Serum concentrations of Cyclophilin A in patients with Nonalcoholic Fatty Liver Disease

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Abstract

Aim : **Nonalcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver disease and its incidence is rising worldwide. Cyclophilin A (CyPA) is a protein, which is secreted under the presence of oxidative stress and hyperglycemia, and it plays role in proinflammatory signal reduction. In this study we investigated serum levels of CyPA in patients with biopsy proven NAFLD and examined their association with clinical and histological phenotypes.**

Methods : **In this study, we identified serum levels of CyPA in patients with NAFLD (n=52) and healthy controls without evidence of any liver disease (n=44). The levels of CyPA were measured by enzyme-linked immunosorbent assay and were compared between two study groups. Furthermore, serum levels of CyPA were assessed in relation to the clinical characteristics of the study participants.**

Results : **Serum levels of CyPA were significantly higher in patients with NAFLD (3,8±2,6 µg/ml, P=0.03) compared to healthy controls (2,8±1,8 µg/ml). Moreover, concentrations of CyPA were 2,8±1,8, 3,4±2,3, and 4,2±2,9 µg/ml in control group, non-diabetic and diabetic NAFLD patients, respectively. The difference between the groups was statistically significant (P=0.04). There was significant correlation between the serum concentrations of CyPA and glucose levels (P=0.01), but there was no significant correlation with other clinical and histologic parameters.**

Conclusion : **Our data suggest that CyPA levels are elevated in patients with NAFLD, especially in patients with diabetes.** (Acta gastroenterol. belg., **2017**, 80, **3-7**).

Key words : nonalcoholic fatty liver disease, cyclophilin a, diabetes mellitus.

Introduction

Nonalcoholic fatty liver disease (NAFLD), is the most common cause of chronic liver disease in most of the world and its incidence is increasing (1). Nowadays NAFLD is recognized as the hepatic component of metabolic syndrome due to common etiological factors in both diseases' pathogenesis including obesity insulin resistance and dyslipidemia. Disease has progressive character and in clinical practice it can present itself in a wide spectrum; from asymptomatic high transaminase levels to end stage liver disease and hepatocellular carcinoma. Although there is an increase in mortality rate associated with liver disease, main cause of mortality is due to cardiovascular disease (CVD) in patients with NAFLD (2).

Cyclophilin A (CyPA) also known as peptidylprolyl isomerase A, is a protein from immunophilin family and is associated with proinflammatory signal transduction. CyPA plays role in tissue damage related to oxidative stress and inflammatory stress by being secreted from both vascular smooth muscle cells (VSMC), and leucocytes and platelets and it is also secreted from monocytes in hyperglycemic states (3). It has function in VSMC and leucocyte activation and migration, expression of proinflammatory cytokines, vessel wall remodeling and in foam cell transformation (3). Recently there have been various studies published investigating the role of CyPA in atherosclerosis, diabetes, hyperlipidemia and CVD (4-6). Nevertheless, there isn't any research regarding the role of CyPA in the pathogenesis of NAFLD. In this sectional, case–control study, we investigated serum levels of CyPA in patients with NAFLD and examined the association between levels of this molecule and clinical, histological and biochemical parameters in this patient group.

Materials and Methods

Study subjects

The study protocol was approved by our local ethics committee and all volunteers gave written informed consent. Fifty-two patients with NAFLD (21 men and 31 women) and 44 healthy control subjects (16 men and 28 women) were included in the study. Patients referred to our clinic in 2014 with ALT elevations for at least 6 months, with no history of any hepatotoxic drugs, hormone replacement therapy or herbal products, without drinking alcohol more than 20g/day, viral hepatitis, autoimmune hepatitis, Wilson's disease, hemochromatosis, alpha-1 antitrypsin deficiency, biliary disease or malignancies were found eligible. An ultrasonography

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(US)-guided liver biopsy was performed to the patients with hepatosteatosis by ultrasonographic examination and those having NAFLD diagnosis were enrolled in the study. The healthy control group consisted of subjects with no illness, no usage of alcohol, drug or herbal substances, no history of previous liver diseases and who was negative for viral hepatitis serology tests and who had normal liver US.

Clinical assessment

A complete physical examination was performed to each subject. Anthropometric assessment of height and weight were measured, body mass index (BMI) (kg/m2) was calculated, and waist circumference (cm) was measured. Blood pressures were measured after ten minutes of rest in a quiet room. Venous blood samples were taken in the morning after twelve hours of fasting. Complete blood count and biochemical parameters were assessed using standard methods. The serum samples were centrifuged for 10 minutes at 2500xg and samples were stored at -80 C^0 until analysis. The Adult Treatment Panel III for metabolic syndrome (7) and American Diabetes Association (8) criteria were used for diabetes mellitus diagnosis. HOMA-IR index [fasting plasma insulin (mU/ml) x fasting plasma glucose (mg/dL)/405.23] was used for determining insulin resistance. All ultrasonographic examinations and US guided percutaneous liver biopsies were performed by the same radiologist.

Serum CyPA levels were measured duplicately using enzyme-linked immunosorbent assay (Blue Gene Biotech Inc, Shanghai, China) kit according to the manufacturer's instructions. The minimum detectable value was 1 μg/ml. The intra-assay and the inter-assay coefficients of variation for CyPA were <10%, respectively. All biochemical tests were performed in a blind manner.

Histological analysis

All of the patients in this study have undergone US-guided percutaneous liver biopsy. The liver specimens obtained were accepted sufficient if the length of the tissues were greater than 2cm and/or showing more than six portal areas in histological examination. Specimens were stained with hematoxylin-eosin, Masson's trichrome and reticulin silver stains. Scoring and evaluation were done by an experienced hepatopathologist blind to the clinical status of the patients. Histological evaluation was done according to the NAFLD scoring system recommended by National Institute of Diabetes and Digestive and Kidney Diseases NASH Clinical Research Network (9). Briefly, hepatic steatosis was graded from 1 to 3 according to the steatosis ratio as 5-33%, 33-66% and >66% representing score 1, 2 and 3 respectively. Lobular inflammation was defined as an overall assessment of all inflammations; no foci as score 0, <2 foci per x200 field as score 1, two-four foci per x200 field as score 2, more than 4 foci per x200 field as score 3. Ballooning scoring is defined as score 0 if there is no ballooning of hepatocytes, score 1 if there are few and score 2 if there are numerous ballooning. Fibrosis was staged as follows: stage 0, no liver fibrosis; stage 1, perisinusoidal or periportal fibrosis; stage 2, perisinusoidal and portal/periportal fibrosis; stage 3, bridging fibrosis and stage 4, cirrhosis. Histologically, total NASH score is calculated as a sum of steatosis (1-3), lobular inflammation (0-3) and ballooning (0-2). It is concluded according to the total NASH score 0-2 as simple steatosis, 3-4 as borderline NASH, or greater were diagnosed as definitive NASH[9].

Data were processed on a personal computer and analyzed using SPSS 16.0 (SSPS Inc., Chicago, IL, USA). Normally distributed continuous variables are presented as mean ± standard deviation; skewed continuous variables were characterized by the medians and interquartile ranges. The student t test was used in the evaluation of the difference between the two averages of the independent groups. Differences in the levels of CyPA among more than two groups were determined by one-way analysis of variance followed by Bonferroni multiple-comparison post-hoc test. Categorical data were analyzed by using the x^2 test. Spearman rank correlation was used to examine the relationship between variables. Multiple linear regression analysis was performed to evaluate the independence of the association between CyPA levels, clinical, biochemical and histological parameters of liver injury in NAFLD patients. P values < 0.05 were considered statistically significant.

Results

The general characteristics of the study participants are shown in Table 1. The two study groups (healthy controls and NAFLD patients) did not differ in terms of age and sex. As expected, BMI, waist circumference, systolic and diastolic blood pressure, C-reactive protein, white blood cells, platelets, hemoglobin, glucose, hemoglobin A1c, HOMA-IR index, total cholesterol and triglycerides, LDL and HDL cholesterol, transaminases, and ferritin levels in patients with NAFLD were significantly higher from those of the controls (Table 1). Seven (13%) patients had simple steatosis, 16 (31%) patients had borderline NASH, and 29 (56%) patients had definite NASH in the NAFLD group (Table 2). Metabolic syndrome was found in 63% and prevalence of diabetes was found 46% of the patients with NAFLD.

NAFLD patients had significantly higher serum CyPA levels (3,8±2,6 μg/ml) than control subjects (2,8±1,8 μg/ml, P=0.03, Figure 1). There were no sex-related differences in PEDF levels both in patients (males : 3,4±2,2; females: 4±2,8 μg/ml ; P=0.27) and in controls (males : 2,4±1,9; females: 2,9±1,7 μg/ml; P=0.42).

Serum CyPA levels were 2,8±1,8, 3,4±2,3, and 4,2±2,9 μg/ml in control group, non-diabetic and diabetic NAFLD patients, respectively. The difference between the groups was statistically significant (P=0.04).

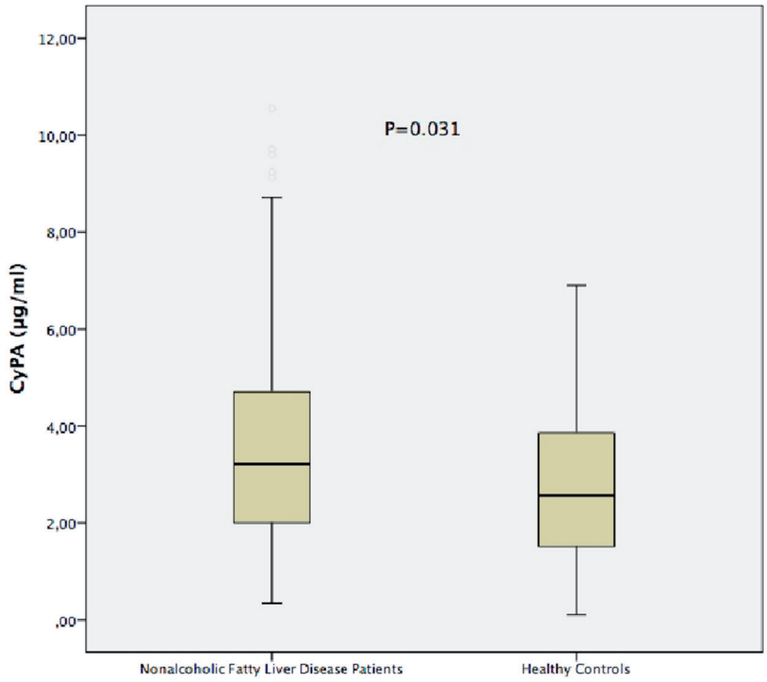

Fig. 1. — Serum cyclophilin A (CyPA) levels in patients with nonalcoholic fatty liver disease and healthy controls groups.

Serum levels of CyPA were 3,8±2,7 and 3,7±2,5 μg/ml in NAFLD patients with or without metabolic syndrome, respectively. The difference between the groups was not statistically significant (P=0.1).

As assessed by one-way ANOVA, serum CyPA levels were not significantly different across the two subgroups in patients with NAFLD (simple steatosis group: 3,9±2; NASH group: 3,8±2,7 μg/ml) compared with healthy controls. In correlation analyses of the entire study cohort there was a significant correlation between serum levels of CyPA and glucose levels (r=0.25, P=0.01), but there were not any statistically significant association with clinical and histopathological parameters. No statistically significant result was obtained from multivariate regression analysis.

Discussion

In this cross-sectional observational study, we observed significantly higher serum concentration of CyPA in patients with NAFLD. This difference was more prominent in the setting of diabetes. Furthermore, there was a statistically significant correlation between serum CyPA and glucose levels whereas there was no association between other clinical and histopathological parameters.

Various studies have shown the role of CyPA in oxidative stress and inflammatory process particularly in the development of vascular damage and endothelial dysfunction (10). In a recent study, serum CyPA levels were found to be significantly high in patients with coronary artery disease (CAD), suggesting CyPA levels as a potent biomarker (11). In another study by Satoh et al. (12) CyPA levels were significantly higher in patients with diabetes compared to control group and also found to be higher in patients with CAD and diabetes compared to diabetic patients without CAD. Moreover, recently increased endothelial dysfunction, atherosclerosis and presence of CAD were observed in patients with NAFLD, supporting the fact that cardiovascular events are the most important cause of mortality in this population (2,13-15). In our study although serum CyPA levels were higher than healthy controls in NAFLD group, we failed to demonstrate a significant difference between simple steatosis and NASH subgroups regarding CyPA levels and also a correlation between NASH scores and CyPA levels. Nevertheless, NAFLD patients with diabetes had significantly higher CyPA levels compared to patients without diabetes. These results suggest CyPA levels in NAFLD cases are not directly linked to hepatic inflammation. There are two possible mechanisms explaining this increase in CyPA levels. One of which is the presence of hyperglycemia in NAFLD patients which increases CyPA expression in monocytes. The significant correlation between glucose and CyPA levels and the fact that NAFLD patients with diabetes having higher CyPA levels in the study support this hypothesis. Another possible mechanism is that rather than hepatic inflammation, vascular inflammation is present in NAFLD patients leading to increased CyPA levels.

Table 1. — **Clinical and biochemical characteristics of the NAFLD patients and healthy controls**

	NAFLD group (n=52)	Healthy controls (n=44)	P value
Gender (males/females)	21/31	16/28	NS
Age (years)	39±10,5	36±7,9	NS
BMI (kg/m^2)	32±6,3	23,8±5	< .001
Waist circumference (cm)	101±9,4	79,4±14,5	< .001
Systolic blood pressure (mmHg)	127,1±21,6	109,6±11,8	< .001
Diastolic blood pressure (mmHg)	81,3±11,9	71,6±8,1	< .001
Sedimentation (mm/hr)	17,1±10,7	17,3±11,6	NS
C-reactive protein (mg/L)	5,1 (3-9,4)	3 (1,8-4,8)	< .001
White blood cells (x10^9/L)	7,33±2,25	6,2±1,76	0.008
Platelets (x 10^9/L)	268,5±76,2	251,4±49,1	< .001
Hemoglobin (g/L)	140,3±18,5	129,7±16,1	0.004
Glucose (mmol/L)	5,3(4,8-5,9)	4,3(3,9-4,8)	< .001
Hemoglobin A1c (%)	6±1	5,3±0,3	0.027
HOMA-IR	2,4 (1,3-3,2)	0,86 (0,5-1,3)	< .001
Total cholesterol (mmol/L)	5,3±1,2	4±1,4	< .001
Triglycerides (mmol/L)	2 (1,2-3,2)	0,9 (0,7-1,1)	< .001
LDL cholesterol (mmol/L)	3,5±0,9	2,7±0,9	< .001
HDL cholesterol (mmol/L)	1,2(1-1,3)	1,3(1-1,5)	0.004
AST (U/L)	39(29,3-45,8)	20(15-24,3)	< .001
ALT (U/L)	56(35,3-75,3)	14(9,8-21,8)	< .001
Ferritin (pmol/L)	74(36,3-116,5)	20,7(10,6-49,2)	< .001
CyPA (µg/ml)	3,8±2,6	2,8±1,8	0.03
Diabetes Mellitus	24 (46%)	0	-
Metabolic Syndrome	33 (63%)	0	-

Data are shown as the mean±standard deviation or median±interquartile ranges.
Normal values in laboratory tests: BMI (body mass index) (18-25 kg/m^2); Sedimentation (0-20 mm/hr); C-reactive protein (<8 mg/L); White blood cell count (4–10 x10^9/L); Platelet (150-400 x10^9/L); Hemoglobin (130–180 g/L in males and 110–160 g/L in females); Glucose (4,4-6,1 mmol/L) (as measured by a fasting blood glucose test); HbA1c (4.3–5.8 proportion of total hemoglobin); Total cholesterol (2.6–5.2 mmol/L); Triglyceride (0.7–1.7 mmol/L); LDL cholesterol (1-3.37 mg/dl); HDL cholesterol (>0.9 mmol/L); AST (5–32 U/L); ALT (5–38 U/L); Ferritin (54–755 µg/L in males and 25–755 µg/L in females); HOMA-IR, CyPA and metabolic syndrome are described in the text.

Some limitations can be referred to this study. This is a cross-sectional case-control study and thus does not able to elucidate the causal relationships between serum CyPA and NAFLD. Also, the number of our study population was relatively small. Third, the study group consisted of only Turkish ethnicity, so our results cannot be extrapolated to populations with other ethnic groups. Finally, the exclusion of NAFLD in control subjects was done by normal hepatic ultrasonography and normal biochemical findings: no liver biopsies were performed due to ethical concerns.

In conclusion, this is the first study that evaluated the serum CyPA levels in patients with NAFLD and we found that serum CyPA levels are significantly increased in patients with biopsy proven NAFLD compared to healthy controls and also this difference was more prominent in the setting of diabetes. However the causality relationship is unknown in this regard for today. This elevation may not be associated with direct hepatic inflammation; it may be associated with the presence of hyperglycemia and/or vascular inflammation. Thus further clinical research studies are needed to clarify these associations.

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