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Serum autotaxin levels in responders to HCV treatment by direct-acting antivirals

Nancy Abdel Fattah Ahmed[1*], Ahmed Galal Deiab[1], Ahmad Shawki Mohammad Hasan[2] and Ahmad Mohamed Yousry Abd Elbaky[3]

Abstract

Background: Hepatitis C virus (HCV) infection is considered one of the main causes of chronic liver disease around the world. Liver biopsy has been believed to be the gold standard for the assessment of the degree of liver fibrosis. Thus, there is a need to improve non-invasive evaluation of liver fibrosis. The aim of the present study was to study the changes in serum levels of ATX (Autotaxin) as a marker of hepatic fibrosis in responders to HCV treatment by DAAs. This prospective study was carried out at hepatology outpatient clinics for HCV treatment in Mansoura Specialized Medical Hospital that involved 54 participants: 34 patients with HCV and 20 controls; ATX was measured for the controls and all patients before and after treatment.

Results: We found a significant higher ATX level in control subjects vs HCV patients, 100% of control subjects had ATX > 97.5 and 58.8% of HCV had ATX ≤ 97.5. Also, a significantly higher ATX after treatment with DAAs as a whole was observed.

Conclusion: The authors concluded that ATX should be considered cautiously as a diagnostic marker for liver fibrosis in Egyptian patients with chronic hepatitis C infection. Although this study yielded negative results, this may be important to prevent duplication of the research efforts.

Keywords: Serum autotaxin, Direct-acting antivirals, Hepatic fibrosis

Background

Hepatitis C virus (HCV) infection is considered one of the main causes of chronic liver diseases around the world. Around the world, there are about 71 million chronically infected persons [1].

Liver biopsy has been believed to be the gold standard for the assessment of the degree of liver fibrosis [2]. Diagnosis of liver fibrosis is commonly built on histological results after liver biopsy. The strategy of using biopsy to stage most cases of liver diseases has many restrictions such as sampling errors [1, 3], intraobserver, and interobserver variation during histological evaluation and hepatic biopsy is an invasive method with accompanied morbidity [3]. Due to these restrictions, the thought of liver biopsy as the "gold standard" has come down to "best available" standard [4].

Autotaxin (ATX) is a member of the ectonucleotide-pyrophosphatase/phosphodiesterase (ENPP) family and considered a secreted glycoprotein [5]. It converts lysophosphatidylcholine (LPC) to the bioactive phospholipid lysophosphatidic acid (LPA) which is a multifunctional bioactive lipid mediator [6]. ATX is a necessary enzyme, which is required for early embryological development [7]. Serum ATX levels may increase during pregnancy [8] and in patients with idiopathic pulmonary fibrosis or some types of cancers [9–11]. In the serum, ATX is found and its metabolism is done by hepatic sinusoidal endothelial cells. Liver fibrosis inhibits metabolism of ATX, resulting in elevation of its serum levels. Due to these findings, ATX may be directly related to liver fibrosis [12].

Serum ATX was correlated to staging of liver fibrosis in patients with chronic hepatitis C (CHC). After comparison with serum hyaluronate and (APRI score), i.e.,

* Correspondence: ziad.emad90@yahoo.com
[1]Internal Medicine Department, Hepatology & Gastroenterology Unit, Mansoura Specialized Medical Hospital, Mansoura University Faculty of Medicine, Mansoura, Egypt
Full list of author information is available at the end of the article

two confirmed markers for liver fibrosis [13], it was found that serum ATX level was the best parameter for predicting cirrhosis in both men and women [14].

This work aimed to study the changes in serum ATX levels as a sign of fibrosis of liver in responders to HCV treatment by DAAs.

Methods

Study design

The present study was prospective in nature and our patients were selected from the hepatology outpatient clinics for HCV treatment in Mansoura Specialized Medical Hospital.

Sample and selection of patients

From 34 patients and 20 controls serum samples have been obtained just before treatment (baseline), and at end of 12 weeks course of treatment for all patients. At − 20 °C until testing, all collected samples have been quickly stored, serum ATX has been measured using Human ENPP-2/ATX Quantikine ELISA Kit (manufactured and distributed by R&D systems, Inc., USA and Canada) according to the recommendations of the manufacturer.

Patients have been followed by investigations just before and 12 weeks after DAAs treatment to determine impact of DAAs on serum ATX levels and its correlation with fibrosis changes. DAAs regimen includes the 12 weeks course of Sofosbuvir and Daclatasvir ± Ribavirin. The goal of treatment is undetectable HCV RNA in plasma or serum by a sensitive assay (lower limit of detection ≤ 15 IU/ml) 12 weeks (SVR12) or 24 weeks (SVR24) after the termination of therapy as EASL, 2018 [1].

For SVR patients, a sample size of 31 achieves 99% power to detect a mean of paired differences of 0.2 with an estimated standard deviation of differences of 0.2 and with a significance level (alpha) of 0.005 using a two-sided paired *t* test.

This study was approved by the ethical committee of Mansoura Faculty of Medicine and its university hospital.

- Medical Research committee has submitted study protocol for approval with code number: MS. 18.09.301.
- Confidentially and personal privacy have been respected in all levels of the study.

Table 1 ATX level regardless of sex in the 2 groups: it showed a statistically significant higher ATX level in control subjects vs HCV patients

Statistic	Group		*Z*	*P*
	Control (*n* = 20)	HCV (*n* = 34)		
Median	192.1	85.8	− 2.508	**0.012**
IQR	143.2–239.1	59.7–255.2		

Data expression: median (IQR). *P* value: Mann-Whitney *U* test

Table 2 ATX cut-off value to discriminate HCV from control: it showed that ATX ≤ 97.5 has 100% specificity in discriminating HCV and control but it is not sensitive

Cut-off	AUC	95% CI	SE	*P*	SN	SP	PPV	NPV
≤ 97.5	0.706	0.563–0.849	0.073	**0.012**	59%	100%	100%	59%

AUC area under the curve, *SE* standard error, *SN* sensitivity, *SP* specificity, *PPV* positive predictive value, *NPV* negative predictive value

- Collected data has not been used for any other purpose.

Inclusion criteria

All responders to HCV treatment by DAAs who are clinically and virologically improved (DAAs regimen includes the 12-weeks course of Sofosbuvir and Daclatasvir + Ribavirin) and for the controls included were free of chronic liver diseases who are matched for age and sex.

Exclusion criteria

- Patients with history of cancers and other causes of liver disease.
- Previous liver transplantation.
- Patients co-infected with HIV or HBV.
- Other organ failure (heart failure and renal failure).

Laboratory investigations

Fasting blood glucose, liver function tests:(AST, ALT, ALP, total bilirubin, direct bilirubin, total albumin), kidney function tests: (serum creatinine and urea), serum AFP, serum ATX, PT, INR, CBC, HCV Ab, and HBsAg.

Statistical analysis

Sample size was calculated using PASS software (Hintze, J.; 2011. PASS 11. NCSS, LLC. Kaysville, UT, USA. www.ncss.com).

Data were entered and analyzed using IBM-SPSS software (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 25.0. Armonk, NY, USA: IBM Corp.)

Repeated-measures graph was created by GraphPad Prism software for Windows (version 6.01) (Tables 1, 2, 3, 4, 5, 6 and 7).

Table 3 ATX cut-off distribution in HCV and control: it showed that 100% of control subjects had ATX > 97.5 and 58.8% of HCV had ATX ≤ 97.5; a difference which is statistically significant

ATX	Group		χ^2	*P*
	Control (*n* = 20)	HCV (*n* = 34)		
> 97.5	20 (100%)	14 (41.2%)	18.685	**< 0.0005**
≤ 97.5	0 (0%)	20 (58.8%)		

Data expression: frequency (%), *P* chi-square test

Table 4 ATX before and after DAAs treatment: it showed a statistically significant higher ATX after treatment with DAAs as a whole. By stratifying the cases according to sex, this statistical significance exists only in male patients but not female patients

Group	Timing		*Z*	*P*
	Before	After		
Whole group (*n* = 34)	85.8 (59.7–255.2)	249.9 (178.6–453.1)	− 3.308	**0.001**
Male (*n* = 17)	66.5 (52.5–85.8)	182.5 (148.9–243.8)	− 3.101	**0.002**
Female (*n* = 17)	139 (84.2–403.4)	420.4 (256–541.8)	− 1.681	0.093

P value Wilcoxon signed-ranks test

Results

Discussion

The present study included 54 individuals and they were subdivided into groups:

- ***HCV group*** included 34 patients with chronic HCV.
- ***Control group*** included 20.

Our study showed a statistically significant higher ATX level in control subjects vs HCV patients as Yamazaki and his colleagues 2017 [15] who found that median ATX concentrations were essentially higher in patients than in healthy persons in contrast to Fujimori et al. 2018 [16] who found elevated serum/plasma ATX concentrations in hepatic patients.

Also, in accordance with our findings, Ezzat and his colleagues found elevated levels of serum autotaxin among controls higher than HCV patients (Ezzat et al. 2013 [17]).

That could be explained by the differences between populations according to molecular basis for serum ATX expression. There is contradiction in our findings because of liver bilharziasis among Egyptian patients with chronic HCV. There are different findings in our study due to genetic difference between the studied Egyptian patients and other foreign patients in previous studies. Serum ATX expression may be affected by different patterns of immune expression such as environmental stress, genetic predisposition, and bilharziasis (Ezzat et al. 2013 [17]).

Table 5 Laboratory data before and after treatment: it showed a statistically significant improvement (decrease) in AST, ALT, and APRI score, but no statistically significant change in platelet count and FIB4 score

Parameter	Before	After	*Z*	*P*
AST	33 (24–44)	24.5 (22–30)	− 3.662	**< 0.0005**
ALT	37 (22–64)	24.5 (21–29)	− 3.583	**< 0.0005**
Platelet count	175 (124–228)	177.5 (145–219)	− 0.479	0.632
APRI score	0.52 (0.25–0.85)	0.37 (0.24–0.55)	− 3.889	**< 0.0005**
FIB4 score	1.7 (0.91–2.72)	1.6 (0.87–2.26)	− 1.564	0.118

Data expression: median (IQR). *P* Wilcoxon's signed-ranks test

Table 6 APRI and FIB4 scores before and after treatment: *it* showed a statistically significant improvement in APRI score (but not FIB4 score) after treatment with DAAs

Score	Before	After	*Z*	*P*
APRI:			− 2.828	**0.005**
< 0.5	16 (47.1%)	22 (64.7%)		
0.5–1.5	16 (47.1%)	12 (35.3%)		
>1.5	2 (5.9%)	0 (0%)		
FIB4:			− 1.633	0.102
< 1.45	14 (41.2%)	16 (47.1%)		
1.45–3.25	15 (44.1%)	15 (44.1%)		
> 3.25	5 (14.7%)	3 (8.8%)		

Data expression: frequency (%). *P* Wilcoxon's signed-ranks test

In the present study, we found a statistically significantly higher ATX after treatment with DAAs as a whole.

We also found a statistically significant improvement in APRI score and non-statistically significant improvement in FIB4 score after treatment with DAAs.

In the present study, there was a statistically significant improvement (decrease) in AST, ALT after DAAs treatment as Khan and his colleagues 2017 [18] found that hepatic enzymes, frequently increased in chronic hepatitis C patients, tend to decrease to baseline during hepatitis C virus treatment.

Limitations

Not all patients agreed to be in a research and came for follow-up easily.

Conclusion

Overall, it can be concluded that ATX should be considered cautiously as a diagnostic marker for liver fibrosis in Egyptian patients with chronic hepatitis C infection. Although this study yielded negative results, this may be important to prevent duplication of the research efforts.

Table 7 Correlation between ATX and baseline parameters: it showed only a statistically significant correlation between ATX and sex. No correlation exists between ATX and other parameters

Parameter	Correlation coefficient	*P* value
Sex	0.435	**0.010**
Age	0.104	0.557
AST	0.086	0.628
ALT	0.005	0.978
Platelet count	− 0.062	0.728
APRI score	0.109	0.541
FIB4 score	0.079	0.657

Correlation coefficient: r_{pb} for sex and r_s for other parameters. *P* point bi-serial for sex and Spearman's correlation for other parameters

Recommendations

- Larger sample sizes, longer study period and different ethnicities are necessary to confirm these findings.
- That our promising results need further confirmation.
- Liver biopsy is recommended as the gold standard method for determining fibrosis.

Abbreviations

DAAs: Direct-acting antivirals; APRI score: (AST)-to-platelet ratio index; SVR: Sustained virologic response; HIV: Human immunodeficiency virus; HBV: Hepatitis B virus; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; AFP: Alpha-fetoprotein; PT: Prothrombin time; INR: International normalized ratio; CBC: Complete blood count; HCV Ab: Hepatitis C virus antibody; HBs Ag: Hepatitis B surface antigen; IFN: Interferon; FIB4: Fibrosis index based on the 4 factors

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Authors' contributions

NAFA: manuscript review, design, manuscript editing, publishing, and final revision (CA). AGDA: idea of the study, data collection and follow-up. ASMH: laboratory studies. AMYA: literature search, clinical and statistics. All authors have read and approved the manuscript.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding on reasonable request.

Ethics approval and consent to participate

Study protocol was investigated and approved by the Medical Ethics Research Team, Faculty of Medicine, Mansoura University. Every case, after guaranteeing privacy, has given informed written consent (code number MS.18.09.301).

Consent for publication

Agreement of Ministry of Health and population and National committee for control of viral hepatitis.

Competing interests

The authors declare that they have no competing interests.

Author details

[1]Internal Medicine Department, Hepatology & Gastroenterology Unit, Mansoura Specialized Medical Hospital, Mansoura University Faculty of Medicine, Mansoura, Egypt. [2]Clinical Pathology Department, Mansoura University Faculty of Medicine, Mansoura, Egypt. [3]Master Second Candidate Internal Medicine, Sherbeen Hospital, Ministry of Health Dakahlia, Dakahlia, Egypt.

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