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ASSESSMENT OF SERUM AUTOTAXIN AS NOVEL BIOMARKER OF LIVER CIRRHOSIS

By

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ABSTRACT

Background: Autotaxin and its product lysophosphatidic acid are considered to be involved in the development of liver cirrhosis and elevated levels of serum autotaxin have been found in patients with hepatitis C virus associated liver disease.

Objective: The clinical role of systemic autotaxin in the stages of liver cirrhosis was unknown. So, in our study we investigated the utility of serum autotaxin level as a marker of liver cirrhosis.

Methods: Patients with liver cirrhosis were prospectively enrolled. Blood samples drawn at the day of inclusion in the study were assessed for autotaxin content by an enzyme-linked immunosorbent assay. Autotaxin levels were correlated with liver cirrhosis. The diagnostic value of autotaxin was investigated by analyses.

Results: 60 subjects were enrolled. Cirrhotic group had highest level of serum Autotaxin (105.86 ± 14.85 mg/l, p1<0.001) compared to hepatitis C virus non cirrhotic group (81.98 ± 11.31 mg/l, p2<0.001) and to control group (42.52 ± 4.95 mg/l, p3<0.001) with statistically significant difference and also hepatitis c non cirrhotic group had higher level compared to Control group by using of one-way ANOVA test.

Conclusion: Our findings supported that serum Autotaxin level is a valuable test for detection of cirrhosis and associated with the severity of liver cirrhosis.

Keywords: Liver cirrhosis, Autotaxin, hepatitis C virus.

INTRODUCTION

Liver cirrhosis is the final common pathological pathway of liver damage arising from a wide variety of chronic liver diseases *(Qua CS et al., 2011)*.

The most common causes of liver cirrhosis are alcohol abuse, chronic infections with hepatotropic viruses, namely hepatitis B (HBV) and hepatitis C (HCV) viruses and non-alcoholic steatohepatitis. Patients suffering from liver cirrhosis are at risk of decompensation which is associated with impaired prognosis. Cirrhosis specific complications that may arise include ascites, spontaneous bacterial peritonitis (SBP), hepatorenal syndrome (HRS), gastrointestinal bleeding, hepatic encephalopathy (HE) and hepatocellular carcinoma (HCC) *(Cárdenas A et al., 2011)*.

Liver biopsy is recommended as the gold standard method for determining fibrosis stage prognosis and therapeutic indications in patients with chronic liver disease. However, liver biopsy is an invasive procedure *(Palmeri ML et al., 2011)*.

Approximately 1–3% of patients require hospitalization for complications, and a quarter of them report pain after percutaneous liver biopsy. The diagnostic accuracy of liver biopsy for assessment of hepatic fibrosis is influenced by the quality of the biopsy samples. In addition, there are many absolute or relative contraindications to liver biopsy, including severe coagulopathy *(Boursier J et al., 2010)*.

These limitations have led to the development of serum biomarkers for the assessment of liver fibrosis in recent years. Serum biomarkers are categorized into direct and indirect biomarkers whether or not they reflect extracellular matrix (ECM) turnover. Direct biomarkers have clinical values involving both the evaluation of liver fibrosis and monitoring the behavior of fibrogenesis and ECM metabolism *(Papastergiou V et al., 2012)*.

The autotaxin level may be a useful biomarker to select treatment therapy for ascites, hepatic encephalopathy, and varix ruptures. And the assessment for the complications of LC is especially valuable in helping to make treatment decisions *(Ge PS et al., 2016)*.

However, serum serum autotaxin in liver cirrhosis and its prognostic value has not yet been investigated *(Pleli T et al., 2014)*.

AIM OF THE WORK

To assess the value of autotaxin as potential biomarker for detection of liver cirrhosis.

PATIENTS AND METHODS

This prospective cross sectional analytic study included 60 patients divided in 3 groups: liver cirrhosis patients, Hepatitis C patients with no cirrhosis and normal subjects recruited from Matria Teaching Hospital. All patients supplied informed consent before participating in this study.

Collection of blood samples:

Blood samples were collected from Matria Teaching Hospital and serum was separated by centrifuging of clotted blood at 4000 rpm at 4 ^{o}C for 10 minutes and then stored at - 80 ^{o}C until testing of biochemical parameters. Additional blood samples were collected in tubes containing sodium citrate centrifuged at 4000 rpm for 10 minutes and then plasma was collected immediately for testing clotting parameters.

All patients were subjected to the following:

- Complete blood picture.
- Liver function tests: AST, ALT, serum total proteins, serum albumin, total and direct bilirubin.
- Prothrombin time and INR.
- Kidney function tests: BUN, creatinine.
- HBsAg.
- HCV antibodies using ELISA technique
- Special test: Fibrosis 4 score "It is a noninvasive scoring system based on

several laboratory tests that help to estimate the amount of scarring in the liver, the formula is: (age x AST)/ (platelets x square root of [ALT])".

- Measurement of serum Autotaxin using enzyme linked immunosorbent assay (ELISA).
- Abdominal Ultrasonography scan for patient and controls.

Statistical Analysis:

1. Chi-square test: For categorical variables, to compare between different groups
2. Monte Carlo correction: Correction for chi-square when more than 20% of the cells have expected count less than 5
3. Student t-test: For normally distributed quantitative variables, to compare between two studied groups
4. F-test (ANOVA): For normally distributed quantitative variables, to compare between more than two groups, and Post Hoc test (Tukey) for pairwise comparisons
5. Pearson coefficient: To correlate between two normally distributed quantitative variables
6. Wilcoxon signed ranks test: For abnormally distributed quantitative variables, to compare between two periods
7. Receiver operating characteristic curve (ROC): It is generated by plotting sensitivity (TP) on Y axis versus 1-specificity (FP) on X axis at different cut off values. The area under the ROC curve denotes the diagnostic performance of the test. Area more than 50% gives acceptable performance and area about 100% is the best performance for the test. The ROC curve allows also a comparison of performance between two tests.

RESULTS

This study was conducted to assess the value of serum Autotaxin as potential biomarker for detection of liver cirrhosis. This study is a case control study that included 60 subjects divided in 3 groups: Group (A) liver cirrhosis patients, Group (B) Hepatitis C patients with no cirrhosis and Group (C) normal subjects recruited from Matria Teaching Hospital.

This table shows no statistically significant difference between groups as regards the age and the majority of cases were male **(Table 1)**.

Table (1): Comparison between the three studied groups according to demographic data

	Group A (n =20)		Group B (n = 20)		Group C (n = 20)		Test of sig.	p
	No.	%	No.	%	No.	%		
Sex								
Male	15	75.0	18	90.0	19	95.0	c^2=3.290	^{MC}p= 0.252
Female	5	25.0	2	10.0	1	5.0		
Age (years)								

Min. – Max.	35.0 – 59.0	29.0 – 57.0	29.0 – 56.0	F= 2.595	0.083
Mean ± SD.	51.40 ± 5.55	47.55 ± 7.62	46.75 ± 7.35		
Median (IQR)	52.5 (49.0 – 54.5)	49.50 (42.5 – 53.5)	46.50(44.0 – 52.0)		

This table shows that all enrolled subjects were HBs Ag negative and cirrhotic group were half cirrhotic due to HCV and half due to other etiologies **(Table 2)**.

Table (2): Comparison between the three studied groups according to HBs Ag and HCV Abs

	Group A (n =20)		Group B (n = 20)		Group C (n = 20)		c^2	p
	No.	%	No.	%	No.	%		
HBs Ag	0	0.0	0	0.0	0	0.0	–	–
HCV Abs	10	50.0	20	100.0	0	0.0	48.574*	<0.001*

This table shows that half of Cirrhotic group were Child Pugh Score C, other half of Cirrhotic group were child Pugh score B. Hcv group non cirrhotic were all from Child Pugh type A **(Table 3)**.

Table (3): Comparison between the two studied groups according to Child Pugh Score (CPS)

Child Pugh Score (CPS)	Group A (n =20)		Group B (n = 20)		c^2	p
	No.	%	No.	%		
A	0	0.0	20	100.0	40.000*	<0.001*
B	10	50.0	0	0.0		
C	10	50.0	0	0.0		

This table shows that Fibrosis 4 score were higher in Cirrhotic group than other two groups with statistically significant difference by comparison between groups **(Table 4)**.

Table (4): Comparison between the three studied groups according to fibrosis 4 score

Fibrosis 4 Score	Group A (n =20)	Group B (n = 20)	Group C (n = 20)	H	p
Min. – Max.	3.31 – 13.61	1.42 – 2.86	0.34 – 2.61	48.275*	<0.001*
Mean ± SD.	6.98 ± 3.41	2.0 ± 0.51	0.89 ± 0.56		
Median (IQR)	6.13 (4.32 –9.68)	1.76 (1.63 –2.43)	0.74 (0.56 –0.97)		
Sig.bet.Grps	p_1<0.001*,p_2<0.001*,p_3=0.003*				

This table shows that Cirrhotic group had highest level of serum Autotaxin compared to HCV non cirrhotic group and to Control group with statistically significant difference and also HCV non cirrhotic group had higher level compared to Control group by using of one-way ANOVA test **(Table 5)**.

Table (5): Comparison between the three studied groups according to serum autotaxin

Serum Autotaxin	Group A (n =20)	Group B (n = 20)	Group C (n = 20)	F	p
Min. – Max.	81.40 – 130.30	62.0 – 101.60	32.50 – 52.50		
Mean ± SD.	105.86 ± 14.85	81.98 ± 11.31	42.52 ± 4.95	164.614*	<0.001*
Median (IQR)	105 (92.2 –119.9)	82.05 (72.45 –91.9)	42.50 (38.9 –46.2)		
Sig.bet.Grps	p_1<0.001*,p_2<0.001*,p_3<0.001*				

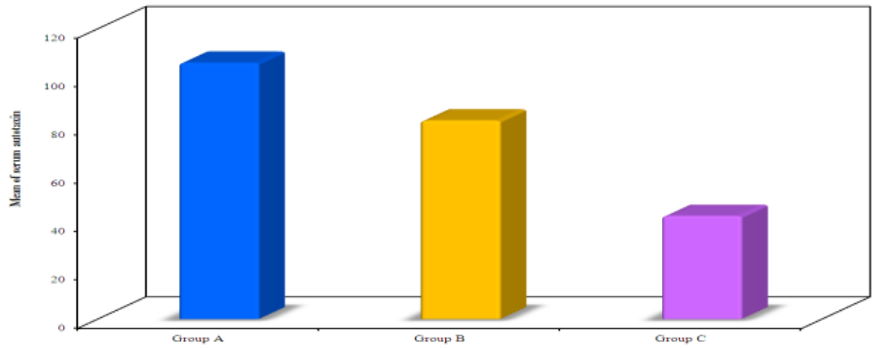

Figure (1): Comparison between the three studied groups according to serum autotaxin

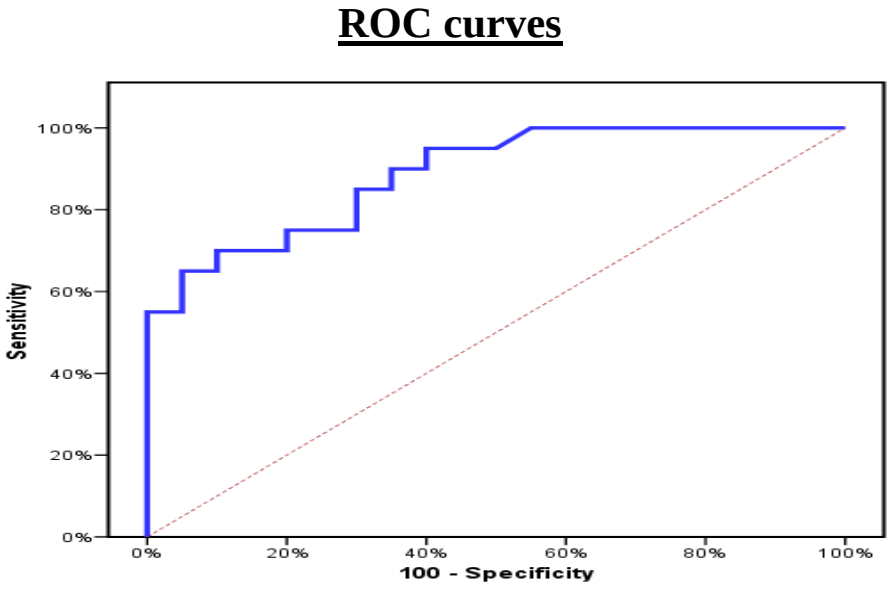

Figure (2): ROC curve serum autotaxin to predict cirrhosis cases vs HCV cases

Table (6): Agreement (sensitivity, specificity) for serum autotaxin to predict cases (vs control)

	AUC	P	95% C.I	Cut off	Sensitivity	Specificity	PPV	NPV
Serum autotaxin	0.886*	<0.001*	0.788 –0.984	**>87.7**	90.0	65.0	72.0	86.7

AUC: Area Under a Curve, p value: Probability value, CI: Confidence Intervals
NPV: Negative predictive value, PPV: Positive predictive value
*: Statistically significant at $p \leq 0.05$

DISCUSSION

Liver cirrhosis is the result of several kinds of chronic liver damage. Cirrhosis is a diffuse hepatic process characterized by fibrosis and the conversion of normal liver architecture into structurally abnormal nodules. The progression to cirrhosis is very variable and may occur over weeks or many years. Around 80-90% of the liver parenchyma needs to be destroyed before there are clinical signs of liver failure *(Tsochatzis EA, et al., 2014)*.

Pleli et al reported serum levels of Autotaxin levels from subjects with liver cirrhosis were elevated compared to healthy control subjects, and serum Autotaxin levels correlated with the Child-Pugh score in predicting the severity of the disease. That is, dysfunction of endothelial cells from the progression of fibrosis lead to reduced Autotaxin clearance and increased serum Autotaxin *(Pleli T, Martin D., 2014)*.

Aim of the work: to assess the value of serum Autotaxin as potential biomarker for detection of liver cirrhosis.

For this purpose, 60 patients were subjected to history taking, full clinical examination, full laboratory assessment for liver function and abdominal ultrasound examination and serum Autotaxin.

In our study, we found that the majority of patients with liver cirrhosis were males (75%). This agrees with the study done by Jennifer and Marion, who found that the majority of cases of liver disease due to viral hepatitis are males *(Guy J and Peters MG et al., 2013)*.

High percentage of patients with liver cirrhosis gave history of Gastrointestinal bleeding (80% LC, 0% HCV), Hepatic encephalopathy (40%LC, 0% HCV) and ascites (80%LC, 0%HCV) in comparison with patients with HCV without cirrhosis. Also, Child Pugh was higher in liver cirrhosis patients (Child C was 50% in LC and Child B 50%) in comparison with HCV group (Child C &B score in HCV group was 0%).

These results agree with the study done by Pleli T who found serum levels of ATX levels from subjects with LC were elevated compared to healthy control subjects, and serum ATX levels correlated with the Child-Pugh score in predicting

the severity of the disease *(Pleli T et al., 2014)*.

Our study showed that liver enzymes (Alanine transaminase and Aspartate transaminase) were high in cirrhotic group with statistically significant difference by comparison with other groups. This result partially agrees with the study done by Khattab, et al., in 2015 which found that AST increases in patients with liver cirrhosis in comparison to patients with HCV without cirrhosis and there's positive correlation between level of AST and histopathological status of the liver while ALT showed no correlation *(Khattab, et al., 2015)*.

Total protein and serum albumin were low in cirrhotic group with statistically significant difference in comparison with HCV group. This agrees with the study done by Nagao Y and Sata Min 2010 which showed that albumin level decreases in patients with liver cirrhosis in comparison with HCV due to impairment of liver function and signifies albumin as a predictor of mortality in patients with chronic liver disease *(Nagao Y and Sata M, 2010)*.

Our study showed that Platelets were low in cirrhotic group with statistically significant difference in comparison with HCV group and control group. The results agree with the study made by Mitchell O et al., who found that Thrombocytopenia in chronic liver disease may be explained by suppression of platelet production by the bone marrow as a result of viral infection, alcohol consumption, iron overload, and medications. Splenic sequestration of platelets due to hypersplenism may be another cause of the reduction in the platelet numbers *(Mitchell O et al., 2016)*.

Regarding serum Autotaxin levels, this study showed highly significant serum levels of Autotaxin between Cirrhotic group as compared to Control group with cutoff point>87.7ng/mland the sensitivity was 90% and specificity 60%.

This agrees with the study done by Pleli T, et al., which showed the similar result in 2014 *(Pleli T, et al.,2014)*. And concluded that Serum ATX is an indicator for the severity of liver disease and the prognosis of cirrhotic patients.

This study revealed positive correlation between serum Autotaxin and Both Child Pugh score and FIB 4 score suggesting that serum Autotaxin is associated with the severity of liver cirrhosis. This study came in line with the study of **Pleli T et al., (2014)** who stated that patients with higher Child-Pugh stage had higher Autotaxin levels.

This study showed that the levels of serum Autotaxin increased significantly in the patients of liver cirrhosis. The extent of this increase seemed to be correlated with degree of liver cirrhosis and disruption of normal liver function. This study was in agreement with the results of Pleli T et al., who reported that In conclusion we showed in this study for the first time that elevated serum ATX levels are associated with the stage of liver cirrhosis, the prevalence of esophageal varices, portal hypertensive gastropathy and hepatic encephalopathy *(Pleli T et al., 2014)*. Additionally, serum Autotaxin may offer a new therapeutic strategy for the management and treatment of cirrhotic liver damage.

CONCLUSION

Our findings supported that serum Autotaxin level is a valuable test for detection of cirrhosis and associated with the severity of liver cirrhosis.

RECOMMENDATIONS

Measurement of serum Autotaxin is good predictor of cirrhosis and may be associated with the stage of cirrhosis. This may help in many circumstances of assessment of cirrhosis with emergent novel biomarkers.

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تقييم مستوي الأتوتكسين كعلامة بيولوجية تشخيصية فى مرضى التليف الكبدى

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خلفيـــة البحـــث: إن التليـف الكبـدي يعـد النتيجـة للعديـد مـن أمـراض الكبـد المزمنـة و مـع تقـدم مرحلـة التليـف يكـون المـريض عرضـة للوفـاة نتيجـة المضـاعفات كالأستسـقاء و الغيبوبة الكبدية و نزيف دوالى المرئ.

الاوتوتاكسـين هـو انـزيم مفـرز تـم اكتشـافه فـي الاصـل مـن خلايـا سـرطان الجلد البشري.

الاوتوتاكسـين لـه وظيفـه انزيميـه مهمـه فـي تحويـل ليـزو فوسـفاتيديل كـولين الـي حمـض ليـزو فوسـفاتيدك،الذي لـه ادوار فسـيولوجيه مختلفـه فـي هجـره الخلايـا، تكـوين الخلايـا العصـبيه، تكـوين الاوعيـه، تقلـص العضـلات الملسـاء، تـراكم الصـفائح الدمويه، وشفاء الجروح.

يقلـل تليـف الكبـد مـن القـدره علـي ايـض الاوتوتاكسـين، ممـا يـؤدي الـي زيـاده في مستوي الاوتوتاكسين في الدم.

ثبـت ان الاوتوتاكسـين فـي الـدم كمؤشـر لتحديـد مرحلـه التليـف فـي مرضـي الالتهـاب الكبـدي المـزمن. بالاضـافه الـي ذلـك يقتـرح ان يكـون الاوتوتاكسـين مفيـدا كمؤشر علي شده امراض الكبد ولتحديد توقعات مرضي تليف الكبد.

الهـدف مـن البحـث: هـو التحقـق مـن العلاقـة بـين مسـتوي الاوتوتاكسـين فـي الـدم و بـين تليف الكبد و تقييم فعاليته في تشخيص التليف الكبدي.

المرضـي و طـرق البحـث: أجريـت هـذه الدراسـة علـي عشـرين مـريض تليـف كبـدي و عشـرين مصـاب بفيـروس سـي بـدون تليـف كبـدى و عشـرين مـن الأصـحاء، و تـم اجـراء الفحوصـات المعمليـه الكاملـه والاوتوتاكسـين فـي الـدم واشـعه الموجـات الفـوق

صـــوتيه لجميـــع المرضـــي، و تـــم إجـــراء تقســـيم مرضـــى التليـــف الكبـــدي حســـب شـــدة التليف الكبدي في مقارنة مع مستوى الاوتوتاكسين في الدم.

نتـــائج البحـــث: أظهـــرت النتـــائج أرتفاعـــا ملحوظـــا لمســـتوى الاوتوتاكســـين فـــي الـــدم مـــع مجموعـــة مرضـــي التليـــف الكبـــدي مقارنـــة مـــع مرضـــي فيـــروس كبـــدي ســـي بـــدون تليـــف كبـــدي و الأصـــحاء، و أظهـــرت أيضـــا أزديـــاد نســـبة مســـتوى الاوتوتاكســـين فـــي الـــدم بمرضـــي التليـــف الكبـــدي المصـــحوب بمضـــاعفات فـــي المراحـــل المتـــأخرة ممـــا يعطـــى مؤشـــرا قويـــا علـــى فاعليـــة و دلالـــة اســـتخدام الاوتوتاكســـين فـــي تشـــخيص التليـــف الكبدي.

الإســـتنتاج: يوصـــي باســـتخدام مســـتوي الاوتوتاكســـين فـــي الـــدم لتقيـــيم شـــدة التليـــف الكبدي و متابعته و تشخيصه و كذا فحص اولي للتشخيص.