVR12	20 (19-23)	30 (21-39)
	SVR24	18 (16-22)	29.5 (25-37.5)
	P-value	**<0.0001*** **0.01****	**<0.0001*** 0.5**
Albumin (g/dL), mean (SD)	Baseline	4.26 (0.44)	3.65 (0.52)
	SVR12	4.01 (0.30)	3.70 (0.44)
	SVR24	4.02 (0.22)	3.63 (0.45)
	P-value	**0.0002*** 0.2**	0.6* 0.3**
Bilirubin (mg/dL)	Baseline	0.55 (0.4-0.7)	0.75 (0.5-1)
	SVR12	0.4 (0.3-0.6)	0.7 (0.5-0.8)
	SVR24	0.4 (0.3-0.6)	0.7 (0.5-1.1)
	P-value	0.1* 0.97**	0.08* 0.56**
INR	Baseline	1 (1-1.1)	1.11 (1-1.19)
	SVR12	1 (1-1.03)	1.1 (1-1.2)
	SVR24	1 (1-1.07)	1.12 (1.05-1)
	P-value	0.5* 0.79**	0.59* 0.05**
White blood cell ($x10^3/mm^3$)	Baseline	6.25 (5-8.4)	5.25 (4.3-6.95)
	SVR12	6.74 (5-8.6)	5.8 (4.3-7.1)
	SVR24	6.31 (4.8-7.5)	5.83 (4.3-6.74)
	P-value	0.2* **0.04****	0.5* 0.06**
Hemoglobin (g/dL), mean (SD)	Baseline	14.26 (1.60)	13.41 (1.43)
	SVR12	13.73 (1.48)	12.98 (1.56)
	SVR24	13.74 (1.34)	13.29 (1.43)
	P-value	**0.004*** 0.9**	**0.04*** 0.1**
Platelets ($x10^3/mm^3$)	Baseline	240.5 (183-289)	114 (94-135)
	SVR12	225 (192-264)	114 (81-165)
	SVR24	235 (200-280)	110.5 (89.5-135)
	P-value	0.8* 0.1**	0.9* 0.7**
Creatinine (mg/dL)	Baseline	0.80 (0.7-1)	0.87 (0.7-1)
	SVR12	0.8 (0.61-0.95)	0.8 (0.7-1.03)
	SVR24	0.8 (0.7-0.95)	0.85 (0.66-1.01)
	P-value	**0.04*** 0.6**	0.9* 0.3**
AFP (ng/dL)	Baseline	3.8 (2.3-5.7)	10.1 (7.8-16.5)
	SVR12	3 (2.1-6.1)	6.6 (5-9.1)
	SVR24	2 (1-5)	5.3 (4-8.6)
	P-value	0.09* **<0.0001****	**0.0001*** **0.3****
FIB-4	Baseline	1.20 (0.8-1.83)	4.97 (3.89-6.05)
	SVR12	0.98 (0.77-1.33)	2.94 (1.8-2.64)
	SVR24	0.96 (0.72-1.15)	3.01 (2.1-4.43)
	P-value	**0.0003*** 0.4**	**0.0009*** 0.4**
APRI	Baseline	0.4 (0.3-0.6)	1.45 (1-2.1)
	SVR12	0.2 (0.2-0.3)	0.7 (0.4-1)
	SVR24	0.2 (0.1-0.3)	0.6 (0.5-0.9)
	P-value	**<0.0001*** 0.1**	**<0.0001*** 0.2**
TE values (kPa)	Baseline	6.4 (5.6-8.9)	23.9 (17.2-33.6)
	SVR12	-	-
	SVR24	6.4 (4.8-8.1)	22.5 (16.8-31.9)
	P-value	**0.02#**	**0.0007#**
ATX **(**pg/ml)	Baseline	210 (150-280)	250 (150-365)
	SVR12	-	-
	SVR24	333 (200-479)	250 (150-365)
	P-value	**0.006#**	0.75#

Data are expressed either as mean (SD) or median (IQR). Abbreviations: AFP: alpha-fetoprotein; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; APRI: Aspartate aminotransferase-to-platelet ratio index; ATX: autotaxin; FIB-4: Fibrosis 4 score; INR: International normalized ratio; kPa: kilopascals; TE: Transient elastography. SVR12: sustained virological response at week 12 after end of therapy. SVR24: sustained virological response at week 24 after end of therapy. Statistically significant values are in bold.
*p for baseline vs SVR12. **p for SVR12 vs SVR24. #p for baseline value vs SVR24.

Figure 1. Illustrating the expression of ATX

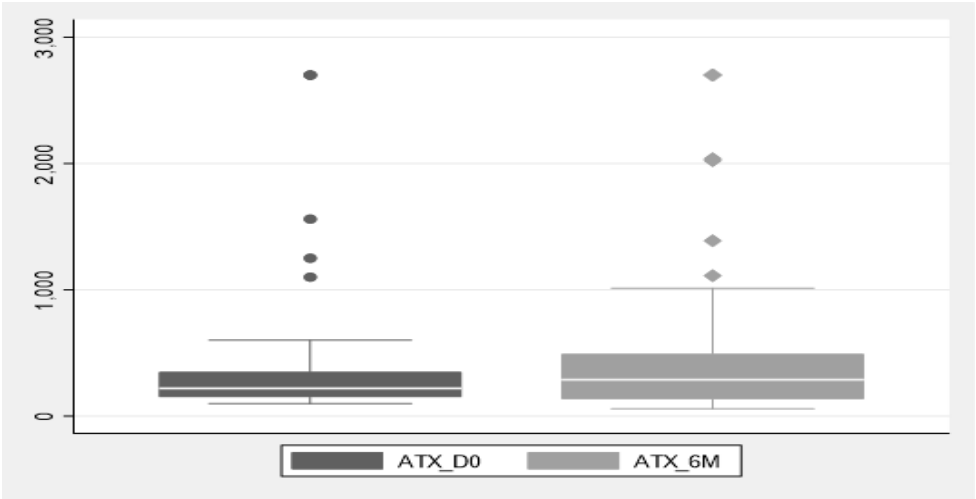


Figure 2. ROC curves of ATX, FIB-4 & APRI for advanced fibrosis at baseline

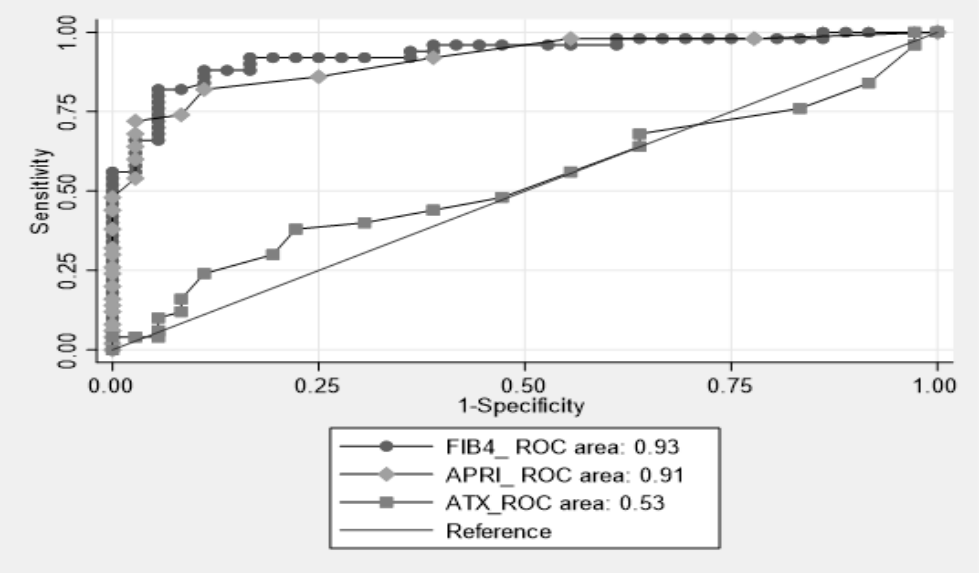
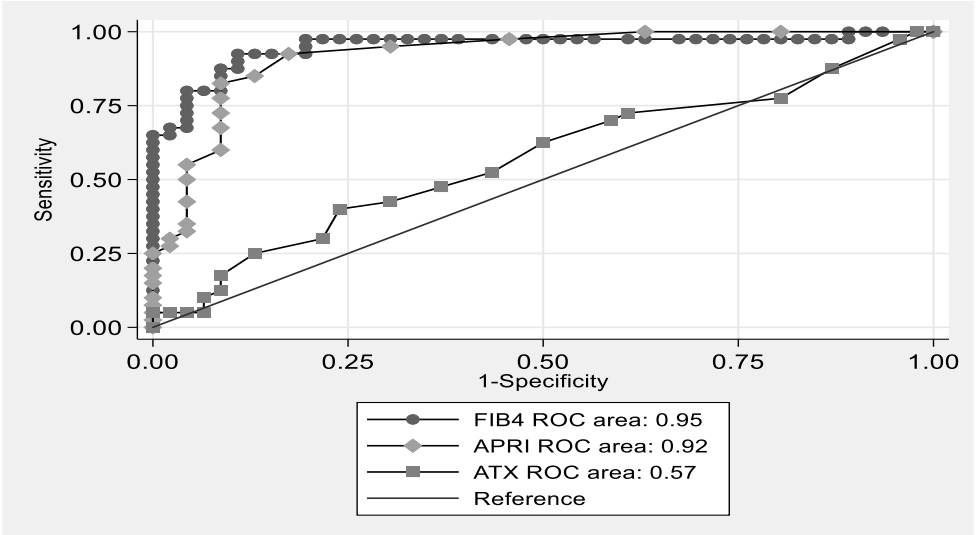


Figure 3. ROC curves of ATX, FIB-4 & APRI for cirrhosis at baseline



Discussion

A significant global health issue that affects 1% of people worldwide is chronic HCV infection [25]. Due to the high incidence of schistosomiasis and the widespread use of risky intravenous injections for its treatment between the 1950s to 1980s, Egypt had the highest frequency of HCV infection. [26]. The NCCVH set a treatment program combining both SOF and DAC ± weight-based dose of RBV for 12 weeks. More than 2 million patients were treated by 2018 and cure rates were above 90% [26]. **Elsharkawy et al.** observed that in cirrhotic individuals, SOF-based therapy results in a statistically significant improvement in hepatic fibrosis parameters using both TE and FIB4 [27].

The degree of hepatic fibrosis regression must be evaluated in order to identify those people who are still at a high risk of developing HCC. Therefore, to accomplish this purpose, straightforward and trustworthy non-invasive techniques are needed. [20]. The current study aims at evaluating the changes of serum levels of ATX before treatment with DAAs for chronic HCV patients as well as six months after treatment to clarify the role of ATX as a biochemical indicator for assessing fibrosis of liver and to compare its sensitivity and specificity to the well-known FIB4 and APRI.

ATX has been studied as a potential prognostic marker of disease activity and an innovative non-invasive indicator for liver fibrosis. It aims to meet the requirements of the ideal marker for liver fibrosis, which is to be highly specific and sensitive to distinguish between different stages of fibrosis. Additionally, it must be easily accessible, safe, affordable, and repeatable in order to evaluate and track the development of liver disease, particularly after treatment regimens [28]. On the other hand, there is little data regarding whether and how ATX levels change during and after DAA treatment [29]. **Pleli et al.** (2014) found a correlation between serum ATX concentrations and the severity of hepatic fibrosis. This is because endothelial cell dysfunction caused by progression of fibrosis impairs ATX clearance [30]. Liver sinusoidal endothelial cells are known to have phenotypic changes over the process of liver fibrosis, including the loss of different receptors and sinusoidal endothelial fenestrae, which causes the sinusoids to become capillarized and limit various substances' absorption [31]. In agreement with this assumption, we noticed in our study a growing pattern of serum ATX with the progression of stage of hepatic fibrosis, given the higher ATX level at baseline in the cirrhotic group of patients.

It was found that there is a correlation between the histological stage of liver fibrosis and elevated levels of serum ATX and plasma LPA in chronic HCV-related liver fibrosis. Furthermore, more information was required to demonstrate whether the elevated levels of ATX and LPA in the blood during liver injury is a result of the injury or its cause [32]. Since it has been demonstrated that LPA can significantly increase following sample preparation unless temperature is properly regulated, we examined serum ATX in our study rather than LPA. The presence of synthetic ATX and its substrate lysophosphatidyl choline, which can cause a large production of LPA in plasma [33]. LPA should be evaluated in plasma to determine its true clinical importance because it is also released by platelets, among other sources [34]. While serum ATX can be tested in serum and is temperature stable [35].

We compared ATX to the fibrosis parameters FIB-4 and APRI at baseline and found that both FIB-4 and APRI scores were statistically significant in detecting liver fibrosis at baseline (p<0.0001), with AUC of 0.93 and 0.91, respectively while ATX values were insignificant with AUC of 0.526 (p=0.3), thus matching with a meta-analysis of 40 studies where the investigators concluded that an APRI score greater than 1.0 had a sensitivity of 76% and a specificity of 72% for predicting cirrhosis.

They concluded that an APRI score greater than 0.7 had a sensitivity of 77% and a specificity of 72% for predicting significant hepatic fibrosis [22]. In our study the median value of APRI score was 1.45 in the cirrhotic group of patients (p<0.0001).

The FIB-4 score was developed by Sterling et al, 2006 to assess fibrosis in HIV/HCV co-infected patients; at a cutoff value of 3.25, they found that 87% of patients were correctly classified with an AUC of 0.765 for significant fibrosis [6]. In our study the median value of FIB-4 score was 4.97 in the cirrhotic group (p<0.0001).

In the present study, we found comparatively greater ATX levels at SVR24 post-DAA therapy, although it was not of statistical significant (p=0.2). There is contradiction to the results of the study conducted by **Yamazaki et al,** 2018 who investigated the changes of ATX after 24

weeks post DAAs therapy and found a decrease in ATX levels, which suggested a reduction of necro-inflammatory activity. As a result, they came to the conclusion that the ATX levels at SVR24 continued to improve, which would indicate an early reversal of hepatic fibrosis and the resolution of inflammation that happened shortly after beginning DAA treatment. [20].

This contradiction in our findings regarding ATX performance could be explained by the differences between populations according to molecular basis for serum ATX expression. Hepatic schistosomiasis among Egyptian HCV patients as well as genetic difference between the Egyptian patients and the other races in previous studies may be the reason behind this contradiction. Hence, Different immunological expression patterns linked to schistosomiasis, genetic susceptibility, and environmental stress may have an impact on serum ATX expression [31].

Conversely, our findings aligned with those of a 2013 study by **Ezzat et al.,** which involved 68 HCV patients and involved subcutaneous pegylated interferon alpha-2a plus oral ribavirin at the time of treatment. The researchers concluded that there was insignificant difference in the mean values of ATX among the various grades of liver fibrosis [31].

In accordance with our findings, a study conducted in 2020 by **Ahmed et al.** found that responders to DAAs treatment had altered serum levels of ATX. Their study involved 54 participants and ATX was measured for all patients before and after treatment. Their results showed a significantly increase ATX levels following DAAs treatment [36].

Contrarily to the results of our study, Another Egyptian study was conducted by **Saleh et al,** 2020 to evaluate serum ATX levels at the beginning of treatment and SVR12 weeks in 48 chronic HCV patients. They evaluated ATX in comparison to FIB-4 score and APRI to detect stage F3–4 fibrosis. The findings revealed that serum ATX levels dramatically decreased in 47 individuals following SVR12 (p<0.001). The diagnostic performance of FIB-4 and APRI scores at baseline and SVR12 was outperformed ATX for the identification of grade F3–4 fibrosis in their study [37].

The most significant findings of the current study are the non-significant higher baseline ATX in the cirrhotic group of patients as well as the non-significant higher levels of ATX in the chronic HCV patients at SVR24. Based on these findings, we can therefore draw the conclusion that FIB-4 and APRI are effective biochemical markers for predicting liver fibrosis in individuals with HCV infection, and that ATX should be used cautiously as a diagnostic marker for liver fibrosis in patients with chronic HCV infection. Eventually, In order to ascertain whether the improvement in liver fibrosis parameters and the decrease in the likelihood of developing HCC are reflected in consecutive ATX assessments, we advise doing longer follow-up studies following DAA medication.

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Competing interests:

None to be declared.

Authors' contribution

All authors have substantially contributed to the conception and design, acquisition of data, data analysis and interpretation. All authors have agreed on the content of the manuscript.

AM: Fibroscan operator, manuscript writing; NN: Laboratory assessment and interpretation; ZAL: statistical analysis; YM, Master: data collection and acquisition; HEG: Study design, conception and manuscript revision; REE: Data analysis and interpretation and manuscript revision.

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