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Plasma pentraxin-3 is associated with endothelial dysfunction in non-alcoholic fatty liver disease

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Abstract. – OBJECTIVE: Pentraxin-3 (PTX-3) is an acute-phase protein belonging to the PTX family. It has been reported that PTX-3 is significantly associated with obesity, metabolic syndrome, and cardiovascular diseases (CVD). Non-alcoholic fatty liver disease (NAFLD) is strongly associated with atherosclerosis and CVD. In this study, we aimed to investigate the relationship of PTX-3 with circulating markers of endothelial dysfunction and atherosclerosis in patients with NAFLD.

PATIENTS AND METHODS: Seventy patients with biopsy-proven NAFLD and seventy healthy controls were enrolled in the study. Plasma asymmetric dimethylarginine (ADMA), adiponectin, and PTX-3 levels were determined using enzyme-linked immunosorbent assay (ELISA). High-sensitivity C-reactive protein (hsCRP) serum levels were measured with the immunoturbidimetric assay. Insulin resistance was estimated using the HOMA-IR index.

RESULTS: PTX-3 and hsCRP levels were higher and adiponectin levels were lower in the NAFLD group compared to the healthy controls ($p < 0.001$ for all). In correlation analysis, a significant association was observed between PTX-3 and ADMA levels ($r = 0.423$, $p < 0.001$).

CONCLUSIONS: Our study demonstrated for the first time that increased circulating PTX-3 is strongly associated with endothelial dysfunction in subjects with NAFLD. However, large prospective studies are needed to establish the independent predictive value of PTX-3 for CVD endpoints in this clinically relevant condition.

Key Words
Pentraxin-3, Endothelial dysfunction, Non-alcoholic fatty liver disease

Introduction

Non-alcoholic fatty liver disease (NAFLD) is increasingly diagnosed worldwide and is considered to be the commonest liver disorder in clinical practice[1]. It comprises a spectrum of conditions ranging from simple steatosis (SS) to non-alcoholic steatohepatitis (NASH). SS is benign, whereas NASH is characterized by hepatocyte injury, inflammation, and fibrosis, which can lead to cirrhosis, liver failure, and hepatocellular carcinoma[2]. The pathogenesis of NAFLD is thought to be related to insulin resistance and oxidative stress. Accumulating evidence indicates that NAFLD is associated with obesity and type 2 diabetes mellitus (T2DM) and may serve as a predictor of cardiovascular diseases (CVD)[3]. The possible mechanisms linking NAFLD and CVD include inflammation, oxidative stress, hyperlipidemia, and insulin resistance[4].

There is increasing evidence that the pathophysiology linking obesity and insulin resistance includes inflammatory pathways. Hence, several proteins have been identified in these inflammatory pathways related specifically to excess adiposity[5]. Pentraxin-3 (PTX-3) and C-reactive protein (CRP) are members of the pentraxin superfamily. CRP is an acute-phase protein produced by the liver, and its level increases in a variety of infections and immuno-inflammatory diseases[6]. High-sensitive CRP (hsCRP) is clinically useful for differentiating between SS and NASH. Furthermore, it seems that high concentrations of hsCRP are associated with extensive

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liver fibrosis in NASH[7]. PTX-3 is thought to be a specific marker of localized vascular inflammation. Hence, unlike the CRP, PTX-3 is produced at sites of inflammation by cells such as vascular endothelial cells, smooth muscle cells, and macrophages, cells that are directly involved in atherosclerosis. Hence, in line with these experimental findings, circulating PTX-3 levels have been linked to obesity, atherosclerosis, and inflammation in clinical studies[8].

Today, there is very scarce data regarding the role of PTX-3 in the pathogenesis of NAFLD. Moreover, no study to date has investigated the role of PTX-3 between NAFLD, endothelial dysfunction, and atherosclerosis. Thus, in the present study, we aimed to investigate plasma PTX-3 concentrations in subjects with biopsy-proven NAFLD who had no additional disorders such as morbid obesity, T2DM, and hypertension. In addition, the relationship of PTX-3 with adiponectin, asymmetric dimethylarginine (ADMA), insulin sensitivity, and liver histology were also investigated.

Patients and methods

Subjects

A total of 70 male subjects with NAFLD and 70 healthy male individuals participated in the study. The study group was composed of a selected sample of male outpatients recruited from among 270 patients with histologically proven NAFLD who attended the outpatient clinic of our gastroenterology department at the Gulhane School of Medicine, Ankara, Turkey. Inclusion criteria were the following: persistently (at least 6 months) elevated aminotransferases; presence of ultrasonographic brightness in liver without any other liver or biliary tract disease; and liver histology compatible with a diagnosis of NASH or SS. Exclusion criteria were as follows: a history of alcohol consumption of more than 40 g/wk, as assessed by a detailed interview extended to family members; a body mass index (BMI) ≥35 kg/m^2; positive serum markers of viral, autoimmune, or celiac disease; abnormal copper metabolism or thyroid function tests; a diagnosis of diabetes mellitus (fasting plasma glucose ≥126 mg/dL or ≥200 mg/dL at 2 hours on a standard oral glucose tolerance test, OGTT); serum total cholesterol (TC) ≥250 mg/dL; serum triglycerides (TG) ≥400 mg/dL; and exposure to occupational hepatotoxins or drugs known to be steatogenic or to affect glucose and lipid metabolism. The control group was matched for age and consisted of 70 healthy male volunteers with normal liver ultrasonography and normal liver function tests.

All participants provided a detailed medical history and underwent a clinical examination. The anthropometric variables (weight and height) of all the participants were measured with a calibrated scale after the patients had removed their shoes and any heavy clothing. BMI was calculated by dividing weight in kilograms by height in meters squared (kg/m^2). Waist circumference (WC) was measured as the mid-point between the lower costal margin and the level of the anterior superior iliac crests. Blood pressure measurements were obtained using standard manometers.

Written informed consent was obtained from all participants. The study was approved by the local Ethics Committee of the Gulhane School of Medicine, and all participants gave their consent to the study, which was conducted according to the Helsinki Declaration Biological measurements.

Biochemical Analysis

All venous blood samples were collected from an antecubital vein, between 08:00 and 09:00 a.m. after an overnight fast of 12 hours. The blood samples were centrifuged for 15 minutes at 3000 rpm, aliquoted, and immediately frozen at −80 ºC until assessment time. All samples were run in the same assay.

Serum glucose, TC, TG, HDL-C, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), uric acid, bilirubin, and gamma-glutamyl transpeptidase (GGT) levels were measured by the enzymatic colorimetric method via an Olympus AU2700 auto analyzer and reagents (Olympus Diagnostics Hamburg, Germany). Low-density lipoprotein (LDL) cholesterol was calculated using Friedewald's formula[9]. The serum basal insulin levels were measured in duplicate using the chemiluminescence method (Roche Diagnostics GmbH, Mannheim, Germany). Insulin resistance was calculated using the homeostasis model assessment of insulin resistance (HOMA-IR) index[10] using the formula [HOMA-IR = fasting insulin (μU/mL) × fasting glucose (mg/dL)/405]; this index has been shown to be well correlated with the results of the euglycemic–hyperinsulinemic clamp method to determine IR[11]. Low HOMA-IR values indicate high insulin sensitivity, whereas high HOMA-IR values indicate low insulin sensitivity.

PTX-3 levels were determined using the enzyme-linked immunosorbent assay (ELISA) method (Cat. No: CK-E90303, Hangzhou Eastbiopharm Co., Ltd, China) intra-assay coefficient of variation (CV) and inter-assay CV were <8% and <10%, respectively, and assay range was between 0.1 ng/mL and 30 ng/mL with sensitivity of 0.05 ng/mL. Plasma ADMA levels were measured by ELISA (ADMA direct ELISA kit, Immunodiagnostic AG, Bensheim, Germany) [detection limit of ADMA assay = 0.04 μmol/L]. Intra-assay CV ranged from 5.8% to 7.9%, while inter-assay CV ranged from 7.6% to 10.8% for the ADMA assay. Measurements were carried out using an ELISA BioTek Synergy HT plate reader (BioTek Instruments Inc., Winooski, VT, USA). Serum adiponectin levels were also determined using the ELISA method (Human Adiponectin ELISA Kit, Cat. No: E09; Reutlingen, Germany) Intra-assay CV ranged from 2.35% to 4.66%, while inter-assay CV ranged from 5.7% to 6.72% for adiponectin. The minimum detectable concentration for adiponectin was 0.6 ng/mL. Measurements were carried out using an ELISA BioTek Synergy HT plate reader (BioTek Instruments Inc., Winooski, VT, USA). High-sensitivity C-reactive protein (hsCRP) level was determined in serum using the immune turbidimetric fixed rate method with a biochemical auto-analyzer (Olympus AU 2700, Olympus Diagnostics, Hamburg, Germany). Intra-assay CV and inter-assay CV were 5.8% and 3.1%, respectively. The minimum detectable concentration for hsCRP was 0.07 mg/L.

Liver Histology

All liver tissue samples were obtained by the same investigator from the right lobe of the liver through the intercostal route using a 16-gauge semi-automatic percutaneous liver biopsy needle via ultrasound guidance. Mean liver biopsy sample size was 21 ± 5 mm in our study. An experienced hepatopathologist blinded to patient details scored liver biopsy specimens using the classification of Kleiner et al[12]. Patients were subdivided into three histological groups: SS (steatosis in the absence of inflammation and hepatocyte ballooning degeneration), borderline NASH (steatosis with minimal, rare inflammation and hepatocyte ballooning), and NASH (steatosis with inflammation and hepatocyte ballooning, often with fibrosis). Briefly, liver tissues were stained with hematoxylin–eosin (H&E), reticulin, and Gomori trichrome stains and scored. All cases showed macrovesicular steatosis affecting at least 5% of hepatocytes and these were classified as steatosis. In addition to steatosis, the minimum criteria for the diagnosis of steatohepatitis included the presence of lobular inflammation and either ballooning cells or perisinusoidal/pericellular fibrosis in zone 3 of the hepatic acinus. Steatosis was graded on a four-point scale: grade 0, steatosis involving <5% of hepatocytes; grade 1, steatosis involving up to 33%; grade 2, steatosis involving 33% to 66% and grade 3, steatosis involving >66%. Lobular inflammation was graded on a four-point scale: grade 0, no foci; grade 1, less than two foci per 200× field; grade 2, two to four foci per 200× field; grade 3, more than four foci per 200× field. Hepatocyte ballooning was graded from 0 to 2: 0, none; 1, few balloon cells; and 2, many/prominent balloon cells. Liver fibrosis stage was evaluated on a four-point scale: stage 0, no fibrosis; stage 1, perisinusoidal or periportal fibrosis; stage 2, perisinusoidal and portal/periportal fibrosis; stage 3, bridging fibrosis; and stage 4, cirrhosis. NAFLD activity score (NAS), which is the total of steatosis (scale from 0 to 3), lobular inflammation (scale from 0 to 3), and hepatocellular ballooning (scale from 0 to 2) scores, as defined by Kleiner et al[12] Moreover, an overall diagnostic categorization was determined for each case as SS (NAS: 0-2), borderline NASH (NAS: 3-4), or definite NASH (NAS: 5-8).

Statistical Analysis

Results are reported as the mean ± SD and median (min-max). The Kolmogorov-Smirnov test was used to determine the distribution characteristics of variables and Levene's test was used to evaluate the equality of variance. Differences between groups were tested for significance by independent-sample *t*-tests and Mann-Whitney U tests, as appropriate. The relationship between variables was analyzed using Spearman's rho and Kendall's tau-B correlations. Variables that were significantly different between two groups were analyzed using multivariate analysis. Also, multivariate logistic regression analysis was used to assess the association between PTX-3, ADMA, adiponectin, and histopathological findings. The statistical analysis was carried out using Statistical Package for the Social Sciences (SPSS), version 15.0 (SPSS Inc., Chicago, IL, USA). Differences and correlations were considered significant at $p < 0.05$.

Results

The histological features of NAFLD patients are summarized in Table I. According to study results, 10/70 (14.3%) of the patients had SS,

Table I. Histological features of 70 patients with NAFLD.

Histology	
NAS, n (%)	
0-2	10 (14.3)
3-4	20 (28.6)
5-8	40 (57.1)
Lobular inflammation, n (%)	
0	5 (7.1)
1	36 (51.4)
2	29 (41.4)
Steatosis, n (%)	
0	0 (0)
1	17 (24.3)
2	33 (47.1)
3	20 (28.6)
Hepatocellular ballooning, n (%)	
0	10 (14.3)
1	42 (60.0)
2	18 (25.7)
Fibrosis, n (%)	
0	31 (44.3)
1	36 (51.4)
2	3 (4.3)

Data are expressed as the number of cases (%).
NAFLD: Non-alcoholic fatty liver disease, NAS: NAFLD activity score.

20/70 (28.6%) had borderline NASH, and 40/70 (57.1%) had NASH. Demographic characteristics and the laboratory data of these three groups are shown in Table 2. Age, BMI, and WC levels were higher in NAFLD group when compared to the healthy controls ($p < 0.05$). In addition to demographic findings, levels of glucose, lipid parameters, AST, ALT, GGT, uric acid, and HOMA-IR indexes were significantly higher in subjects with NAFLD than the controls ($p < 0.05$). PTX-3 and hsCRP levels were higher and adiponectin levels were lower in the NAFLD group when compared to the healthy controls ($p < 0.001$ for all). This difference remained significant when the findings were adjusted according to the BMI, WC, glucose, lipid levels, and IR. However, no difference was found for PTX-3 levels among the histological subgroups.

The clinical and biochemical characteristics of NAFLD patients with ($n = 39$) and without ($n = 31$) fibrosis are shown in Table III. Age, AST, ALT, total bilirubin, and direct bilirubin levels were higher in subjects with fibrosis in comparison to levels for those without fibrosis. However, there were no statistically significant differences between the two groups regarding PTX-3, hsCRP, ADMA, adiponectin, and the other remaining parameters.

Finally, we also investigated the association between PTX-3 and the other study parameters. Using correlation analysis, PTX-3 was positively correlated with ADMA in subjects with NAFLD ($r = 0.423$, $p < 0.001$) (Figure 1). On the other hand, PTX-3 was not found to be associated with histological findings such as steatosis, ballooning degeneration, lobular inflammation, or fibrosis.

Discussion

In this study examining associations between circulating PTX-3 with liver histology and endothelial dysfunction in subjects with biopsy-proven NAFLD, higher PTX-3 levels were significantly correlated with ADMA, a well-known marker of endothelial dysfunction. To the best of our knowledge, this is the first study to show that PTX-3 is significantly associated with endothelial dysfunction in this clinically relevant condition.

As far as we know, there are only three studies that report circulating PTX-3 levels in subjects with biopsy-proven NAFLD. In the first study, Yoneda et al. investigated the plasma levels of PTX-3 in 70 patients with NAFLD (28 without NASH and 42 with NASH) compared to 10 healthy controls. The plasma PTX-3 levels were significantly higher in the patients with NASH than in the non-NASH and healthy control groups. In addition, plasma PTX-3 was closely associated with the stages of liver fibrosis[13]. Maleki et al[14] analyzed the plasma hsCRP and PTX-3 levels in 32 patients with biopsy-proven NAFLD and 34 healthy controls. The HsCRP levels were higher in subjects with NASH than those without NASH, and HsCRP levels were also increased with higher levels of fibrosis. However, PTX-3 had no efficacy as a tool to differentiate different levels of NAFLD and fibrosis. Lastly, a new study published by Boga et al[15] evaluated plasma PTX-3 levels in 70 patients with histologically verified NAFLD (56 with NASH, 14 without NASH) and 12 healthy controls. PTX-3 levels were significantly higher in subjects with NAFLD when compared to the controls, as well as in the NASH subgroup compared to the non-NASH subgroup. A significant association was observed between PTX-3 NAS and degree of fibrosis. In the present study, plasma PTX-3 levels were found to be significantly elevated in subjects with NAFLD when compared to healthy controls. This difference remained significant when the findings were adjusted according to BMI, WC, glucose, lipid lev-

Table II. Comparison of anthropometric and laboratory features of subjects with SS, borderline NASH, NASH and controls.

Variable	SS (n= 10)	Borderline NASH (n= 20)	NASH (n= 40)	Controls (n= 70)	*p*-value
Age (years)	39 (31-43)	32 (25-37)	34 (28-38)	29 (26-33)	0.001
BMI (kg/m^2)	28.5±3	28.7±2.9	28.6±3	24.4±2.5	<0.001
WC (cm)	99±6	107±7	100±6	86±7	<0.001
FPG (mg/dL)	91±6	94±14	96±9	81±10	<0.001
TC (mg/dL)	203 (182-230)	206 (161-216)	198 (166-233)	174 (154-200)	0.081
TG (mg/dL)	223 (117-372)	168 (110-224)	193 (137-239)	109 (79-157)	<0.001
HDL-C (mg/dL)	37 (33-49)	41 (36-44)	41 (38-45)	43 (38-50)	0.167
LDL-C (mg/dL)	122 (106-148)	140 (99-158)	126 (78-147)	110 (94-133)	0.239
AST (IU/L)	40 (37-51)	44 (35-57)	54 (44-63)	21 (18-25)	<0.001
ALT (IU/L)	67 (51-95)	93 (68-121)	105 (76-130)	21 (15-28)	<0.001
GGT (IU/L)	63 (31-109)	48 (37-69)	64 (50-84)	21 (18-26)	<0.001
Uric acid (mg/dL)	5.9 (5.1-7)	6.2 (5.4-7)	6 (5.5-6.9)	5.1 (4.7-5.8)	<0.001
Total bilirubin (mg/dL)	0.72 (0.57-0.95)	0.76 (0.56-1.05)	0.98 (0.7-1.33)	0.68 (0.51-0.92)	0.006
Direct bilirbin (mg/dL)	0.18 (0.10-0.22)	0.15 (0.10-0.19)	0.15 (0.12-0.26)	0.14 (0.1-0.18)	0.323
Insulin (μU/mL)	18.3 (15.9-27)	11.7 (8-21.4)	14.5 (10.2-21.8)	11.9 (10.2-16.8)	0.046
HOMA-IR	3.84 (3.49-6.89)	3.09 (1.79-4.90)	3.78 (2.61-4.77)	2.44 (1.98-3.25)	0.002
Pentraxin-3 (ng/mL)	6.92 (1.52-16)	6.26 (2.85-13.98)	6.46 (1.32-16)	0.94 (0.87-1.79)	<0.001
ADMA (μmol/L)	0.57 (0.23-0.79)	0.63 (0.37-0.79)	0.72 (0.21-0.79)	0.65 (0.24-0.79)	0.988
Adiponectin (μg/mL)	2.72 (2.10-5.42)	2.48 (2.14-3.52)	3.77 (2.72-4.96)	5.84 (4.33-7.86)	<0.001
hsCRP (μg/mL)	2.49 (1.14-3.90)	1.13 (0.65-2.82)	2.39 (0.79-3.77)	0.46 (0.40-0.60)	<0.001

Data are expressed as the mean ± SD, median (25th-75th interquartile range) or number of cases (%) as appropriate. P values were calculated using Kruskal-Wallis and ANOVA test as appropriate.
NAFLD: non-alcoholic fatty liver disease, NASH: non-alcoholic steatohepatitis, SS: simple steatosis BMI: body mass index, WC: waist circumference, FPG: fasting plasma glucose, TC: total cholesterol, TG: triglyceride, HDL-C: high density lipoprotein cholesterol, LDL-C: low density lipoprotein cholesterol, AST: aspartate aminotransferase, ALT: alanine aminotransferase, GGT: γ-glutamyltransferase, HOMA-IR: homeostasis model assessment of insulin resistance, ADMA: asymmetric dimethylarginine, hsCRP: high sensitive C-reactive protein.

els, and IR. However, no difference was found for PTX-3 levels among the histological subgroups. In addition, no significant association was found between PTX-3 and histological findings. We suggest that the discrepancies in the findings of these studies were likely due to differences in the populations of patients enrolled, specifically with respect to the age and number of participants. Nonetheless, our findings provide further support that circulating PTX-3 levels are elevated in subjects with NAFLD.

NAFLD is significantly associated with subclinical atherosclerosis and endothelial dysfunction independent of the classical cardiovascular risk factors, i.e., obesity and metabolic syndrome (MetS)[15,16]. Hence, a large body of evidence suggests that subjects with NAFLD have a significantly increased CVD risk[17,18]. A general agreement emerging from these studies indicates that patients with NASH are at higher risk of CVD than those with SS, emphasizing the role of chronic inflammation in the pathogenesis of atherosclerosis in NAFLD. However, the pathophysiologic mechanisms underlying the evolution from NAFLD to atherosclerosis and cardiovascular events remain to be determined. Endothelial dysfunction is one of the earliest markers of atherosclerosis and plays an important role in the development of atherosclerotic diseases[19,20]. Endothelial function can be investigated by measuring circulating markers produced by the endothelium such as ADMA, an endogenous competitive inhibitor of nitric oxide synthase; plasminogen activator inhibitor-1, a prothrombotic factor that inhibits fibrinolysis; and

Table III. The clinical and laboratory characteristics of NAFLD patients with and without fibrosis.

Variable	Fibrosis 0 (n =31)	Fibrosis 1-4 (n =39)	*p*-value
Age (years)	32±6	35±8	0.045
BMI (kg/m^2)	28.3±2.9	28.8±3	0.514
WC (cm)	100±6	101±6	0.499
FPG (mg/dL)	95±11	94±11	0.774
TC (mg/dL)	199±33	189±56	0.369
TG (mg/dL)	207±94	193±115	0.576
HDL-C (mg/dL)	39±6	44±16	0.086
LDL-C (mg/dL)	132±67	122±45	0.438
AST (IU/L)	46 (37-54)	51 (42-61)	0.042
ALT (IU/L)	87±30	107±41	0.028
GGT (IU/L)	67±44	68±33	0.936
Uric acid (mg/dL)	5.9±1	6.3±1	0.129
Total bilirubin (mg/dL)	0.74 (0.55-0.98)	0.99 (0.70-1.25)	0.004
Direct bilirubin (mg/dL)	0.15 (0.10-0.19)	0.15 (0.12-0.30)	0.016
Insulin (µU/mL)	16.6±10.2	17.5±12.3	0.742
HOMA-IR	3.9±2.4	4.1±2.8	0.755
PTX-3 (ng/mL)	7.46±6.20	8.40±6.27	0.533
ADMA (µmol/L)	0.51±0.27	0.57±0.26	0.367
ADİPONECTİN (µg/mL)	3.69±1.65	4.50±6.23	0.493
hsCRP (µg/mL)	2.06±1.65	2.82±2.50	0.146

Data are expressed as the mean ± SD, median (25th-75th interquartile range) or number of cases (%) as appropriate. *p*-values were calculated using Student's t-test, Mann Whitney U test and Chi Square as appropriate.
BMI: body mass index, WC: waist circumference, FPG: fasting plasma glucose, TC: total cholesterol, TG: triglycerides, HDL-C: high-density lipoprotein cholesterol, LDL-C: low density lipoprotein cho-lesterol, AST: aspartate aminotransferase, ALT: alanine aminotransferase, GGT: γ-glutamyltransferase, HOMA-IR: homeostasis model assessment of insulin resistance, PTX-3: Pentraxin-3, ADMA: asymmetric dimethylarginine, hsCRP: high sensitive C-reactive protein.

intra-cellular adhesion molecule-1 and vascular cell adhesion molecule-1, both of which reflect low-grade chronic inflammation of the endothelium[21-23].

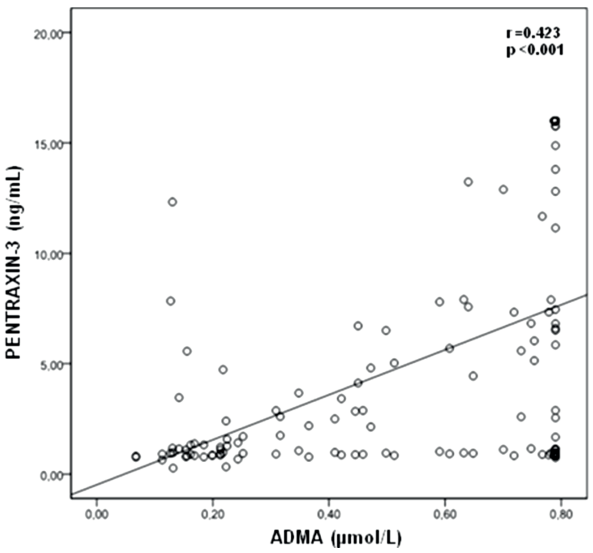

Figure 1. Correlation of PTX-3 with ADMA in patients with NAFLD.

PTX-3, a newly identified acute-phase reactant that resembles CRP both in structure and function[13], has been found to be produced by vascular smooth muscle cells, endothelial cells, and fibroblasts.[24,25] Plasma PTX-3 levels have recently been found to be elevated in patients with acute myocardial infarction, unstable angina pectoris, and heart failure[26,27]. It is also expressed in advanced atherosclerotic lesions and is associated with MetS and CVD risk factors[28]. To the best of our knowledge, this is the first study to evaluate the relationship between PTX-3 and endothelial dysfunction in NAFLD. Our findings showed a significant association between ADMA and PTX-3 levels in male subjects with biopsy-proven NAFLD who have no T2DM, hypertension, and morbid obesity when compared to healthy male controls. The abnormalities in glucose and blood pressure that can potentially affect endothelial function are frequently accompanied by NAFLD[29,30]. Therefore, we think that including only subjects free from these confounding factors is an important feature of the present investigation. Moreover, it is well known that circulating PTX-3 levels are easily affected by these metabolic confounders[30-32].

This study has some limitations. Firstly, because our study was cross-sectional, the causative nature of the associations cannot be established. Further prospective studies should be arranged to clarify the cause-and-effect relationship and test whether quantification of PTX-3 levels could provide additional information beyond the currently recognized risk factors to predict future cardiovascular events in subjects with NAFLD. Secondly, because of the small sample size and the strict inclusion criteria, the findings obtained are not representative for all subjects with NAFLD. However, we think that the design of our work was a requirement for the goals of the study to be achieved. Lastly, even if HOMA-IR had a reasonable linear correlation with the glucose clamp technique and was used in several population studies, another limitation of this work is due to the fact that the HOMA-IR index does not help to clarify whether insulin resistance is central or peripheral.

Conclusions

We report for the first time that elevated circulating PTX-3 in NAFLD is significantly associated with endothelial dysfunction. Our findings suggest that PTX-3 may contribute to atherosclerotic disease progression and enhanced cardiovascular risk in subjects with NAFLD. However, large prospective studies are needed to establish the independent predictive value of PTX-3 for CVD endpoints in this clinically relevant condition.

Conflict of Interests

The Authors declare that they have no conflict of interests.

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