Original Article

Autotaxin as a marker for liver fibrosis in chronic HCV patients

Clinical Pathology

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ABSTRACT:

Background: Chronic hepatitis C is a major health problem. Noninvasive markers were developed to overcome liver biopsy complications. Autotaxin (ATX) is a lysophospholipase D enzyme that regulates lysophosphatidic acid (LPA) blood concentrations. Studies have suggested that the phenotypic changes during liver fibrosis may lead to changes in ATX level in the serum.

Objectives: to determine the serum Autotaxin levels and its correlation to the liver fibrosis and whether it can be used as a non-invasive diagnostic biomarker of fibrosis in Egyptian Chronic viral hepatitis C individuals.

Methodology: This study was conducted on 40 patients divided into 2 groups: Group I comprised of 20 patients with chronic viral hepatitis C patients in compensated stage and Group II comprised of 20 patients with chronic viral hepatitis C patients in decompensated stage together with 20 apparently healthy controls group. All patients were subjected to the following: complete history taking، complete clinical examination، routine lab investigations، abdominal ultrasound and measurement of serum ATX level detected by ELISA technique.

Results: 40 patients were enrolled. Serum ATX was significantly higher according to liver fibrosis worsen stage (p=0.002)، ATX was significantly higher in decompensated chronic hepatitis C patients (CHC) compared to patients with compensated CHC and controls with mean values (646.7 ± 247.4)، (615.3 ± 266) and (469.9 ± 62.3) ng/ml respectively (p=0.005).

Conclusion: Autotaxin was shown to be a potential serum marker for estimating the degree of fibrosis in patients with chronic HCV.

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INTRODUCTION

The hepatitis C virus (HCV) is the principal risk factor for chronic hepatitis, liver fibrosis, and hepatocellular carcinoma (HCC) affecting more than 170 million individuals all over the world and is the leading reason of liver transplantations in the USA and the Europe [1]. Morbidity and death correlated with chronic HCV are a significant public health concern in Egypt with a reported national incidence of viral chronic HCV of approximately 15 percent. Of instance, genotype 4 accounts for over 90 % of cases in Egypt [2].

Chronic HCV infection continues to be a major health hazard, considering developments in antiviral therapy (DAA) with elevated virological reaction rates [2].

Cirrhotic hepatic failure, and hepatic cell carcinoma as well as esophageal and gastric varices are also symptoms of this viral infection [3].

For treatment strategies, the step of evaluation of liver fibrosis in cases with chronic viral HCV is significant. Liver biopsy is the conventional and most reliable procedure for the assessment of the stage of hepatic fibrosis, but it has several risks since it is intrusive, sampling errors and other critical unusual errors [4].

The need for non-invasive biomarkers of course of the disease is therefore needed. Examples of non-invasive hepatic fibrosis indicators such as the Fibrosis-4 index

(Fib-4), Aspartate aminotransferase ratio (AST), Forn index, wave Elastography (SWE), and Color velocity of Doppler are useful in the clinical context for the evaluation of liver fibrosis compared to hepatitis C-patients' liver biopsy [5].

Autotaxin (ATX) is a lysophospholipase D enzyme that regulates lysophosphatidic acid (LPA) blood concentrations [6]. ATX and LPA also have been shown to be a possible therapeutic target and have been documented to be inducing inflammation, proliferation, migration, and angiogenesis in many cancers, such as melanoma, glioblastoma, breast and lung cancer and follicular lymphoma [7]. Physiologically, the enzyme is extracellular and is metabolized by liver, so fibrosis contributes to serum elevation, meaning that ATX is specifically correlated with liver fibrosis [8]. The purpose of this work was to determine the serum Autotaxin levels and its correlation to the liver fibrosis and whether it can be used as non-invasive diagnostic biomarker of fibrosis in Egyptian Chronic HCV Patients.

SUBJECTS and METHODS

▪ **Study design, setting, and time:**
A hospital-based, comparative analytical study design was chosen to perform this research at the outpatient clinic and inpatient department of Hepatogastroenterology and Infectious Diseases, Al-Zahraa University Hospital in the period from December 2017 to December 2018.

▪ **Administrative design:**
Approvals to conduct the study were obtained from the department of Hepatogastroenterology and Infectious Diseases, Al-Zahraa University hospital.

▪ **Study population:**
This study done on 40 patients who were diagnosed clinically and laboratory with chronic viral hepatitis C infection. Twenty cases apparently healthy individuals were selected as controls. They were divided into:

1. **Group I:** include 20 patients with compensated chronic HCV infection. They were 14 men and 6 women. Their ages ranged from 50 to 69 years.
2. **Group II:** include 20 decompensated chronic HCV infections. They are 8 males and 12 females. Their ages ranged from 40 to 77 years.
3. **Group III:** include 20 apparently healthy control persons of age and sex not matched with patients.

The inclusion criteria were: Adult patients with chronic HCV with or without hepatic fibrosis. The exclusion criteria were Co infection with other hepatitis viruses, Liver cancer, Pregnancy, Chronic renal failure and Alcohol or drug intake.

▪ **Ethical consideration:**
Study aims were explained to the participants after obtaining informed verbal consent, Study protocol was approved by 2 local Ethical Committees of Al-Azhar Faculty of Medicine for Girls, Cairo.

All patients and controls subjected to:
1. Full history taking with stressing on history of pain, fatigue, anorexia, weight loss and bleeding tendency, if these symptoms were present the patient was considered in decompensated stage and if these symptoms were absent the patient was considered in compensated stage. Generally speaking compensated Liver disease means that the liver is scarred but still able to perform most of its basic functions at some level as the liver still has enough healthy cells to perform its function but decompensated liver disease means that the liver can no longer function properly and the patient will begin to experience more severe symptoms. History of bilharziasis, blood transfusion or operation also Family history of HCV.
2. Complete abdominal examination and abdominal ultrasonography.
3. Laboratory investigations: Complete Blood Count (CBC) using Coulter Counter T890 (Coulter LH 750 analyzer, Berlin,Germany), Estimation of prothrombin time (PT) immediately on automated blood coagulation analyzer Sysmex CA1500 (Siemens AG, Erlangen, Germany), Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST),Total bilirubin (T. bil.) and serum albumin (s.alb.) were performed using Hitachi 911 auto-analyzer (Roche-Hitachi, Japan.),Hepatitis markers: Hepatitis C antibody (HCV Ab) done by Enzyme- Linked ImmunoSorbant Assay ELISA technique, kits of Ortho (for HCV Ab), The Fibrosis 4 index (FIB-4) index (FIB-4 is a non-invasive scoring system based on many laboratory tests which help estimate the degree of hepatic scarring) and used for fibrosis grading (mild fibrosis less than 1.5 ,moderate fibrosis from 1.5-3.5 and sever fibrosis more than 3.5) :Formula: (Age x AST) / (Platelets x sqr (ALT))[21] and measurement of serum Autotaxin by ELISA technique using available kits cat. No: E4178HU from Bioassay Technology Laboratory Company, China. The serum ATX levels in healthy subjects (males, 0.656 mg/l; females, 0.852 mg/l [23].

Statistical analysis
Data analysis was performed utilizing statistical package for the social sciences version20. Data were presented as mean± standard deviation (SD) for quantitative variables and frequency and percentage for qualitative variables. Groups' comparison was done using independent sample t-test for quantitative data and Yates's chi-square (χ^2) or Fischer's (F) tests, as appropriate. Receiver operated characteristic (ROC) curve was constructed with Area under curve (AUC) is the definite integral of a curve that describes the variation of a material concentration in blood plasma as a function of time. It provides a valuable way to assess the sensitivity and specificity for quantitative diagnostic tool that categorize cases into one of two

groups. P-value <0.05 was considered statistically significant difference for t-test, χ2, and F tests.

RESULTS

The three groups regarding sex showed no significant difference but there was a significant difference in age between group I&II as compared to group III as shown in (table 1). There was significant difference in history of previous bilharzial infection and family history of HCV between group I and II as compared to group III. No significant difference was seen in blood transfusion or operation given past histories between group I&II in comparison to group III as shown in (table 2).

ATX levels in the studied groups. ATX were significantly greater in decompensated chronic hepatitis C patients (CHC) compared to patients with compensated CHC and controls with a mean value (646.7 ± 247.4), (615.3 ± 266) and (469.9 ± 62.3) ng/ml respectively as demonstrated in (table 3).
Overall, Serum ATX was significantly higher according to liver fibrosis worsen stage as shown in (table 4).

Correlations between ATX and laboratory investigations in patient groups revealed no significant correlation between ATX and other studied parameters (Liver function tests and CBC) in both patient's groups as shown in (table 5).

Correlation between Fibrosis index-4 and lab investigations in patient groups in group I showed significant association between Fib-4 and (ALT, AST, PLT, Hb and PT) but no significant correlation between Fib-4 and total bilirubin, albumin. In group II it showed significant correlation between Fib-4 and (ALT, AST, Hb and PLT) but no significant correlation between Fib-4 and total bilirubin, albumin and WBCs as shown in (table 6).

ROC curve analysis showed that the calculated values of ATX for AUC, optimal cutoff value, sensitivity, specificity, positive predictive value, and negative predictive value for each fibrosis stage are listed in (table 7). The optimal cutoff values that best predicted fibrosis at cut off 484.15 ng/ml had the best sensitivity and specificity as shown in (table 7) and Figure 1.

Table (1): Characteristics of subjects of the studied groups

		Group I (20)	Group II(20)	Group III (20)	Fischer's (F) test	P-value
Age\yrs.	Mean ± SD	51 ± 10.7	52.6 ± 12.7	44 ± 10.7	1.9	0.016*
	Range	33 – 67	24 – 76	24 – 60		
Sex	Female	40% (8)	35% (7)	40% (8)	0.11	0.059
	Male	60% (12)	65 (13)	60% (12)		

SD: Standard deviation, F : Fischer's (F) test, *: p value < 0.05

Table (2): Past history of the studied groups

	Group I	Group II	Group III	Chi-square test & p-value	
	n (%)	n (%)	n (%)	X²	P-value
Bilharziasis	10 (50%)	7 (35%)	0 (0%)	13	0.001*
Blood Transfusion or operation	10 (50%)	8 (40%)	8 (4·%)	0.54	0.85
Family history of HCV	12 (60%)	11 (55%)	5 (25%)	11	0.005*

HCV: hepatitis C virus, X^2 : Chi-square test), *: p value < 0.05

Table (3) Fibrosis markers in the studied group

Fibrosis markers	Group I (n= 20)	Group II (n= 20)	Group III (n= 20)	F test	P-value
Autotaxin (ng/ml)	615.3 ± 266	646.7 ± 247.4	469.9 ± 62.3	5.89	0.005*
FIB-4	1.58 ± 0.64	2.84 ± 1.41	0.88 ± 0.29	27.4	0.001*

FIB-4: fibrosis index -4, *: significant, F: Fischer's (F) test, *: p value < 0.05

Table (4) Autotaxin level and Fibrosis index -4 values among cases according to severity of fibrosis

		Mild (n=14)	Moderate (n=18)	Sever (n=8)	F test	P-value
Autotaxin (ng/ml)	Mean	469.9 ± 62.3	615.7 ± 247.4	646.3 ± 266	3.93	0.002*
	Range	400-518	430-710	470-870		
FIB-4	Mean	1.26 ± 0.07	2.14 ± 0.57	4.2 ± 1.11	23.7	0.001*
	Range	0.65-1.40	1.54-3.15	3.5-6.90		

FIB-4: fibrosis index -4, F: Fischer's (F) test, *: p value < 0.05

Table (5) Correlation between Autotaxin and lab investigations in patients groups

Group		PLT	HB	WBCs	AST	ALT	Albumin	T. bilirubin	PT
Group I	r	0.009	0.119	0.111	0.210	0.077	0.009	-0.327	0.143
	P	0.969	0.617	0.642	0.375	0.746	0.969	0.160	0.549
Group II	r	0.087	0.204	-0.084	0.204	0.141	0.066	-0.074	0.059
	P	0.716	0.389	0.726	0.389	0.554	0.784	0.756	0.804

PLT: platelets, HB: hemoglobin, WBCS: white blood cells, AST: Alanine aminotransferase (ALT) Aspartate aminotransferase, PT: prothrombin time

Table (6) Correlation between Fibrosis index-4 and lab investigations in patients' groups

Group		PLT	HB	WBCs	AST	ALT	Albumin	T. bilirubin	PT
Group I	r	0.576	-0.111	-0.065	0.694	0.337	0.047	0.250	.400
	P	0.008*	0.640	0.785	0.001*	0.146	0.845	0.081	0.287
Group II	r	0.716	-0.475	0.302	0.504	0.456	-0.054	0.257	0.602
	P	0.001*	0.034*	0.195	0.023*	0.043*	0.821	0.274	0.001*

PLT: platelets, HB: hemoglobin, WBCS: white blood cells, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, PT: prothrombin time, *: p value < 0.05

Table (7): The calculated values of ATX for AUC, optimal cutoff value, sensitivity, specificity, positive predictive value, and negative predictive value

	Cut off point	AUC	Sensitivity	Specificity	PPV	NPV
Autotaxin	484.15	0.776	80%	70%	63.6%	84.2%

NPV: Negative predictive value***PPV**: Positive predictive value***AUC**: Area under curve
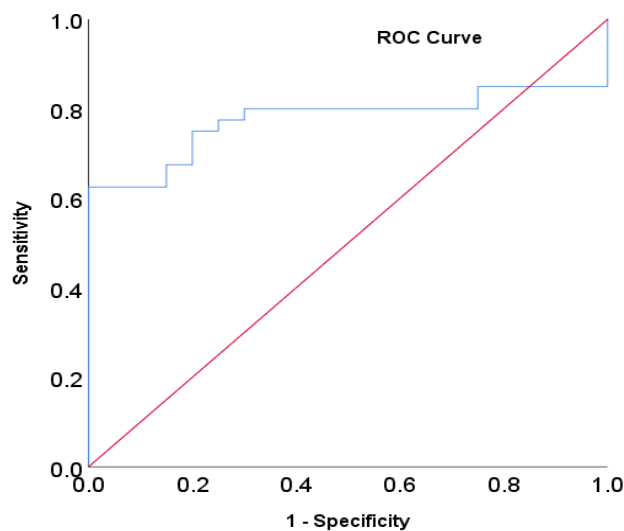

Figure1. Receiver operating characteristic curves for autotaxin (ATX) for the estimation of hepatic fibrosis

DISCUSSION

The most popular HCV genotype in Egypt is the HCV genotype 4, which is > 85% of the total in Egypt [11, 12]. The evaluation of the stage of hepatic fibrosis in chronically HCV cases is important for treatment interventions [13]. The liver biopsy is the most effective and reliable procedure for the evaluation of the amount of liver fibrosis, but has several problems [4, 14]. The search for non-invasive biomarkers to improve the diagnosis is also important. Examples for non-invasive indicators of liver fibrosis such as Fibrotic index 4, AST / Platelet Ratio (APRI), Forn Index, Wave Elastography (SWE) and Doppler color velocity are useful for clinical evaluation of hepatic fibrosis in chronic HCV patients [5, 15]. Autotaxin (ATX) hydrolyzes lysophosphatidyl choline into a multi-functional, bioactive mediator of the lipid lysophosphatiditic acid (LPA) [6].

In our study, history of schistosomal infection was positive in 50% of group I and in 35% of group II. Schistosomiasis decreases human immunity, which could result in increasing the viral load in the blood after acute infection of both hepatitis B and C. whereas; anti-schistosomal therapy which taken parentally may lead to transmission of hepatitis viruses through improperly sterilized glass syringes [18]. In our study, past history of blood transfusion was reported in 50% of group I and in 40% of group II. This finding was in consistent with a study By El Shafie et al [19] to identify the possible risk factors among HCV patients, 9.4% of CHC patients gave history of blood transfusion in the past, Schistosomiasis, and blood transfusion.

Comparing the three studied groups regarding autotoxin level revealed that serum level of ATX was higher in decompensated chronic HCV cases compared to patients with compensated chronic HCV and control groups with a mean value (646.7 ± 247.4), (615.3 ± 266) and (469.9 ± 62.3) ng/ml respectively. Our study showed that autotoxin level for diagnosis of hepatic fibrosis at cut off 484.15 ng/ml had the best sensitivity (80%) and specificity (70%) as shown in Table7. This came in agreement with Yamazaki et al [20] their results indicated that, Autotaxin level was significantly elevated among cases with decompensated chronic HCV in comparison to healthy controls with high sensitivity and specificity.

Incidentally, there was no significant association between ATX and lab parameters. Such findings did not match Yamazaki et al [20] who reported that the ATX levels were positively associated with laboratory parameters and the histological degree of involvement. The amount of serum ATX was also increased with liver fibrosis, including hepatitis and liver regeneration [21].

In our sample, there was a highly significant positive association between Fib-4 and ALT, AST, PLTs, Hb and PT and a very significant negative correlation among Fib-4 and Total Bilirubin, Albumin and WBCS in Group II as shown in Table 6, which agreed with Yen et al [22] who stated that FIB-4 is linked to Alanine aminotransferase (ALT) and Aspartate aminotransferase (AST), Prothrombin time and Platelet level.

Our analysis identified levels of ATX in hepatic decompensation patients to be considerably greater than the amount of ATX in compensated patients (646.7 ± 247.4 ng / ml vs. 615.3 ± 266 ng / ml, P = 0.025). These findings were compatible with Pleli [1] who showed that levels of ATX were strongly associated with the phase of Child-Pugh and MELD. There was a substantial difference between patient groups according to fibrosis level at ATX level even in Fib-4 among patient groups according to the fibrosis level which was accepted with Ikeda and Yatomi [9], who noticed that the levels of autotoxin were associated to the fibrosis phase as elevation of ATX

level may be due to lower clearance of serum ATX because ATX is rapidly absorbed by liver sinusoidal cells or increased output under other circumstances, such as tumors, so lowered clearance of ATX via diseased liver fibrosis or harm to the liver may account for elevated serum ATX level among patients with significant liver fibrosis [9], this fits with our research.

CONCLUSION
ATX level has shown a direct association with stage of fibrosis in patients with chronic HCV also ATX can be used to determine the intensity of liver disorders and to evaluate the prognosis of liver cirrhosis, we found that using both ATX and FIB-4 assessments strengthens the sensitivities and diagnostic specificity of fibrosis in Egyptian chronic viral hepatitis C.

Conflict of interest: No conflict of interest.

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الملخص العربي

الاوتاكسين كوسيلة في قياس نسبة التليف في المرضي المصابين بالتهاب الكبدي المزمن سي

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ملخص البحث

الخلفية: يشكل الالتهاب الكبدي الفيروسي المزمن (سي) مشكلة صحية كبيرة، وقد تم الاعتماد طويلا على الفحص النسيجي لعينة الكبد كوسيلة لتقييم درجة التليف ، ولكن نظرا لما تحمله من مخاطر وضعت العديد من الوسائل غير التداخلية للتغلب على تلك المضاعفات مثل قياس نسبة الاوتوتاكسين في الدم .

الاوتوتاكسين انزيم يتواجد في الدم مسؤول عن تحلل ليسوفوسفاتيديل كولين لإنتاج حمض الليسوفوسفاتيديك (LPA) احد الدهون النشطة بيولوجيا متعددة الوظائف وقد اثبت الدراسات ان مرض تليف الكبد قد يظهر زيادة مستويات الاوتوتاكسين في الدم.

الهدف: تهدف الدراسة إلى تحديد مستويات الاوتوتاكسين في الدم وارتباطه بتليف الكبد وما إذا كان يمكن استخدامه كمؤشر تشخيصي غير تداخلي للتليف في مرضى الذين يعانون الفيروس الكبدي الوبائي المزمن سي في مصر.

الطرق: أجريت هذه الدراسة في جامعة الازهرطب بنات القاهره، حيث شملت ٤٠ مريضا مصاب بالالتهاب الكبدي الفيروسي المزمن (سي) وتم تقسيمهم الي تلاث مجموعات كلاتي :

المجموعة الأولى: تضم ٢٠ مريضاً يعانون من مرضى التهاب الكبد الوبائي المزمن في مرحلة التعويض.

المجموعة الثانية: شملت ٢٠ مريضا يعانون من مرضى التهاب الكبد الوبائي المزمن في المرحلة اللاتعويضية. المجموعة الثالثة (المجموعة الضابطة): تضم ٢٠ شخص عادي خالي من الامراض. وقد تم اجراء الآتي للمرضى: التاريخ المرضى الكامل والفحص السريري الشامل والفحص المعملي المعتاد و اشعه تليفزيونيه على البطن و قياس نسبة الاوتوتاكسين في الدم باستخدام تحليل الايلازا.

النتائج: وجدت دلالة احصائية ايجابية بين نسبة الاوتوتاكسين ودرجه التليف بالكبد وزياده قيمته بزياده درجه النشاط. وجدت دلالة احصائية ايجابية بين نسبة الاوتوتاكسين ودرجه التليف في المرضى في مرحلة اللاتعويضية.

الأستنتاجات: الاوتوتاكسين يعتبر عامل تشخيص فعال لدرجة التليف لدى مرضى التهاب الكبد الوبائي المزمن .

الكلمات المفتاحية: الاوتوتاكسين، تليف الكبد، التهاب الكبد الوبائي سي.

الباحث الرئيسي

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