Al-Azhar Med. J . (Medicine) *Vol. 51 (1), January, 2022, 495-506*
DOI: 10.21608/amj.2022.212640
https://amj.journals.ekb.eg/article_212640.html

STUDY OF PENTRAXIN-3 LEVELS IN NONALCOHOLIC FATTY LIVER DISEASE (NAFLD) AMONG EGYPTIAN PATIENTS

By

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ABSTRACT

Background: Pentraxin-3 (PTX-3) (an acute-phase protein) is a member of the long pentraxin protein family. It has been reported that PTX-3 is significantly associated with obesity, metabolic syndrome and cardiovascular diseases.

Objective: To study plasma Pentraxin-3 levels in nonalcoholic fatty liver disease (NAFLD) among Egyptian patients.

Patients and Methods: Fifty Egyptian patients divided into three groups: Group I: 20 non-alcoholic fatty liver disease (NAFLD) patients with Simple Steatosis (non-NASH), Group II: 20 NAFLD patients with Steatohepatitis (NASH), and Group III: 10 healthy subjects as controls (age and sex matched).

Results: Pentraxin-3 was found significantly higher in NASH group than non-NASH group, and also significantly higher in non-NASH group than control group. It was 5.65 (4.1 – 7.15) in NASH, 1.7 (0.85 – 2.5) in non-NASH and 0.85 (0.6 – 1.1) in control group.

Conclusion: Patients with NASH showed increased level of pentrxin-3.

Keywords: NASH, pentrixin-3.

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is a common medical condition worldwide and its prevalence has increased notably in the past few years due to the increases in prevalence of obesity and metabolic syndrome *(Hadizadeh et al., 2017)*. This disease includes a broad range of hepatic disorders from simple fat accumulation in hepatic cells (simple steatosis) to hepatic tissue inflammation and fibrosis (Steatohepatitis; NASH) and finally cirrhosis and even hepatocellular carcinoma *(Pulzi et al., 2011)*.

Nonalcoholic fatty liver disease (NAFLD) is characterized by excessive fat infiltration of the liver in absence of significant alcohol intake or secondary causes for steatosis *(Korean Association for the Study of the Liver. KASL clinical practice guidelines, 2013)*.The incidence of NAFLD is rapidly increasing, with huge clinical and economic burdens *(Younossi and Henry, 2016)*. Development of NAFLD is a complex process that includes genetic susceptibility

and environmental exposures *(Sookoian and Pirola, 2016).*

Pentraxin-3 (PTX-3) (an acute-phase protein) is a member of the long pentraxin protein family *(Kadir et al., 2016)*. It has been reported that PTX-3 is significantly associated with obesity, metabolic syndrome and cardiovascular diseases *(Gurel et al., 2016).*

Pentraxins are proteins formed by 5 monomers that form a ring in radial symmetry. They are a class of pattern recognition receptors. Among pentraxins, the main ones are pentraxin-3, CRP and serum amyloid P component. PTX3 is a long-chain pentraxin considered an acute phase marker produced mainly by endothelial and vascular smooth muscle cells at the site of inflammation. It is also produced by macrophages, fibroblasts, neutrophils, epithelial cells, dendritic cells and other cell types both near and far from the inflammation site *(Zhang et al., 2012).*

PTX3 has been recognized as an independent marker of inflammation associated with various disorders such as atherosclerosis, cancer, respiratory diseases and CNS diseases in which increased levels are related to the risk of the disease or its progression *(Rajkovic et al., 2016).* However the role of PTX3 in the hepatic disorders such as NAFLD needs more clarification.

The present study aimed to study plasma pentraxin-3 level in non-alcoholic fatty liver disease among Egyptian patients.

PATIENTS AND METHODS

The current study was implemented in coordination with the guidelines of the Declaration of Helsinki. Ethical approval was gained according to the recommendations of Ethics Unit, Faculty of Medicine, Al-Azhar University, Cairo, Egypt. The clinical steps and possible adverse events were plainly demonstrated for all candidates. All patients or their legal trustee assigned an informed consent before the enrollment in the study. The study was implemented throughout the period between at Bab El-Sharia University Hospital, Al-Azhar University Hospitals.

Fifty Egyptian patients divided into three groups:

Group I: Twenty NAFLD patients with Simple Steatosis (non-NASH),

Group II: Twenty NAFLD patients with Steatohepatitis (NASH),

Group III: Ten healthy subjects as controls (age and sex matched) were included in the current study.

On other hand, the following patients were excluded from the study patients with other chronic liver disease, Patients with any clinical evidence of hepatic decompensating, Patient on medication known to cause hepatic affection, Current or past consumption of alcohol, Patients with a history of treatment with statins, Patients with other chronic illness, Patients with malignancy or history of malignancy.

All subjects will be subjected to the following: Detailed history taking (with special emphasis on: Age, sex. History of alcohol and drug use. Previous history of chronic liver disease). Full clinical examination including (Measurements of waist circumference and height, body weight, body mass index (BMI) was

calculated according to BMI= weight (kg)/ height2 (m), Abdominal examination and other systems examination). Laboratory investigations including (CBC, ESR, serum creatinine, FBG, TC, TG, LDL-C, HDL-C and CRP. Liver functions tests: ALT, AST, ALP and GGT, serum albumin, PT, INR, serum bilirubin (total and direct). HBsAg, HCV-Ab, ANA and Pentraxin -3 level assessment in serum by ELISA).

Abdominal ultrasound for Grading of Non-Alcoholic Fatty Liver Disease *(Sudhir Navale et al., 2019)*.

Liver biopsy with histopathological examination for patient groups *(Takahashi and Fukusato, 2014)*.

Statistical analysis:

Continuous-normally distributed variables were reported in the form of mean, and standard deviation (SD) and compared by one-way ANOVA test or by Kruskal-Wallis test whereby continuous non-normally distributed data were notified using median and range. Besides that, categorical variables were expressed using number, and percentage and were compared by Chi2 test Correlation analysis was conducted using Spearman's rank correlation coefficient for categorical data. The significance was established when $P< 0.05$. Statistical analysis was performed using SPSS software version 23 for Windows (SPSS Inc., Chicago, IL, USA).

RESULTS

Fifty Egyptian Patients was selected from internal medicine department of Sayed Galal university hospital, inpatient and outpatient clinics. Patients divided into three groups Group I: 20 NAFLD patients with Simple Steatosis (non-NASH), Group II: 20 NAFLD patients with Steatohepatitis (NASH), Group III: 10 healthy subjects as controls (Age and sex matched) were included in the current study. The age of NASH group ranged between 27 – 65 with a Mean ± SD of 52.25 ± 9.82 of them 16 subjects (80.0%) were males & the remaining 4 subjects (20.0%) were females, and the age of non-NASH group and control group ranged between 25 – 65 and 24 – 62 respectively.

Subsequently, there was a statistically significant difference found between the three studied groups regarding weight, BMI and WC ($p< 0.0001$ for each parameter). Similarly, comparison between the three groups regarding the laboratory variables showed a statistically significant difference in ALT, AST, total bilirubin, GTT, CRP, ESR, cholesterol, LDL-C, TG and pentrixin-3.While there was no statistically significant difference found between the three studied groups regarding age, sex, WBC, HB, PLT, PT, INR and HDL-C **(Table 1)**.

The level of ALT and AST was found higher in NASH group than non-NASH group and control group while no statistically significant difference found between Non NASH group and control group regarding ALT and AST levels. The cholesterol, LDL and triglyceride levels were found statistically significant higher in NASH and non-NASH group than the control group but with no statistically significant difference between NASH and non-NASH groups. There was statistically significant difference found between the three studied groups regarding the level of pentraxin-3. Pentraxin-3 was found significantly higher in NASH group than non-NASH group and also significantly higher in non-NASH group than control group **(Table 1)**.

Table (1): Clinical and biochemical characteristics of the study population

Variables \ Groups	NASH group (No. = 20)	Non-NASH group (No. = 20)	Control group (No=10)	P-value
	Mean ± SD/ Median(IQR)	Mean ± SD/ Median(IQR)	Mean ± SD/ Median(IQR)	
Age (years)	52.25 ± 9.82	48.15 ± 10.95	46.30 ± 10.75	0.276
BMI (%)	32.48 ± 3.87	30.33 ± 2.56	25.79 ± 2.56	<0.001
WC (cm)	100.30 ± 5.97	94.20 ± 4.05	89.20 ± 1.55	<0.001
WBC (mic /L)	5550 (4890 – 6345)	5540 (4660 – 6470)	5275 (4800 – 6200)	0.910
HB (g/dl)	12.68 ± 1.37	12.87 ± 1.39	13.07 ± 1.00	0.740
PLT (mic /L)	27.25 (19 – 34.9)	27.25 (19 – 34.75)	29.75 (24 – 37)	0.594
PT (Seconds)	12.66 ± 0.63	12.77 ± 0.54	12.72 ± 0.60	NS
INR	0.97 ± 0.16	0.98 ± 0.12	0.95 ± 0.13	NS
ALT (IU/L)	62.55 ± 13.31	33.45 ± 3.80	27.10 ± 5.36	<0.001
AST (IU/L)	55.75 ± 12.09	29.05 ± 3.72	24.40 ± 5.58	<0.001
BIL.T (mg/dl)	0.95 ± 0.22	0.82 ± 0.21	0.62 ± 0.22	<0.001
BIL.D (mg/dl	0.2 (0.2 – 0.3)	0.2 (0.2 – 0.25)	0.2 (0.1 – 0.2)	0.425
ALB (g/dl)	3.96 ± 0.36	4.04 ± 0.43	4.17 ± 0.45	0.405
ALK.P (IU/L	83.60 ± 19.39	74.50 ± 16.10	67.10 ± 11.91	0.039
GGT (IU/L)	56.5 (43 – 69.5)	46 (36 – 55.5)	21.5 (18 – 24)	<0.001
CRP (mg/L)	4.7 (3.95 – 5.8)	3 (2 – 4)	2.95 (2 – 4)	<0.001
ESR (mm/hr)	19.77 ± 3.43	15.72 ± 3.36	12.40 ± 4.17	<0.001
CHOL (mg/dl)	276.80 ± 43.90	268.90 ± 35.04	197.1 ± 29.26	<0.001
HDL-C (mg/dl)	44.35 ± 8.80	44.35 ± 8.70	43.30 ± 10.74	0.052
LDL-C (mg/dl)	199.95 ± 37.27	189.95 ± 32.22	154.60 ± 35.87	0.006
TG (mg/dl)	175 (142.5 – 215)	160 (135 – 182.5)	117.5 (105 – 130)	0.021
PENTRAXIN-3 (ng/ml)	5.65 (4.1 – 7.15)	1.7 (0.85 – 2.5)	0.85 (0.6 – 1.1)	<0.001

BMI=body mass index, WC= waist circumference, WBC=white blood cells, HB=hemoglobin, PLT=platelets, PT=prothrombin time, INR=international normalized ratio, ALT=alanine transaminase, AST=aspartate transaminase, BIL T=bilirubin (total), BIL D=bilirubin (direct), ALB=Albumin, ALK P=alkaline phosphatase, GGT=gamma glutamyltransferase, CRP=c reactive protein, ESR=erythrocyte sedimentation rate, CHOL=cholesterol, TG= triglyceride,, LDL= low density lipoprotein, HDL= high density lipoprotein

As regard U/S results for grading of Non-Alcoholic Fatty Liver Disease it show that in NASH group 6 patients (30%) grade 1, 8 patients (40%) grade 2 and 6 patients was grade 3.on other hand all non-NASH patients was grade 1 by U/S **(Table 2)**.

Table (2): U/S results in the three studied groups

U/S \ Groups	NASH		Non NASH		Control group	
	No.	%	No.	%	No.	%
Grade 1	6	30.0%	20	100.0%	0	0.0%
Grade 2	8	40.0%	0	0.0%	0	0.0%
Grade 3	6	30.0%	0	0.0%	0	0.0%
Normal	0	0.0%	0	0.0%	10	100.0%

Comparison between NASH group and non-NASH group regarding histopathological results show that in NASH group 6 patients (30%) was stage 1,6 patients (30%) stage 2, 2 patients (10%) stage 3 and 6 patients (30%) was stage 4. Also histopathology of Non-NASH group show that 9 patients (45%) was stage 0, 7 patients (35%) stage 1 and 4 patients was stage 2 **(Table 3)**.

Groups / Histopathology	NASH No.= 20	Non NASH No.= 20	P- value
Stage 0	0 (0.0%)	9 (45.0%)	0.000
Stage 1	6 (30.0%)	7 (35.0%)	0.735
Stage 2	6 (30.0%)	4 (20.0%)	0.465
Stage 3	2 (10.0%)	0 (0.0%)	0.146
Stage 4	6 (30.0%)	0 (0.0%)	0.007

Receiver operating characteristic curve (ROC) was constructed to assess accuracy of pentraxin-3 level to detect non-NASH group, it shows that the best cut off point found between control group and non-NASH group regarding pentraxin 3 was found > 1.3 with sensitivity of 55%, specificity 100% and area under curve (AUC) of 74.7% **(Table 4 and Figure 1)**.

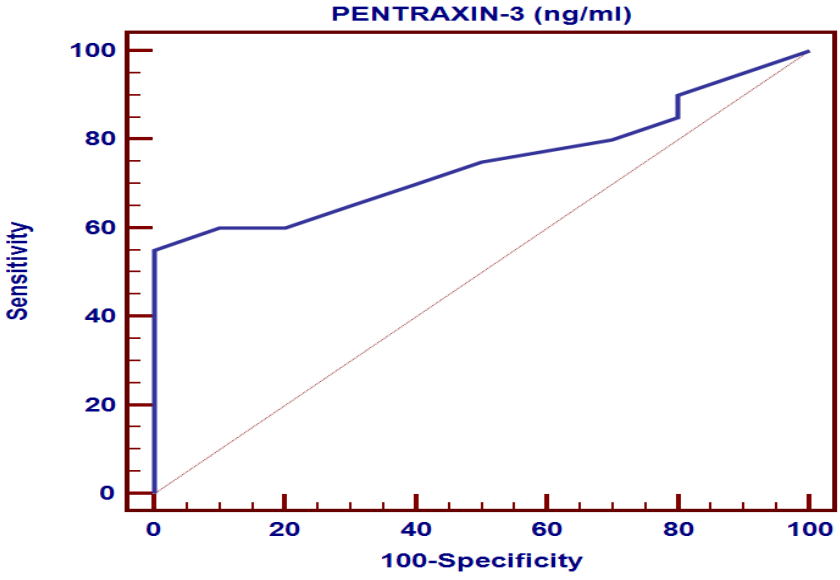

Figure (1): Receiver operating characteristic curve (ROC) was constructed to assess accuracy of pentraxin-3 level to detect non-NASH group

Table (4): Sensitivity, specificity and cutoff value of pentrixin-3 to detect non-NASH group

Cut off point	AUC	Sensitivity	Specificity	+PV	-PV
>1.3	0.747	55.00	100.00	100.0	52.6
AUC= area under curve, **PPV**= positive predictive value, **NPV**= negative predictive value.					

Receiver operating characteristic curve (ROC) was constructed to assess accuracy of pentraxin-3 level to detect NASH group, it shows that the best cut off point found between non-NASH and NASH groups regarding pentraxin 3 was found >3.2 with sensitivity of 95%, specificity 100% and area under curve (AUC) of 99.4% **(Table 5 and Figure 2)**.

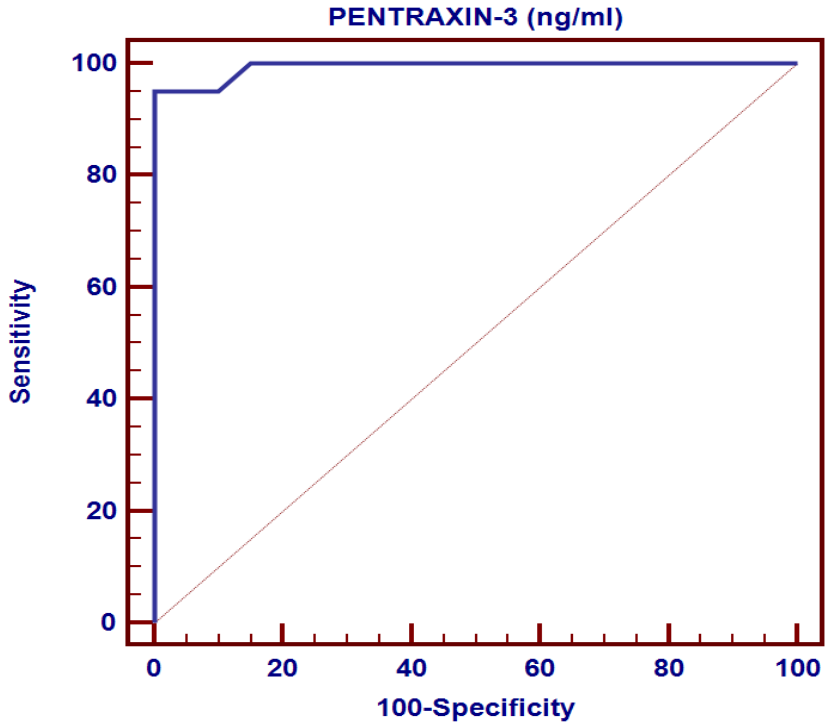

Figure (2): Receiver operating characteristic curve (ROC) was constructed to assess accuracy of pentraxin-3 level to detect NASH group

Table (5): Sensitivity, specificity and cutoff value of pentrixin-3 to detect NASH group

Cut off point	AUC	Sensitivity	Specificity	+PV	-PV
>3.2	0.994	95.00	100.00	100.0	95.2
AUC= area under curve, **PPV**= positive predictive value, **NPV**= negative predictive value.					

Pentraxin-3 level was positively correlated with weight, BMI, WC, ALT, AST, total bilirubin, GGT, CRP, ESR, cholesterol, LDL and TG, while no statistically significant correlation found between pentraxin-3 and the other studied parameters **(Table 6)**.

Table (6): Correlation of penttraxin-3 level with the other studied parameters in all the studied cases

Pentraxin (ng/ml) / Parameters	r	P- value
Age (Y)	0.110	0.446
Wt (kg)	0.761**	<0.001
Ht (m)	-0.211	0.142
BMI (%)	0.825**	<0.001
WC (cm)	0.666**	<0.001
WBC (per /mic L)	0.060	0.678
HB (g/dl)	-0.134	0.353
PLT (per /mic L)	0.037	0.796
PT (Seconds)	-0.084	0.560
INR	0.054	0.713
ALT (IU/L)	0.817**	<0.001
AST (IU/L)	0.800**	<0.001
BIL.T (mg/dl)	0.381**	0.006
BIL.D (mg/dl)	0.156	0.280
ALB (g/dl)	-0.213	0.138
ALK.P (IU/L)	0.212	0.140
GGT (IU/L)	0.525**	<0.001
CRP (mg/L)	0.790**	<0.001
ESR (mm/hr)	0.810**	<0.001
CHOL (mg/dl)	0.693**	<0.001
HDL-C (mg/dl)	0.026	0.860
LDL-C (mg/dl)	0.627**	<0.001
TG (mg/dl)	0.670**	<0.001

DISCUSSION

The evidence obtained in the current study showed that Pentraxin-3 was found significantly higher in NASH group than non-NASH group, and also significantly higher in non-NASH group than control group. Our results were concomitant with previous studies. the study of *Boga et al. (2015)* demonstrated that markedly higher PTX3 levels in NAFLD patients compared with controls, *Hamza et al. (2016)* showed increased PTX3 in patient with NAFLD, and *Afifi et al. (2018)* notified that serum PTX3 level was higher in NAFLD group than control group.

Our study showed a significant association between pentraxin-3 level and weight, BMI, ALT, AST, CRP, ESR, cholesterol, LDL and TG while no statistically significant correlation found between pentraxin-3 and the other studied parameters.

This study revealed that there was a statistically significant difference between three studied groups regarding ALT and AST. The level of ALT and AST was found higher in NASH group than non-NASH group and control group, while no statistically significant difference found between non NASH group and control group regarding ALT and AST levels. This result was in agreement with that reported by *Sanyal et al. (2015)* who found that NAFLD was significantly

associated with higher alanine aminotransferase (ALT) and gamma-glutamyl transferase (GGT), but not ALP levels in impaired glucose tolerance and T2DM patients.

Our study showed that there was a statistically significant difference found between the three studied groups regarding CRP, ESR, weight, BMI and WC. While no statistically significant difference found between the three studied groups regarding height of the studied patients. *Rui Fan et al. (2018)* found that, the participants with fatty liver had higher BMI values. Their study showed that higher BMI (overweight/obesity) was significantly associated with fatty liver risk, among which the risk of fatty liver in overweight population was 3.55 times that of the normal population, and the obese population was 7.59 times that of the normal population. Furthermore, in dose-response analysis, BMI was statistically significantly associated with fatty liver risk in a nonlinear fashion.

Our study showed that the stage of histopathological results was found higher in NASH group than non-NASH group. In the study of *Boga et al. (2015)*, plasma PTX3 level are higher in patients with NASH than in those with a more benign form of NAFLD, namely non-NASH. Also, higher plasma PTX3 levels were associated with a higher fibrosis grade.

The current study showed that there was no statistically significant difference found between the three studied groups regarding HDL, while there was a statistically significant difference found between them regarding cholesterol, LDL and triglyceride levels. The cholesterol, LDL and triglyceride levels were found statistically significant higher in NASH and non-NASH group than the control group. *GUREL et al. (2016)* stated that PTX3 levels were positively correlated TG levels, but no significant correlation between PTX3 levels and others in their study.

CONCLUSION

Patients with NASH showed increased pentrixin-3 levels. Also, non-NASH group showed increased pentrixin-3 levels compared to control group.

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دراسة مستوى البنتراكسين-3 فى المرضى المصريين المصابين بالتدهن الكبدى الغير كحولى

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خلفيــة البحــث: يعتبــر البنتراكســين3 بــروتين حــاد المرحلــة وهــو عضــو مــن عائلــة النتراكســين طويــل البــروتين، وقــد تــم الإخبــار بــأن البنتراكســين3 يــرتبط بشــكل ملحــوظ مــع الســمنة ومتلازمــة التمثيــل الغــذائي وأمــراض القلب والأوعية الدموية.

الهــدف مــن البحــث: دراســة مســتوى البنتراكســين3 فــي المصــل لــدى الرضى المصريين المصابين بالتدهن الكبدي الغير كحولي.

المرضــى وطــرق البحــث: أجريــت هــذه الدراســة علــى خمســين مريضــا مــن المرضــى المصــريين وتــم تقســيمهم إلــى ثــلاث مجموعــات . المجموعــة(1) مكونــة مــن 20 مــريض مــن مرضــى التــدهن الكبــدي الغيــر كحــولي ذوي التــدهن البســيط والمجموعــة(2) مكونــة مــن 20 مــريض مــن مرضــى التــدهن الكبــدي الغيــر كحــولي ذوي الإلتهــاب الكبــدي التــدهني والمجموعــة (3) مكونــة مــن 10 أشــخاص أصــحاء كمقــيلس للتحكــيم (وتــم التنــاظر بــين السن والجنس).

نتــائج البحــث: وجــد أن مســتوى البنتراكســين 3 أعلــى بصــورة ملحوظــة فــي مجموعــة المرضــى المصــابين بالإلتهــاب الكبــدي التــدهني أكثــر مــن مجموعــة المرضــى الغيــر مصــابين بالإلتهــاب الكبــدي التــدهني، وبصــورة ملحوظــة أيضــا وجــد أنــه أعلــى فــي مجموعــة المرضــى الغيــر مصــابين

بالإلتهاب الكبدي التدهني أكثر من مجموعة الأصحاء(مقياس التحكيم). فنسبته كانت 65,5(1,4- 15,7) في مرضى الإلتهاب الكبدي التدهني الغير كحولي ، و 7,1 (85, - 5,2) في المرضى الغير مصابين بالإلتهاب الكبدي التدهني، و 85,0(6,0 – 1,1) في مجموعة وسطاء التحكيم.

الاستنتاج: تبين من خلال الدراسة أن المرضى المصابين بالإلتهاب الكبدي التدهني يعلو لديهم مستوى البنتراكسين3 بصورة ملحوظة.

الكلمات الدالة: البنتراكسين-3، التدهن الكبدى الغير كحولى.