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Serum Pentraxin 3 Level in Egyptian Patients with Nonalcoholic Fatty Liver Disease and Type 2 Diabetes

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Keywords:

Non-invasive diagnosis of NAFLD, Pentraxin, Non Alcoholic Fatty Liver disease, Type 2 diabetes mellitus.

Abbreviations:

NAFLD: Nonalcoholic fatty liver disease; T2DM: Type 2 diabetes mellitus; PTX3: Pentraxin 3; MAFLD: Metabolic dysfunction-associated fatty liver disease; BMI: Body mass index

1. Abstract

1.1. Background: Nonalcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver injury and is strongly associated with type 2 diabetes mellitus (T2DM). The classical gold standard for diagnosing NAFLD is liver biopsy. However, liver biopsy has significant limitations. Pentraxin 3 (PTX3) is an acute-phase reactant and an essential component of innate immunity. According to some research, plasma PTX3 level is a marker for NAFLD. In addition, higher PTX3 serum levels were found in patients with T2DM compared with those with normal blood glucose. We aimed to assess the value of serum Pentraxin 3 level as a non-invasive biomarker of NAFLD in patients with and without type 2 DM.

1.2. Results: Pentraxin 3 at cut-off value >2.3 ng/mL (that predicts NAFLD in DM patients) shows a sensitivity of 87.5%, specificity of 93.75%, Positive Predictive Value of 93.3%, and Negative Predictive Value of 88.2% with an accuracy of 87.8%. Pentraxin 3 at cut-off value > 2.05 ng/mL (that predicts NAFLD patients) shows a sensitivity of 87.5%, specificity of 96.87%, Positive Predictive Value of 96.6%, and Negative Predictive Value of 88.6% with an accuracy of 92.9%.

1.3. Conclusion: Pentraxin 3 is a sensitive and specific promising biomarker for the detection of liver injury in NAFLD when suspected in patients with and without DM.

2. Introduction

Non-alcoholic fatty liver disease (NAFLD) is defined as the ectopic accumulation of fat in the liver (hepatic steatosis) in the absence of other causes of secondary liver fat accumulation (such as excessive alcohol consumption, certain drugs, and viruses). [1] However, large population-based studies clearly demonstrate that, despite considerable phenotypic heterogeneity, most diseases currently designated as NAFLD are related to so-called metabolic factors including overweight, visceral obesity, insulin resistance, and dyslipidemia. In response to this, it has recently been termed metabolic dysfunction-associated fatty liver disease (MAFLD). [2] With the rising prevalence of obesity and metabolic syndrome, NAFLD is quickly becoming the most common liver disease worldwide. [3] The global prevalence of NAFLD in the general population is estimated to be around 29%. [4] South America (35.3%) and the Middle East (31.8%) have the highest prevalence of NAFLD. [5] NAFLD is strongly linked to metabolic syndrome and type 2 diabetes. It has been reported that up to

70% of T2DM patients have NAFLD. [6] The classical gold standard for diagnosing and staging NAFLD is liver biopsy. [7] However, due to the significant limitations of liver biopsy, such as pain, the risk of severe complications, sampling error, cost, and patient unwillingness to undergo invasive testing, [8] the importance of using simple non-invasive diagnostic and prognostic biomarkers has emerged. Many studies demonstrated that serological markers are beneficial as non-invasive tools for the diagnosis of NAFLD. [9] Pentraxin 3 is an acute-phase reactant and an essential component of innate immunity. PTX3 shares structural and functional homology with C-reactive protein. [10] According to mounting evidence, the pathophysiology linking obesity to insulin resistance and metabolic syndrome includes inflammatory pathways. [11] Although hepatocytes do not express PTX3, hepatic progenitor cells isolated from the livers of human patients who had fractional hepatectomy express PTX3 at levels 20 times higher than essential hepatocytes, implying that several cells in the liver tissue produce PTX3. [12] Thus, elevated liver PTX3 is a potential biomarker of primary local inflammation and severe histological liver damage. [13] As NAFLD is a chronic inflammatory condition, plasma PTX3 levels may serve as a marker for the disease. [14] Patients with T2DM had higher serum PTX3 levels than those with normal blood glucose levels. [15]

We aimed to assess the value of serum Pentraxin 3 level as a non-invasive biomarker of NAFLD in patients with and without type II DM, as both NAFLD and type 2 diabetes are major health issues in all the whole world including Egypt.

3. Materials and Methods

This case-control study included 96 subjects (aged between 25-55 years) who presented to the GIT clinic and internal medicine clinic between June 2021 and June 2022 and written informed consent was obtained from all participants before recruitment. Participants were divided into 32 patients with T2DM and NAFLD, 32 patients with T2DM without NAFLD, and 32 healthy subjects without DM or NAFLD with normal liver enzymes.

Subjects with any amount of alcohol consumption or history of alcohol consumption or steatogenic medications (amiodarone, valproic acid, corticosteroids and tetracyclines), patients known to have acute or chronic decompensated liver disease, patients with acute or chronic inflammatory or infectious processes that might increase plasma PTX3 levels, such as asthma, vasculitis, or autoimmune rheumatic disease and devastating diseases and any patient on any medication that affects serum Pentraxin 3 level as (amiodarone, diltiazem, tamoxifen, statins and glucocorticoids) were excluded. NAFLD patients were diagnosed based on a history, physical examination, abdominal ultrasonography, Fibroscan with controlled attenuation parameter (CAP), and Fatty Liver Index. All studied subjects underwent a detailed Full history taking, clinical examination with stress on weight and height which were measured in light clothing without shoes, waist circumference, and body mass index (BMI) which was calculated by dividing the weight by the square of the height (kg/m2) and laboratory tests including CBC, blood sugar, ALT, AST, Serum creatinine, viral markers (HBsAg, anti-HCV Ab) were assayed using an enzyme immunoassay (EIA) Kit (Abbott, Axyam USA), lipid profile, Fatty liver index score, and a venous blood sample was withdrawn for serum Pentraxin. All blood samples for Pentraxin 3 were collected from an antecubital vein. The blood was drained into a tube containing ethylene diaminetetraacetate and samples were centrifuged for 15 minutes at 1000g. Then the plasma was removed immediately and stored frozen at 80C until analyzed. Plasma PTX3 levels were measured by Human Pentraxin ELISA Kit (Aviscera Bioscience Inc.) using the quantitative sandwich enzyme immunoassay technique. Intra-assay and inter-assay coefficients of variation were 4%–6% and 8%10% respectively. The minimum detectable concentration of PTX3 was 0.02 ng/mL.

Transient elastography (Fibroscan) with controlled attenuation parameter was performed by one experienced operator for all cases for the assessment of liver condition, and patients with hepatic steatosis were divided into three grades according to steatosis grade. [16] Also, Ultrasonography by TOSHIBA SSA-700A (Apilo 5) was performed to assess the liver condition. The Fatty Liver Index (FLI) used waist circumference, BMI, triglycerides, and GGT as predictors of hepatic steatosis, with a model score of 0-100. A score of <30 indicates a minimal risk of hepatic steatosis, while a score of >60 indicates fatty liver disease. Patients in group 1 had a FLI score greater than 30, while group 2 and group 3 had FLI scores less than 60. [17]

4. Statistical Analysis

Data were collected then coded then we used the Statistical Package for Social Sciences (SPSS) version 25. Data was summarized using both frequency (count) and relative frequency (percentage) for categorical data. Standard diagnostic indices including sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic efficacy were calculated as described by Galen. [18] For comparing categorical data, a Chi-square ($\chi 2$) test was performed. The exact test was used instead when the expected frequency was less than 5 [19] and a probability (p) value of less than 0.05 was considered statistically significant.

5. Results

The study was conducted on 96 Egyptian adults (aged between 25-55 years) divided into three groups: 32 patients with T2DM and NAFLD (group 1), 32 patients with T2DM and without NAFLD (group 2), and 32 healthy subjects (group3). Demographic features show that the age of patients in group 1 ranges from 30 – 55 years old (46 ± 6), and male cases represent 62.5% but female cases represent 37.5%. Cases in Group 2 range in age from 25 to 55 years (45 9), with male cases accounting for 59% and female cases accounting for 41%. While in the control group (group 3), the age of cases ranges from 25 – 55 years (43 ± 8), and male cases represent 50%, while female cases represent 50%. In terms of biochemical and molecular parameters, diabetics with NAFLD differed significantly from the

other two groups in terms of lipid profile, while there were significant differences between diabetics without NAFLD and healthy controls as regards cholesterol and LDL (Table 1). Our findings revealed a significant increase in Pentraxin 3 levels in the NAFLD patients, as there were significant differences between diabetic patients and controls and also between diabetics with and without NAFLD (Table 2 and Figure 1). Our results showed that there was no correlation between serum Pentraxin 3 level and degree of steatosis (Table 3). The findings revealed a significant positive correlation between Pentraxin 3 and LDL, Triglycerides, and ALT. In the study, there was also a significant negative correlation between Pentraxin 3 and HDL. No significant correlation was found between Pentraxin3 and other labs and investigations (Table 4). In the receiver operating curve (ROC) between groups 1 and 3 (that predicts NAFLD patients), Pentraxin 3 at the cut-off value > 2.05 ng/mL shows sensitivity 87.5%, specificity 96.87%, Positive Predictive Value 96.6%, and Negative Predictive Value 88.6% with an accuracy of 92.9% and between group 1 and group 2 (that predicts NAFLD in DM patients), Pentraxin 3 at the cut-off value >2.3 ng/mL shows sensitivity 87.5%, specificity 93.75%, Positive Predictive Value 93.3%, and Negative Predictive Value 88.2%. with an accuracy of 87.8% (Table 5 and Figures 2 & 3).

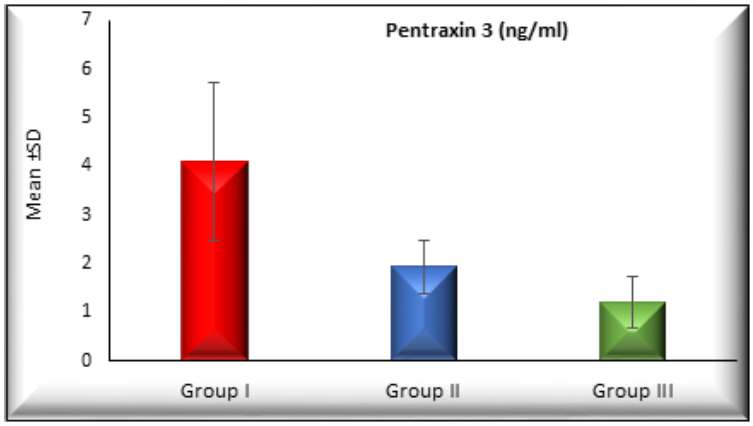

Figure 1: The column chart shows a comparison between mean levels and SD of Pentraxin 3 (ng/ml) in the studied groups. Pentraxin 3 level was higher in group 1 than in group 2 and higher than in group 3.

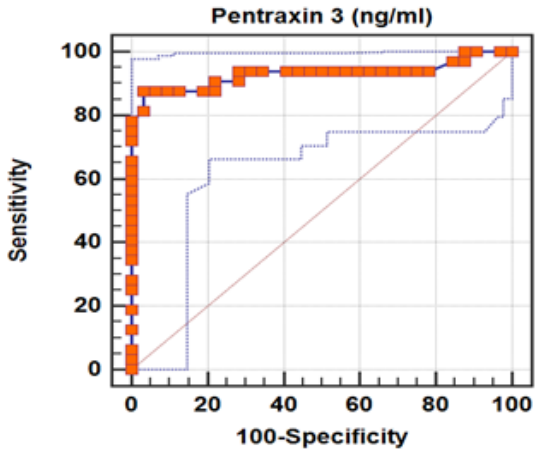

Figure 2: Roc curve of Pentraxin 3 to predict NAFLD patients at the cutoff value > 2.05 ng/mL (that predicts NAFLD patients) shows a sensitivity of 87.5%, specificity of 96.87%, Positve Predictive Value of 96.6%, and Negative Predictive Value of 88.6% with an accuracy of 92.9%.

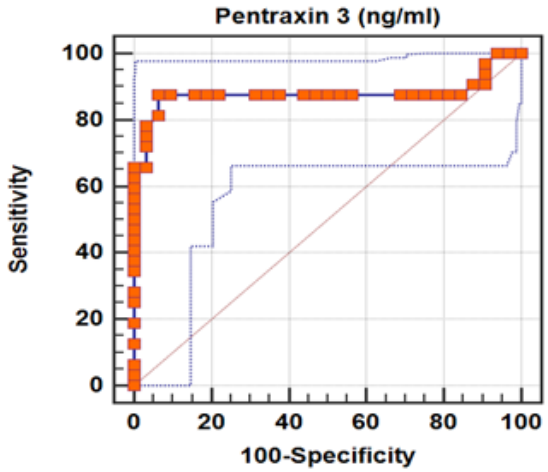

Figure 3: Roc curve of Pentraxin 3 to predict NAFLD in DM patients at the cutoff value >2.3 ng/mL shows a sensitivity of 87.5%, specificity of 93.75%, Positive Predictive Value of 93.3%, and Negative Predictive Value of 88.2% with an accuracy 87.8%.

Table 1: Comparison between the studied groups regarding lipid profiles.

		Groups			ANOVA	
		Group I	Group II	Group III	F	P-value
Cholesterol	Range	99 - 285	123 - 256	88 - 250	27.178	<0.001*
	Mean ±SD	212.156 ± 36.143	185.188 ± 32.442	146.563 ± 38.478		
HDL	Range	32 - 60	35 - 67	36 - 65	11.82	<0.001*
	Mean ±SD	39.906 ± 5.456	47.188 ± 7.99	47.406 ± 7.374		
LDL	Range	55 - 190	65 - 170	34 - 160	35.672	<0.001*
	Mean ±SD	145.344 ± 29.727	110.031 ± 29.498	80.844 ± 32.459		
Trig	Range	99 - 325	78 - 265	89 - 198	9.156	<0.001*
	Mean ±SD	183.625 ± 50.811	155.031 ± 40.424	141.563 ± 24.937		
			TUKEY'S Test			
	I&II		I&III		II&III	
Cholesterol	0.009*		<0.001*		<0.001*	
HDL	<0.001*		<0.001*		0.991	
LDL	<0.001*		<0.001*		<0.001*	
Trig	0.015*		<0.001*		0.0376	

Table 2: Comparison between the studied groups regarding serum Pentraxin 3

Pentraxin 3 (ng/ml)	Groups			ANOVA	
	Group I	Group II	Group III	F	P-value
Range	0.7 - 6.6	0.54 - 3.7	0.5 - 2.5	67.381	<0.001*
Mean ±SD	4.092 ± 1.625	1.934 ± 0.55	1.202 ± 0.525		
	TUKEY'S Test				
	I&II		I&III	II&III	
Pentraxin 3	<0.001*		<0.001*	0.016*	

Table 3: Comparison between different steatosis grade groups regarding Pentraxin 3

		Pentraxin 3		ANOVA	
		N	Mean ± SD	F	P-value
	G1	8	3.955 ± 1.551	3.603	0.148
Fibroscan Steatosis Grade	G2	12	3.334 ± 1.978		
	G3	12	4.691 ± 1.345		

Table 4: Correlation of Pentraxin to investigations.

Correlations	Pentraxin	
	r	P-value
Age	0.16	0.205
BMI	0.147	0.246
WC	0.048	0.705
TLC	0.08	0.529
HB	-0.015	0.909
PLT	-0.058	0.651
RBS	0.03	0.816
FBS	0.212	0.092
HbA1c	0.003	0.979
Cholesterol	0.247	0.052
HDL	-0.352	0.004*
LDL	0.353	0.004*
Trig	0.254	0.043*
PT	0.014	0.912
INR	0.003	0.979
S.ALB	0.085	0.506
AST	0.021	0.869
ALT	0.278	0.026*
GGT	0.269	0.053
T. Bilirubin	0.16	0.206
D. Bilirubin	0.233	0.063
Creat	0.1	0.43
FLI	0.135	0.256

Table 5: Diagnostic performance of Pentraxin 3 in differentiation of patients with NAFLD and without NAFLD

ROC curve between Group I and Group II (predict NAFLD in DM patients)						
	Cutoff	Sens.	Spec.	PPV	NPV	Accuracy
Pentraxin 3 (ng/ml)	>2.3	87.5	93.75	93.3	88.2	87.80%
ROC curve between Group I and Group III (predict NAFLD patients in healthy people)						
	Cutoff	Sens.	Spec.	PPV	NPV	Accuracy
Pentraxin 3 (ng/ml)	>2.05	87.5	96.87	96.6	88.6	92.90%

6. Discussion

NAFLD is the most common cause of chronic liver disease worldwide, as its prevalence is increasing at approximately the same rate as obesity. [20] NAFLD is strongly associated with metabolic syndrome and type 2 diabetes mellitus. [6] The classical gold standard for diagnosing NAFLD is liver biopsy. [7] However, liver biopsy has significant limitations. [8] Pentraxin 3 is an acute-phase reactant and an essential component of innate immunity. [10] As NAFLD is an ongoing inflammatory condition, Plasma PTX3 level could serve as a marker for NAFLD. [14] Boga et al. (2015) demonstrated higher PTX3 levels in NAFLD patients compared with controls (4.1 ± 2.3 vs. 1.3 ± 0.8 ng/mL, P < 0.001). [14] Karamfilova et al., (2022) reported Higher PTX3 serum levels in patients with T2DM compared with those with normal blood glucose (2.32 ± 0.93 vs. 1.88 ± 0.90 ng/mL, P = 0.028). [15] In the present study, in group 1, there were more males with NAFLD (n = 20, 62.5%) than females (n = 12, 37.5%), Sayiner et al. (2016) reported that several studies provide data to suggest a higher prevalence of NAFLD in males. [21] Regarding the Age and sex, there were no statistically significant differences between the studied groups which means that both age and sex were matched. Our results showed significant differences in lipid profile (cholesterol, HDL, LDL, and triglycerides) between diabetics with NAFLD and the other two groups. Furthermore, there were significant differences in cholesterol and LDL between diabetics without NAFLD and healthy controls, which was in agreement with Peng et al. (2017) who found a significant positive association between dyslipidemia and NAFLD in adult males. [22] This study found a significant increase in Pentraxin 3 levels in NAFLD patients, with Pentraxin 3 levels higher in diabetics with NAFLD than in diabetics without NAFLD and in healthy controls. (4.1 ± 1.6 vs. 1.9 ± 0.6 vs 1.2 ± 0.5 ng/mL, P < 0.001), and that was in agreement with the previous studies which investigated serum Pentraxin 3 as a noninvasive marker of NAFLD. Boga et al. (2015) found higher PTX3 levels in NAFLD patients compared with controls (4.1 ± 2.3 vs. 1.3 ± 0.8 ng/mL, P < 0.001) [14] and in agreement with Trojak et al. (2019) who reported median PTX3 level was 4.264 ng/ml in diabetic patients with NAFLD and 3.773 ng/ml in diabetic patients without NAFLD (P = 0.93). [23] Also PTX 3 level was higher in diabetics without NAFLD than in healthy (1.9 ± 0.6 vs 1.2 ± 0.5 ng/mL, P = 0.016) and that was in agreement with Karamfilova et al., (2022) who reported higher PTX3 serum levels in patients with T2DM compared with those with normal blood glucose (2.32 ± 0.93 vs. 1.88 ± 0.90 ng/mL, P = 0.028). [15] Regarding the correlation between serum PTX 3 level and degree of steatosis, we didn't find a correlation and that was in agreement with Maleki et al. (2014) who investigated Pentraxin 3 in 32 NAFLD cases and 34 controls and liver biopsy was performed for all cases. They concluded that Pentraxin 3 had no efficacy in differentiating different grades of NAFLD. [24] Our results showed a significant positive correlation between Pentraxin 3 and LDL, Triglycerides, and ALT in the study (P = 0.004, 0.043, and 0.026), and showed a significant negative correlation between Pentraxin 3 and HDL (P = 0.004). However, there was no significant correlation found between Pentraxin3 and other labs. These findings were comparable to those reported by Hussein et al. (2022) who reported that Pentraxin 3 level was positively correlated with weight, BMI, WC, ALT, AST, total bilirubin, GGT, cholesterol, LDL, and TG (P < 0.001), while no statistically significant correlation was found between pentraxin 3 and the other studied parameters. [25] On the other hand, the study by Albitar et al. (2019) reported that among the NAFLD patients, there were no correlations between PTX3 and various parameters, including anthropometric parameters. [26] In this study, Roc curve analysis of Pentraxin 3 between group 1 and 3 as a diagnostic test of NAFLD suggested that at the cut-off value > 2.05 ng/mL (that predicts NAFLD patients) shows sensitivity 87.5%, specificity 96.87%, Positive Predictive Value 96.6% and Negative Predictive Value 88.6% with Accuracy 92.9% (Figure 2) and between group 1 and group 2 as a diagnostic test of NAFLD in diabetic patients, Roc curve analysis suggested that at the cut-off value >2.3 ng/mL (that predicts NAFLD in DM patients) shows sensitivity 87.5%, specificity 93.75%, Positive Predictive Value 93.3% and Negative Predictive Value 88.2% with an accuracy of 87.8% (Figure 3). These findings were comparable to those reported by Boga et al. (2015) who reported that The optimal cutoff value for the diagnosis of NAFLD was 2.45 ng/mL with a sensitivity of 91.1%, specificity of 71.4%, PPV of 76.1%, and NPV of 88.9%.and at the cutoff value of 3.43 ng/ mL, the level of specificity was 95% and sensitivity was 68%. [14]

7. Conclusion and Recommendations

Plasma Pentraxin 3 level is a simple, efficient, sensitive, specific, and applicable screening method for early diagnosis of NAFLD in diabetic and non-diabetic patients. The sensitivity and specificity of the Pentraxin 3 have been demonstrated by our study. We recommend using Pentraxin 3 to screen for NAFLD in the general population as well as diabetic patients but with a higher cutoff level. We recommend additional research in larger study populations and different settings to confirm our findings.

8. Declarations

8.1. Ethics Approval and Consent to Participate

The Research Ethics Committee of the Faculty of medicine, Ain Shams University approved the study (FWA 000017585) in June 2021. All patients enrolled for the validation of this study gave a written informed consent.

8.2. Consent for Publication

Written informed consent for publication of their clinical details and/or clinical images was obtained from the patient/parent/guardian/ relative of the patient.

8.3. Availability of Data and Material

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

8.4. Competing Interests

The authors declare that they have no competing interests.

8.5. Funding

The authors have nothing to disclose regarding any financial support.

8.6. Acknowledgements

The authors stated no acknowledgement.

8.7. Authors' Contributions

All authors have seen and approved the content of the final manuscript and have contributed significantly to the work.

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