

## **Diagnostic Value of Serum Autotaxin Levels in Liver Fibrosis in Children with Chronic Hepatitis.**

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### **Abstract**

Liver biopsy is the most accurate examination method to evaluate liver fibrosis stage but is invasive. Non-invasive biomarkers of disease progression are therefore needed. The study aimed to assess the role of serum autotoxin levels in diagnosis of liver fibrosis in children with chronic hepatitis. This study was a case controlled study that was conducted on a total number of 80 children of both sex. 50 children (case group) collected from outpatient pediatric hepatology clinic of Benha University hospitals and National Liver Institute Monoufia University. 30 apparently healthy children (control group) matched the patient group in age and sex. All children subjected to full history taking, physical examination and Laboratory Investigations: CBC, AST, ALT, GGT, alkaline phosphatase, serum bilirubin, serum albumin, PT, PTT, INR, total protein, total serum IgG levels, hepatitis markers (HAV, HBV&HCV), liver biopsy and serum autotaxin level by ELISA. There was statistical significant difference (P value <0.01) among chronic hepatitis group and control group regarding HB, platelet, total protein as they were lower in chronic hepatitis group than control group, while PT, INR, AST, ALT, GGT, ALP and bilirubin (total& direct) were higher in chronic hepatitis group than control group. There was statistical significant difference between studied groups regarding serum autotaxin as it was elevated in chronic hepatitis group. There were statistical significant differences between serum level of autotaxin and fibrosis index, hepatitis activity index and interface hepatitis as it increases with increase fibrosis index, hepatitis activity index and interface hepatitis. The best cut off value of serum autotaxin was 10.45ng/ml for detection of early fibrosis stage with sensitivity 97.6%, specificity 93.8% and area under the curve is 0.961 while the best cut off serum autotaxin for detection of moderate fibrosis was 18.15ng/ml with sensitivity 85%, specificity 93.8% and area under the curve is 0.984. Autotaxin may be a novel non-invasive biomarker for liver fibrosis done on Egyptian children with chronic hepatitis.

**Keywords:** Chronic hepatitis, Liver fibrosis, Autotaxin.

### **1. Introduction**

Chronic liver disease refers to a wide spectrum of disorders characterized by ongoing liver damage with potential for progression to cirrhosis or end-stage liver disease [1]. Chronic liver disease is most often caused by biliary atresia and genetic-metabolic diseases in infants while in older children, it tends to result from viral infection, autoimmune hepatitis, Wilson's disease, alpha 1-antitrypsin deficiency and primary sclerosing cholangitis [2].

Liver biopsy plays an important role in modern clinical practice in diagnosis of different types of chronic liver disease and assessment of disease severity including staging and grading. It is the most accurate examination method to evaluate liver fibrosis stage but is invasive, prone to sampling error and carries the risk of rare but serious complications. Non-invasive biomarkers of disease progression are therefore needed [3].

Autotaxin (ATX) also known as ectonucleotide pyrophosphatase/ phosphodi-esterase (ENPP) 2, is a secreted lyso-phospholipase D (lyso PLD) that belongs to the ENPP family. It plays an important role in converting lysophosphatidylcholine to the bioactive phospholipid lyso-phosphatidic acid (LPA) that stimulates the proliferation and contractility of hepatic stellate cells. Autotaxin was metabolized by liver sinusoidal endothelial cells so in liver fibrosis, there was retarded degradation of circulating ATX due to liver sinusoidal endothelial cell dysfunction from liver fibrosis [4].

### **2. Subjects and methods**

This cross sectional case controlled study was conducted on a total number of 80 children of both sex. 50 children collected from attendance of outpatient pediatric hepatology clinic of Benha University hospitals and National Liver Institute Menoufia University after informed consent. 30 apparently healthy children were selected from general population during the period from January 2018 to May 2018. Laboratory work was conducted in clinical pathology department, Benha University hospitals.

This were divided into:

**Group I (case group):** included 50 children with chronic hepatitis included (28 females/ 22 males) their mean age  $\pm$  SD 7.58 $\pm$ 5.47 years ranged from 6 months to 17 years.

**Group II (control group):** included 30 children apparently healthy matching the case group for age and sex included (17 females/13 males) their mean age  $\pm$  SD 7.12 $\pm$ 5.40 years ranged from 6 months to 17 years.

### **Inclusion criteria**

All children less than 18 years old with chronic hepatitis of different etiology (e.g viral hepatitis, autoimmune hepatitis and cholestasis included extra hepatic biliary atresia, idiopathic neonatal hepatitis and giant cell hepatitis).

### Exclusion criteria

Children with chronic liver diseases with co-morbidities like renal diseases or heart diseases.

### Informed consent

Informed consent was obtained from the parents after had been informed about all study procedures and their consent was obtained prior to children enrollment in the study.

### Ethical approval

This study was approved by the ethical committee of the Faculty of Medicine, Benha University.

### Methods: All enrolled children were subjected to the following:

#### A- Full history taking.

**B- Clinical examination:** Including general examination & abdominal examination.

**C- Abdominal ultrasound:** For assessment of liver span, texture, spleen span and presence of ascites.

#### D- Laboratory investigations

#### 1. Sampling

Venous blood was drawn by aseptic venipuncture using disposable syringe. Blood was divided in different tubes as follows: 1.5 ml on EDTA for CBC, 2.5 ml on Na-citrate for PT, and 3 ml on plain tube that was allowed to clot at room temperature for 30 min then serum was separated by centrifugation for biochemical and serological testing

#### 2. Biochemical and hematological investigations:

**A-** Complete blood count (CBC): was done by automated hematology analyzer (CELL-DYN Emerald, Germany) to determine hemoglobin level (Hb), total leucocytic count (TLC) and platelets count (PLT).

**B-** Prothrombin time (PT), concentration, INR and activated partial thromboplastin time (aPTT) (STA Compact Max, STAGO, France).

**C-** Liver function tests: included serum total and indirect bilirubin, liver enzymes (ALT, AST, GGT, and alkaline phosphatase), serum total

protein and albumin levels (BioSystems A25, Spain).

All biochemical tests were performed as a routine laboratory investigation by appropriate instruments and kits. The upper limits of normal (ULN) for ALT and AST were established as 40 IU/L.

### 3. Viral markers, auto-antibodies and Immunoglobulin G (IgG)

**A-** Hepatitis B surface antigen (HBsAg), anti-HBs antibodies, anti-HB core Ab (IgM and IgG), anti-HCV Ab and anti-HAV Ab (IgM and IgG) were tested by enzyme linked immunosorbent assay (ELISA) kit (Biokit, Spain).

**B-** Patients suspected to have autoimmune hepatitis were tested for serum autoantibodies including: antinuclear antibodies (ANA), anti-smooth muscle antibody (ASMA), liver kidney microsomal antibody-1 (LKM1), and anti-mitochondrial antibody (AMA) by indirect immunofluorescence technique (NOVA Lite, Inova Diagnostics, Spain).

**C-** Immunoglobulin G (IgG): By radial immunodiffusion using (IgG-NLRID, RN004.3, Binding site, Birmingham, UK).

### 4. Ectonucleotide Pyrophosphatase/ Phosphodiesterase 2 (ENPP2) or Autotaxin level

A double-antibody sandwich ELISA (Enzyme Linked Immune Sorbent Assay) was used to detect serum level of Ectonucleotide Pyrophosphatase/ Phosphodiesterase 2 (ENPP2) or Autotaxin using a commercial Human (ENPP2) ELISA Kit for research use only (Cat #: 201804, SunRedBio, China).

### 5. Percutaneous liver biopsy

Ultrasound guided liver biopsy done for all patients using Menghini spiration needle (Hepafix Luer Lock Melsungen AG, 3409 Mel-Sungen, Germany). Adequate core containing at least 5 portal tracts. Biopsy specimen were fixed in formaline and embedded in paraffin. Five cm thick section were cut, mounted on glass slide and stained with hematoxyline and eosin to evaluate histological activity of hepatitis using Ishak scoring index. Also stained with Mason-Trichrome to assess fibrosis stage. Table (1, 2) .

**Table (1)** Modified HAI Grading: Necro-inflammatory Scores

Periportal or Periseptal Interface Hepatitis (piecemeal necrosis) (A)	Score	Confluent Necrosis (B)	Score	Focal (spotty) Lytic Necrosis, Apoptosis, and Focal Inflammation*(C)	score	Portal Inflammation (D)	Score
Absent	0	Absent	0	Absent	0	None	0
Mild (focal, few portal areas)	1	Focal confluent necrosis	1	One focus or less per 10x objective	1	Mild, some or all portal areas	1
Mild/moderate (focal, most portal areas)	2	Zone 3 necrosis in some areas	2	Two to four foci per 10x objective	2	Moderate, some or all portal areas	2
Moderate (continuous around <50% of tracts or septa)	3	Zone 3 necrosis in most areas	3	Five to ten foci per 10x objective	3	Moderate/marked, all portal areas	3

Table (1) Continue

Severe (continuous around >50% of tracts or septa)	4	Zone 3 necrosis + occasional portal-central(P-C) bridging	4	More than ten foci per 10x objective	4	Marked, all portal areas	4
		Zone 3 necrosis + multiple P-C bridging	5				
		Panacinar or multiacinar necrosis	6				

- Total Modified HAI = \_\_\_/18. Total activity scores are defined as follows (1-3, minimal; mild 9-12; 13-18, sever).
  - Does not include diffuse sinusoidal infiltration by inflammatory cells (5).

Table (2) Modified Staging: Architectural changes, fibrosis and cirrhosis

Change	Score
No fibrosis	0
Fibrous expansion of some portal areas, with or without short fibrous septa	1
Fibrous expansion of most portal areas, with or without short fibrous septa	2
Fibrous expansion of most portal areas with occasional portal to portal (P-P) bridging	3
Fibrous expansion of portal areas with marked bridging [portal to portal (P-P) as well as portal to central (P-C)]	4
Marked bridging (P-P and/or P-C) with occasional nodules (incomplete cirrhosis)	5
Cirrhosis, probable or definite	6

Additional features which should be noted but not scored: Intra-acinar fibrosis, perivenular ('chicken wire' fibrosis) and phlebosclerosis of terminal hepatic venules [5].

**The disease severity scoring systems For predicting prognosis of end-stage liver disease, many prognostic models were proposed as:**

**a. PELD score (pediatric end stage liver disease score):**

It is a disease severity scoring system for children under 12 years of age. This score is used for prioritizing allocation of liver transplantsto predict survival. It is calculated according to the following formula :

PELD = 4.80 × Ln[serum bilirubin (mg/dl)] + 18.57 × Ln[INR] - 6.87 × Ln[albumin (g/dl)] + 4.36(if patient < 1 year : score for patients listed for liver transplantation before the patient’s first birthday continue to include the value assigned for age (<1 year) until the patient reaches the age of 24 months) + 6.67(if patient has growth failure (< -2 Standard deviation)). (In abbreviation for natural logarithm). Liver transplantation may be consider when the PELD score exceeds 4, and

liver transplantation may be required immediately when the PELD score exceeds 22 [6].

**b. MELD score (model for end-stage liver disease score):** It is a scoring system for assessing the severity of chronic liver disease. It was found to be useful in determining prognosis and prioritizing for receipt of a liver transplant. It is calculated according to the following formula (6):

MELD = 3.78× Ln[serum bilirubin (mg/dl)] + 11.2× Ln[INR] +9.57×ln[serum creatinine (mg/dl)]+6.43.(In abbreviation for natural logarithm). In interpreting the MELD Score in hospitalized patients, the 3 month mortality is:

- 40 or more — 71.3% mortality
- 30–39 — 52.6% mortality
- 20–29 — 19.6% mortality
- 10–19 — 6.0% mortality
- <9 — 1.9% mortality

**C. Child-Pugh score and classification Table (3, 4 ) [7].**

Table (3)

Measure	1 point	2 points	3 points
Total bilirubin, μmol/L (mg/dl)	<34 (<2)	34-50 (2-3)	>50 (>3)
Serum albumin, g/dl	>3.5	2.8-3.5	<2.8
Prothrombin time, prolongation (s)	<4.0	4.0-6.0	> 6.0
Ascites	None	Mild (or suppressed with medication)	Moderate to Severe (or refractory)
Hepatic encephalopathy	None	Grade I-II	Grade III-IV

Chronic liver disease is classified into Child-Pugh class A to C, employing the added score from above.

**Table (4)** Child-Pugh classification

Points	Class	One year survival	Two year survival
5-6	A	100%	85%
7-9	B	81%	57%
10-15	C	45%	35%

**Statistical methods**

Results were organized, tabulated and statistically analyzed using SPSS software statistical computer package version 16. Qualitative data were expressed as number and percentage. For quantitative data, the mean and standard deviation and median were calculated. The difference between means was statistically analyzed using the student (t) test, Mann-Whitney test and ANOVA test when was suiTable correlation analysis was done using Pearson(r) and Spearman (rho) correlation when was suiTable. Receiver operator characteristic (ROC) curve was used to assess the performance of autotaxin in diagnosis of fibrosis .The accepted level of significance in this work was 0.05( $p \leq 0.05$ ).

P value  $>0.05$  is non-significant (NS)

$P < 0.05$  is significant (S)

$P \leq 0.001$  is highly significant (HS)

**3. Results**

Results in Table(5) showed that there was statistical significant difference between chronic hepatitis patients and control group regarding weight, weight/ centile, height and height/ centile, body mass index (BMI) and BMI/ centile as it was lower in chronic hepatitis group. Results in Table (6) showed that there was statistical significant difference (P value  $<0.01$ ) between chronic hepatitis group and control group regarding HB, platelet, total protein as they were lower in chronic hepatitis group than control group, while PT, INR, AST, ALT, GGT, ALP and bilirubin (total& direct) were

higher in chronic hepatitis group than control group. However, there was no statistical significant difference among chronic hepatitis group and control group regarding TLC, albumin and PTT. Results in Table (7) showed that there was statistical significant difference between studied groups regarding serum autotaxin as it was elevated in chronic hepatitis group. Results in Table (8) showed that there was statistical significant difference between serum autotaxin and sex as it was elevated in female patients. Results in Table (9) showed that there were statistical significant differences between serum level of autotaxin and fibrosis index, hepatitis activity index and interface hepatitis as it increases with increase fibrosis index, hepatitis activity index and interface hepatitis. Results in Table (10) showed that there was positive correlation between serum autotoxin and liver enzymes (PT, PTT, INR, AST, ALT, GGT, ALP and bilirubin (total& direct)). However, there was negative correlation between serum autotaxin and hemoglobin, platelet and total protein. Results in Table (11) showed that there was positive correlation between serum autotoxin and liver span, spleen size, APRI and FIB4. Results in Table (12) showed that The best cut off value of serum autotaxin was 10.45ng/ml for detection of early fibrosis stage with sensitivity 97.6%, specificity 93.8% and area under the curve is 0.961 while the best cut off serum autotaxin for detection of moderate fibrosis was 18.15ng/ml with sensitivity 85%, specificity 93.8% and area under the curve is 0.984.

**Table (5)** Anthropometric measurements between studied groups.

Anthropometric measurements	Chronic hepatitis group[n=50]		Control group[n=30]		T-Test	P value
	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range		
Weight in kg	25.26 $\pm$ 12.93	6-57	31.5 $\pm$ 9	6-38	2.32	$<0.05$
Weight / centile	39.34 $\pm$ 12.7	3-75	46.3 $\pm$ 10.2	3 -90	2.54	$<0.05$
Height in cm	100.50 $\pm$ 32.69	65-140	120 $\pm$ 25	61-160	2.8	$<0.05$
Height / centile	30.62 $\pm$ 18.6	3-75	39.70 $\pm$ 20.6	5-90	U=660	$<0.05$
BMI	17.03 $\pm$ 2.27	11-24	22 $\pm$ 8.3	12-25	4	$<0.001$
BMI / centile	39.22 $\pm$ 26.15	3-88	59.80 $\pm$ 22.77	9-97	3.57	$<0.01$

**Table (6)** Comparison between studied groups regarding some laboratory parameters.

LAB	Chronic hepatitis group[n=50]		Control group[n=30]		T-test	p
	Mean $\pm$ SD	Range	Mean $\pm$ SD	Range		
Hb (g/dL)	10.55 $\pm$ 1.07	8-14	11.60 $\pm$ 1.3	9-14	3.6	$<0.01$
TLC ( $\times 10^3 \mu\text{L}$ )	8.41 $\pm$ 1.48	5.3-11.4	825.36 $\pm$ 273.82	7-13	0.292	$>0.05$
Platelet ( $\times 10^3 \mu\text{L}$ )	242.28 $\pm$ 740.96	70-452	302.40 $\pm$ 101.86	170-568	2.8	$<0.01$
total protein(g/dL)	6.38 $\pm$ 0.63	5.6-6.5	6.97 $\pm$ 0.45	6-7.9	4.5	$<0.001$
Albumin (g/dL)	3.54 $\pm$ 0.50	2.6-5	3.62 $\pm$ 0.26	3.1-4.3	0.844	$>0.05$
PT(Second)	13.90 $\pm$ 1.43	12-18	13.28 $\pm$ 0.92	12-14	2.13	$<0.05$

Table (6) Continue

PTT(Second)	33.99± 3.94	27-44	32.87± 2.06	29-39	1.6	>0.05
INR	1.10± 0.18	1-1.7	1.00± 0.02	1-1.1	3.7	<0.01
AST(IU/L)	110.04 ± 109.21	30-430	33.27 ± 8.86	19-51	U=167.5	<0.001
ALT(IU/L)	102.76 ± 116.24	24-458	37.40 ± 11.57	16-59	U= 476	<0.01
GGT(IU/L)	64.16 ± 44.89	12-156	25.40 ± 11.31	15-65	U= 286	<0.001
ALP(IU/L)	290.74 ± 222.14	85-764	86.77 ± 30.96	51-202	U=140.5	<0.001
Bil.Total (mg/dL)	2.33 ± 1.79	0.6-7.8	0.90 ± 0.13	0.6-1.2	U= 164	<0.001
Bil.Direct (mg/dL)	0.70 ± 1.15	0.01-4.5	0.09 ± 0.04	0.02-0.16	U=380.5	<0.01

Table (7) Comparison between studied groups regarding serum autotoxin level.

	Chronic hepatitis group(n=50)		Control group(n=30)	T Test	P
Autotoxin(ng/ml)	Mean ± SD	19.65 ± 5.56	4.37 ± 1.46	18.4	<0.001
	Range	10.2-28.3	2.10-6.3		

Table (8) Comparison between serum autotoxin and sex, consanguinity, family history of liver disease.

Variables	Autotoxin (ng/ml)		Test	p	
	Mean ± SD	Range			
Sex	Male	10.75± 6.9	2.1-26.3	T =3.12	<0.01
	Female	16.38± 9.14	4.3- 28.3		
Consanguinity	Yes	12.04± 8.43	3.2-27.5	1.04	>0.05
	No	14.46± 8.73	2.1-28.3		
Family history of liver disease	Yes	9.84± 8.32	2.5-25.1	U=265.5	>0.05
	No	14.57± 8.61	2.1-28.3		

Table (9) Comparison between serum autotoxin and liver biopsy.

Liver biopsy	Autotoxin (ng/ml)		F Test	p	
	Mean ± SD	Range			
Fibrosis index (FI)	No fibrosis (0/6)	17 ± 3.9	2.1-23.8	3.48	<0.05
	Mild fibrosis (1-2/6)	20.7 ± 5.4	10.2-28.1		
	Moderate fibrosis (3-4/6)	26.4 ± 4.4	18.1-30.3		
Hepatitis activity index (HAI)	Minimal	17.52±5.6	10.2-27.4	6.77	<0.01
	Mild	20.03±4.1	10.7-27.8		
	moderate	24.38± 4.3	18.1-30.3		
	Negative	12.84 ±5.2	10.2-27.4		
Interface hepatitis	1\4	17.80 ±5	11.2-18.4	5.63	<0.01
	2\4	27.89 ±4.3	18.1-28.3		
Type of cells	Plasma cells	17.7200± 6.02	11.5-24.6	2.21	>0.05
	Esinophils	18.8200±5.36	12.20-25.1		
	Mononuclear	16.6769±5.37	10.2-26.3		
	Giant cells	19.3000±11.45	11.2- 27.4		
	Lymphocytes	21.7680±4.68	10.7-28.3		

Table (10) Correlation between serum autotoxin and some laboratory parameters.

Variables	Autotoxin (ng/ml)	Test	P value
Hb(g/dL)		r= -0.302	<0.01
TLC( $\times 10^3$ $\mu$ L)		r= 0.035	>0.05
platelet( $\times 10^3$ $\mu$ L)		r= -0.319	<0.01
total protein(g/dL)		r= -0.307	<0.01
Albumin(g/dL)		r= -0.025	>0.05
PT (Second)		r= 0.329	<0.001
PTT(Second)		r= 0.241	<0.05
INR		r= 0.408	<0.001
AST(IU/L)		rho= 0.650	<0.001

**Table (10) Continue**

AIT(IU\L)		rho= 0.388	<0.001
GGT(IU\L)	rho= 0.453	<0.001	
ALP(IU\L)		rho= 0.652	<0.001
<b>Total Bil.(mg\dl)</b>		rho= 0.582	<0.001
<b>Direct Bil.(mg\dl)</b>		rho= 0.351	<0.01

**Table (11)** Correlation between serum autotoxin and liver scoring systems, APRI, FIB4, liver span and spleen size.

Variables	Autotoxin (ng/ml)	Test	P value
<b>Liver span</b>		r= 0.520	<0.001
<b>Spleen size</b>		r= 0.518	<0.001
<b>MELD score</b>		r= 0.429	>0.05
<b>PELD score</b>		rho= -0.026	>0.05
<b>APRI</b>		r= 0.261	<0.05
<b>FIB4</b>		r= 0.286	<0.05

**Table (12)** Performance of serum autotoxin in assessment of fibrosis.

Fibrosis Stage	Cut off	AUC	Sensitivity%	Specificity %	PPV%	NPV%
<b>F 1\6</b>	10.45ng/ml	0.961	97.6%	93.8%	81.6	96.8
<b>F 3\6</b>	18.15ng/ml	0.984	85%	93.8%	75	96.8

### 3. Discssion

In the current study, there was statistical significant difference between chronic hepatitis patients and control group regarding weight, weight/ centile, height, height/ centile, body mass index and body mass index/ centile as it was lower in chronic hepatitis group. This data run in accordance with Nunes et al., 2017[8] who studied 131 chronic liver disease patients and found that all anthropometric measurements that include weight, height, body mass index, mid upper arm circumference and triceps skinfold were significantly lower among cases than controls due to poor food intake, disturbances in absorption and digestion of nutritional substances in the gastrointestinal tract and impaired hepatic synthesis of energy substrates and these abnormalities gradually affect their anthropometric parameters. Also Younossi et al., 2018[9] who assessed weight, height and body mass index in adolescents infected with HCV before and after treatment with ledipasvir/ sofosbuvir and found that the patients had poor weight, height and body mass index before treatment and there is improvement after treatment. Also Murray, 2017[10] who studied 12 child infected with HCV and found that the children infected with HCV has low weight, height and body mass index due to malnutrition and growth retardation.

In the current study, there was statistical significant difference between chronic hepatitis patients and control group regarding HB, platelet, total protein as they were lower in chronic hepatitis group than control group, while PT, INR, AST, ALT, GGT, ALP and bilirubin (total& direct) were higher in chronic hepatitis group

than control group. However, there was no statistical significant difference among chronic hepatitis group and control group regarding TLC, albumin and PTT. These results were in agreement with Abu-Taleb et al., 2019 [11] who found that there was elevation in AST, ALT, GGT and bilirubin but HB was low. Also Ahmed et al., 2018 [12] who studied 771 patient with chronic liver disease for evaluating the stage of fibrosis and found that there was elevation in AST, ALT, INR and ALP but platelets and albumin were decreased with increasing fibrosis stage. Also Tsai et al., 2015 [13] who studied 144 positive anti-HCV child and 168 anti-HCV-negative child and found that platelets were lower in positive anti-HCV children than anti-HCV negative children but there was elevation in AST, ALT and bilirubin in positive anti-HCV children.

ALT and AST levels increase with the progression of liver disease as ongoing liver injury increases their release from mitochondria and fibrosis decreases this clearance [14].

In the current study, there was statistical significant difference between chronic hepatitis patients and control group regarding serum autotoxin as it was higher in chronic hepatitis group (mean  $\pm$  SD was 19.65  $\pm$  5.56). These results were in agreement with Yamazaki et al., 2017 [15] who studied serum autotoxin level on 593 chronic hepatitis C patients and found that serum autotoxin levels were significantly higher in patients than in healthy controls. They explained that autotoxin was rapidly taken up by liver sinusoidal endothelial

cells, reduced clearance of autotaxin by the damaged or fibrotic liver may explain the elevated serum autotaxin levels found in patients with liver fibrosis. Also Kremer et al., 2016 [16] who studied serum autotaxin level on 124 child with cholestatic disease and found that serum autotaxin activity was markedly elevated and correlated with itch intensity in children with cholestatic disease. Also Faujimori et al., 2018 [17] who studied 186 patients with non-alcoholic fatty liver disease (NAFLD) and 160 healthy, non-obese individuals were used as controls and found that serum autotaxin levels were significantly higher in patients than in healthy controls. Also She et al., 2018 [18] who studied 20 patient with chronic viral hepatitis B, 20 patient with hepatocellular carcinoma and another 20 healthy controls and found that serum autotaxin levels were significantly higher in patients than in healthy controls and not only chronic viral hepatitis B but also in patients with hepatocellular carcinoma. Also with Pleli et al., 2014 [19] who studied 220 patients with liver cirrhosis and 85 healthy controls and found that that serum autotaxin levels were significantly higher in patients than in healthy controls.

In the current study, there was statistical significant difference between serum autotaxin and sex as it was elevated in female patients. These results were in accordance with Yamazaki et al., 2017 [15] who found that serum autotaxin levels were significantly higher in female patients than male patients as it was related to sex chromosomes. Also Faujimori et al., 2018 [17] who found that that serum autotaxin levels were significantly higher in female patients than male patients in non-alcoholic fatty liver disease (NAFLD). Also with Pleli et al., 2014 [19] found that that serum autotaxin levels were significantly higher in female patients than male patients with liver cirrhosis.

In the present study, there were statistical significant differences between serum level of autotaxin and fibrosis index, hepatitis activity index and interface hepatitis as it increases with increase fibrosis index, hepatitis activity index and interface hepatitis. These findings were in agreement with Yamazaki et al., 2017 [15] who found that there was positive relationship between serum autotaxin levels and fibrosis stages in chronic hepatitis C patients as it was increase with fibrosis severity. Also Wunsch et al., 2016 [20] who found that there was increasing in serum autotaxin levels with severity of liver fibrosis in children with cholestatic liver disease. Also Ando et al., 2018 [21] who found that there was increasing in serum autotaxin levels with severity of liver fibrosis and liver stiffness in chronic hepatitis C patients.

In the current study, there was positive correlation between serum autotoxin and liver enzymes (PT, PTT, INR, AST, ALT, GGT, ALP and bilirubin (total & direct)), liver span, spleen size, APRI and FIB4 while there was negative correlation between serum autotaxin and hemoglobin, platelet and total protein. But there was no statistical significant correlation between serum autotoxin and total leucocytic count and albumin. These

results were in agreement with Faujimori et al., 2018 [17] who found that serum autotaxin was significantly and positively correlated to the factors of age, weight, AST, APRI, FIB4, ballooning grade, fibrosis stage and duration of the disease in patients with non-alcoholic liver disease. Also Wunsch et al., 2016 [20] who found that serum autotaxin was significantly and positively correlated to age, duration of the disease, ALP and AST concentrations as well as with INR values, platelet counts, hemoglobin levels, APRI and FIB4 in children with cholestatic liver disease. Also Yamazaki et al., 2017 [15] who found that autotaxin was significantly positively correlated to AST, ALT, GGT, AFP, APRI and FIB4 while significantly negatively correlated to albumin and platelet count in chronic hepatitis C patients.

Autotaxin was metabolized by liver sinusoidal endothelial cells so in liver fibrosis, there was retarded degradation of circulating ATX due to liver sinusoidal endothelial cell dysfunction from liver fibrosis was considered a main mechanism [22].

In the current study, the best cut off value of serum autotaxin was 10.45ng/ml for detection of early fibrosis stage with sensitivity 97.6%, specificity 93.8% and area under the curve (AUC) is 0.961 while the best cut off value of serum autotaxin for detection of moderate fibrosis was 18.15ng/ml with sensitivity 85%, specificity 93.8% and AUC is 0.984. These results were in agreement with Yamazaki et al., 2017 [15] who found that there was elevation in serum autotaxin level with increasing fibrosis stage in chronic hepatitis C patients with the best cut off value of serum autotaxin was 0.9 mg/l for detection of early fibrosis stage and the best cut off value of serum autotaxin for detection of moderate fibrosis was 1.5 mg/dl. Also with Faujimori et al., 2018 [17] who found that there was elevation in serum autotaxin level with increasing fibrosis stage in patients with NAFLD with the best cut off value of serum autotaxin was 0.73 mg/l for detection of early fibrosis stage and the best cut off value of serum autotaxin for detection of significant fibrosis was 1.19 mg/l.

#### 4. Conclusion

Serum autotaxin may be a good non-invasive biomarker for liver fibrosis in Egyptian children with chronic hepatitis.

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