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Serum C1q/TNF-related protein 9 is not related to nonalcoholic fatty liver disease

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ABSTRACT

Aims: C1q/TNF-related protein 9 (CTRP9) is an adipokine mainly secreted by white adipose tissue and plays protective roles in energy metabolism. However, information regarding the role of CTRP9 in nonalcoholic fatty liver disease (NAFLD) is scarce. Here we aimed to ascertain the clinical relevance between circulating CTRP9 levels and NAFLD through a cross-sectional study.

Methods: The study enrolled 82 NAFLD adults and 79 sex- and age-matched non-NAFLD controls. Serum CTRP9 was measured via ELISA method. Metabolic parameters were also determined.

Results: Although serum CTRP9 level seems to be higher in NAFLD adults, there was no significant difference among the ultrasonographic degrees of NAFLD ($P = 0.275$). Further, after adjustment for BMI in the multinomial logistic regression model, no significant odds ratio difference was observed for NAFLD among the CTRP9 tertiles. Moreover, binary logistic regression models demonstrated that, body mass index (BMI) and alanine aminotransferase (ALT) but not CTRP9 were independent factors related to NAFLD. Besides, serum CTRP9 was positively correlated with BMI, waist circumference, Fasting insulin, HbA1c, and HOMA-IR in all subjects. BMI was the independent factor associated with serum CTRP9.

Conclusions: Serum CTRP9 is not independently related to NAFLD. The association between serum CTRP9 and NAFLD might be due to the influence of obesity.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD), the leading chronic hepatic disease in developed countries, has become a severe health problem worldwide with a high prevalence [1]. NAFLD includes a spectrum of liver disorders ranging from steatosis, steatohepatitis, to cirrhosis and hepatocellular carcinoma [2,3]. It is widely considered that NAFLD is the hepatic manifestation of metabolic syndrome (MS) and insulin resistance [4]. Obesity especially abdominal obesity is frequently present in patients with NAFLD. Although intensive studies have been conducted, the pathogenesis of NAFLD is not fully understood.

Adipokines are bioactive factors secreted by adipose tissue, and play vital roles in the progress of energy expenditure, insulin sensitivity, inflammation, and glucose homeostasis [5–7]. Accumulating findings have confirmed that the alteration of adipokines is implicated in the development of NAFLD [8–11]. Adiponectin is among the adipokines associated with NAFLD [10]. C1q/TNF-related proteins (CTRPs) are secreted proteins which have the same modular organization with adiponectin. Currently, fifteen members named from CTRP1 to CTRP15 are found in the CTRPs family[12]. Various metabolic functions of CTRPs in skeletal muscle, adipose tissue, the liver, the heart, and hypothalamus have been well recognized [12]. Moreover, CTRP1, CTRP3, and CTRP13 have been shown to be related to NAFLD [13–16].

CTRP9, a member of the CTRPs superfamily, is mainly secreted by adipose tissue and shares 54 percent sequence identity with adiponectin at its globular C1q domain [17]. The beneficial cardiovascular effects of CTRP9 have been well established [18–21]. Recently, Jung et al. [22] demonstrated that CTRP9 regulates growth, differentiation, and apoptosis in human keratinocytes through TGFβ1-p38-dependent pathway.

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In terms of metabolism, gain- and loss-of-function studies showed that CTRP9 is beneficial for lipid metabolism, insulin sensitivity, and attenuation of hepatic steatosis in rodent [23,24]. Besides, Jung et al. [25]found that CTRP9-induced autophagy can alleviate hepatic steatosis through relief of endoplasmic reticulum stress. In humans, CTRP9 levels significantly increase in obese individuals and correlate with insulin resistance and obesity-related parameters, whereas decrease after weight loss surgery [26]. In addition, CTRP9 levels are elevated in patients with type 2 diabetes, whereas reduced in MS [27–29]. However, information regarding the relationship between circulating CTRP9 and human NAFLD is rare. Here, we conducted this cross-sectional study to explore the clinical relevance of CTRP9 in human NAFLD.

2. Material and methods

2.1. Subjects and study design

All 82 NAFLD cases and 79 sex- and age- matched non-NAFLD controls were screened from the medical examination center of Xinghua People's Hospital. Abdominal ultrasonic examination was performed in all the participants. NAFLD was diagnosed based on the ultrasonic manifestation. The liver and the kidney cortex presented a same parenchyma echogenicity was defined as normal liver. Steatosis was defined as hyper-echogenic liver with fine, tightly packed echoes. Additionally, the severity (three grades) of NAFLD was defined according to the degree of steatosis [30,31]. The exclusion criteria are as follows: drug-induced liver disease, viral hepatitis, autoimmune hepatitis, severe cardiopulmonary disorders, chronic or acute inflammatory diseases, renal dysfunction, tumor, pregnancy, hereditary disorders, treated with lipid-lowering drugs or systemic corticosteroids, smoking, and excessive alcohol consumption (men: more than 140 g per week; women: more than 70 g per week). Impaired glucose regulation or newly diagnosed type 2 diabetes (haven't received medication affecting insulin secretion or glucose tolerance) and hypertension were not in the exclusion criteria.

2.2. Anthropometric data collection and biochemical measurements

Anthropometric data collection and biochemical measurements were conducted similarly to our previous study [32,33]. Data regarding waist circumference (WC), weight, and height were collected and recorded by two well-trained nurses. Body mass index (BMI) was calculated as weight (kg)/height squared (m^2). Blood pressure measurement was performed 3 times via an electronic sphygmomanometer (Omron). All subjects kept a sitting position for rest at least 10 min before measurement. The average of the three values was recorded.

After overnight fast, blood samples were taken and centrifuged. Fasting glucose, alanine aminotransferase (ALT), aspartate aminotransferase (AST), uric acid, creatinine, and lipid profiles including triglyceride(TG), total cholesterol(TC), low-density lipoprotein cholesterol(LDL-C), and high-density lipoprotein cholesterol(HDL-C) were determined via a Hitachi 7600 analyzer (Hitachi, Tokyo, Japan). High performance liquid chromatography method was used for HbA1c determination (HLC-723G8, TOSOH, Japan). Fasting insulin was measured by an automated immunoassay analyzer (AIA-2000ST, TOSOH, Japan). White blood cell (WBC) counts were quantified using an automatic analyzer (Sysmex KX-21N, Japan). The remaining samples were stored at −80 °C for CTRP9 measurement.

2.3. Indices of insulin resistance

Insulin resistance status was evaluated using homeostasis model assessment of insulin resistance index (HOMA-IR). HOMA-IR formula = [Fasting glucose (mmol/L) × Fasting insulin (mIU/L)]/22.5.

2.4. Measurement of serum CTRP9

Serum CTRP9 was determined using an ELISA kit for human (Catalogue No: CSB-EL003654HU, CUSABIO, China). The measurement was performed in accordance with the manufacturer's instructions. The intra-assay coefficient of variation (CV) was < 10% and the inter-CV was < 8%. The sensitivity of the assay is < 0.078 ng/mL.

2.5. Statistical analysis

SPSS version 23 (IBM, USA) was performed for all statistical analyses. Kolmogorov–Smirnov test and Q-Q plot were used to test distribution characters of data. Normally distributed data were presented as mean ± SD and non-normally distributed data were shown as median (25th-75th). Then independent samples *t* test and Mann-Whitney *U* test were performed to calculate differences between two groups. χ^2 Test was conducted to compare categorical data. Kruskal-Wallis test and one way ANOVA were used for multiple testing among groups. Pearson correlation analysis was used to analyze bivariate correlation between CTRP9 and other variables. To identify independent factors of CTRP9, multiple liner regression analysis was performed. Before correlation and stepwise regression analysis, non-normally distributed data were log-transformed (e.g. log-ALT). To assess the association between CTRP9 and the prevalence of NAFLD, binary and multinomial logistic regression models were performed. *P* value < 0.05 (two side) was considered to be statistically significant.

3. Results

3.1. General characteristics according to NAFLD

The general parameters of all the 161 subjects are shown in Table 1. Sex and age were comparable between the controls and the NAFLD

Table 1
General clinical and laboratory parameter in participants with and without NAFLD.

Variable	Controls	NAFLD	*P* value
N	79	82	
Sex(M/F)	54/25	63/19	0.228
*Age(years)	48.79 ± 10.54	48.24 ± 9.31	0.724
*BMI(kg/m^2)	23.32 ± 3.28	26.32 ± 2.93	< 0.001
*WC(cm)	81.59 ± 7.99	90.82 ± 9.27	< 0.001
*SBP (mmHg)	124.34 ± 15.82	137.89 ± 15.46	< 0.001
*DBP (mmHg)	77.58 ± 11.40	88.34 ± 13.31	< 0.001
#Fasting glucose (mmol/L)	5.15 (4.87–5.54)	5.59 (5.18–6.03)	< 0.001
*Fasting insulin (mIU/L)	5.67 ± 2.82	9.81 ± 5.68	< 0.001
#HbA1c (%)	5.2 (5.0–5.4)	5.4 (5.2–5.6)	0.001
#HOMA-IR	1.17 (0.79–1.82)	2.12 (1.67–3.25)	< 0.001
#TG (mmol/L)	1.22 (0.87–1.71)	1.99 (1.49–2.72)	< 0.001
*TC (mmol/L)	4.54 ± 0.80	4.99 ± 0.98	0.002
*Uric acid(μmol/L)	317.13 ± 79.52	397.46 ± 95.44	< 0.001
*Creatinine(μmol/L)	83.22 ± 16.13	86.09 ± 11.32	0.194
*ALT (U/L)	25.67 ± 12.05	46.51 ± 28.99	< 0.001
#AST (U/L)	24 (20–28)	29.5 (23–38)	< 0.001
*LDL-C (mmol/L)	2.34 ± 0.65	2.51 ± 0.94	0.231
*HDL-C (mmol/L)	1.69 ± 0.35	1.54 ± 0.31	0.010
*White blood cell(×10^9/L)	5.72 ± 1.34	6.26 ± 1.38	0.014
*CTRP9 (ng/ml)	1.39 ± 0.76	1.79 ± 1.06	0.007

BMI body mass index; WC waist circumference; SBP systolic blood pressure; DBP diastolic blood pressure; TG Triglyceride; TC Total cholesterol; ALT alanine aminotransferase; AST aspartate aminotransferase; LDL-C low-density lipoprotein cholesterol; HDL-C high-density lipoprotein cholesterol.
The categorical data were compared with χ^2 test.
* Data normally distributed are shown as mean ± SD. Independent Sample *t* test was performed.
# Data with skewed distribution are shown as median ($25^{th}-75^{th}$). Mann–Whitney *U* test was performed.