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Low serum CTRP3 levels are associated with nonalcoholic fatty liver disease in patients with type 2 diabetes mellitus

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ABSTRACT

Nonalcoholic fatty liver disease (NAFLD) commonly occurs in patients with type 2 diabetes mellitus (T2DM). C1q/TNF-related protein-3 (CTRP3) levels are decreased in type 2 diabetic patients. However, to date, it is unknown whether low CTRP3 level are correlated with the incidence of NAFLD. The aim of this study was to observe this association in Chinese patients with T2DM. Overall, 175 newly diagnosed T2DM were recruited in this study. The subjects were divided into NAFLD group (n = 93) and control group (n = 82). Anthropometric parameters, blood pressure, and several biochemical parameters were measured. The body composition was assessed with the bioelectrical impedance analysis (BIA) method. Insulin level was evaluated by radioimmunoassay. Levels of serum CTRP3 and interleukin-6 (IL-6) were measured by enzyme-linked immunosorbent assay. Our findings demonstrated that type 2 diabetic patients with NAFLD had lower levels of serum CTRP3 than did those without NAFLD ($P = .002$). Serum CTRP3 level was negatively correlated with body mass index ($r = -0.271$, $P = .001$), visceral fat area ($r = -0.285$, $P < .001$), glycosylated hemoglobin A1c ($r = -0.270$, $P < .001$), triglycerides ($r = -0.267$, $P < .001$), CRP ($r = -0.222$, $P = .010$), IL-6 ($r = -0.212$, $P = .008$), and HOMA-IR indices ($r = -0.334$, $P < .001$). When compared with the highest CTRP3 tertile, the odds ratio of the middle tertile for NAFLD incidence was 4.54 (95% CI, 1.53–13.47) and 5.80 (95% CI, 1.60–21.02) for the lowest tertile after adjustment for confounding factors. In summary, low serum CTRP3 is a strong predictor for the prevalence of NAFLD in Chinese patients with newly diagnosed T2DM.

1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is a condition characterized by excessive fat accumulation in the liver with no history of alcohol abuse, that is, the alcohol consumption is less than 20 g per day [1]. In recent years, NAFLD is epidemic worldwide although the mechanisms underlying are not fully understood. The estimated prevalence of NAFLD is around 15–30% in the general population in view of different ethnicities [2–4]. However, when patients with NAFLD are complicated with type 2 diabetes mellitus (T2DM), the incidence will increase rapidly. It has been reported that more than 70% of type 2 diabetic patients suffer from NAFLD at the same time [5,6]. In China, it is also a very serious issue. A study showed that the co-occurrence incidence of NAFLD and T2DM was almost 80% for Chinese population [7]. Diabetes is independently associated with the occurrence and development NAFLD [8]. On the other hand, NAFLD has some detrimental effects on T2DM, which means worse glucolipid metabolism, higher risk of macro- and micro-vascular complications [9], and so on. Therefore, it would be worthwhile to unravel the mechanisms and develop novel pharmaceuticals for the prevention and treatment of NAFLD, especially when it occurs in combination with T2DM.

C1q/TNF-related protein-3 (CTRP3) is a newly identified adipokine, which is considered as one kind of adiponectin paralogs [10]. Interestingly, CTRP3 has many effects resembling adiponectin. It can improve insulin sensitivity, lower glycemic levels, reduce proinflammatory factors, and ameliorate endothelial dysfunction [11,12]. An experimental study reported that CTRP3 overexpression rescued inflammatory responses and oxidative stress in the myocardium of diabetic rats [13]. CTRP3 was also found to reduce the levels of inflammation factors and reactive oxygen species (ROS) induced by high glucose in cardiomyocytes cultured in vitro [13]. Unfortunately, despite so many beneficial effects, there were several studies demonstrating that CTRP3 levels were significantly decreased in both diabetic animal models and patients with T2DM [14,15].

As mentioned above, the strong correlation between T2DM and NAFLD has been recognized for a long time, indicating increased risks of cardiovascular disease (CVD) [16]. However, to date, it remains unclear whether low CTRP3 levels are causally implicated with NAFLD

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of type 2 diabetic patients. Therefore, the present study attempted to reveal the association between serum CTRP3 level and the incidence of NAFLD in a Chinese population with T2DM.

2. Methods

2.1. Study design and population

A total of 175 newly diagnosed T2DM were enrolled in this study during the 3-month period from March 2016 to May 2016 from Endocrinology Department of Wuhan General Hospital of the Chinese People's Liberation Army. Each participant was taken a hepatic ultrasonographic examination. Those with NAFLD were divided into NAFLD group (n = 93). Those without NAFLD were served as control group (n = 82). All the 175 subjects were taken 75gr-oral glucose tolerance test (OGTT) to reveal the glucose tolerance status. According to the 1999 World Health Organization (WHO) agreed cut-off point [17], newly diagnosed T2DM was defined as either fasting glucose (FBG) ≥ 7.0 mmol/L or post-challenge glucose ≥ 11.1 mmol/L or both on two occasions at least 48 h apart. Participants were excluded from the study if they had type 1 diabetes, renal dysfunction, congestive heart failure, coronary arterial disease, malignant neoplasms, or infectious diseases. Also, participants were excluded if they had a history of heavy alcohol consumption (> 20 g/d for women or > 30 g/d for men), any other causes of chronic liver disease such as hepatitis B or C, autoimmune hepatitis or other. All the subjects did not take any medications during the past 6 months.

Some details of each participant were recorded with standardized questionnaires, including medical histories, smoking status, alcohol consumption, and medication use histories. In this study, the subject who daily smoked one or more cigarette for no less than one year was defined as cigarette smokers. Alcohol consumption was defined as a person who reported drinking alcohol at least once a day. Ethical approval for this study was gotten from our hospital's ethics committee and informed consent forms were signed by each participant.

2.2. Anthropometry and laboratory measurements

Anthropometric parameters including height, weight, and waist circumference were measured using the standardized protocols. Body mass index (BMI) was calculated as weight (kg) divided by the square of height (m^2). The body composition of all the subjects were assessed with the bioelectrical impedance analysis (BIA) method using body composition analyzer IOI353 (JAWON, Seoul, Korea) according to the manufacturer's instructions. BIA is used to estimate visceral fat area, body fat mass, and body fat rate in this study. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were determined by a specially assigned nurse using a mercury sphygmomanometer.

The peripheral blood samples were collected after a 10–12 h of overnight fasting. A standardized clinical and laboratory procedure was used to evaluate the biochemical parameters. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), serum creatinine, uric acid, total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL) cholesterol, and low-density lipoprotein (LDL) cholesterol concentrations were assessed using standard enzymatic methods. FBG level was measured by a glucose oxidase procedure. Glycosylated hemoglobin A1c (HbA1c) was measured by high-performance chromatography. C-reactive protein (CRP) was measured by particle enhanced immunoturbidimetric assay. The intra- and inter-assay coefficients of variation (CV) for these assays were < 5%.

Fasting insulin (FINS) level was assayed by radioimmunoassay method. The intra-assay and the inter-assay CV for insulin were < 10% and < 15%, respectively. The formula for the homeostasis model assessment index-insulin resistance (HOMA-IR) is as follows: HOMA-IR = FBG (mmol/L) × FINS (μIU/mL)/22.5. CTRP3 was quantified using the sandwich enzyme-linked immunosorbent assay (ELISA) kits (Meimian Biotechnology, China) with a lower limit of detection of 0.1 ng/mL. IL-6 was also measured by ELISA method (Anoric Biotechnology, China). The lower limit of detection for IL-6 was 1.1 pg/mL. The intra- and inter-assay CVs for CTRP3 and IL-6 both were < 10% and < 15%, respectively.

2.3. Statistical analysis

Statistics analyses were conducted using the SPSS statistical software (Version 17.0, SPSS Inc., Chicago, IL, USA). Data distributions were determined using the Kolmogorov–Smirnov test. Normally distributed data were shown as mean ± standard deviation (SD). Non-normally distributed data, such as ALT, AST, CRP, IL-6, TG, and CTRP3, were presented as median (interquartile range). These parameters were log transformed before further analysis. Categorical variables were presented as numbers and percentages. Data were compared between the NAFLD group and control group with independent-samples *t* test. Spearman correlation analyses were performed to determine the associations between serum CTRP3 level and other variables. Multivariate logistic regression models, according to serum CTRP3 tertiles (≤6.19, 6.20–14.16 and ≥14.17 ng/mL), were built to estimate the odds ratios (ORs) for NAFLD. In these models, potential confounding factors were controlled, including age, gender, smoking, alcohol consumption, BMI, HbA1c, TG, Il-6, and HOMA-IR. A P value < .05 was considered statistically significant.

3. Results

3.1. The baseline characteristics of all the subjects

Table 1 shows the baseline characteristics of the two groups. Subjects in NAFLD group had higher BMI, waist circumference, visceral fat area, body fat mass, body fat rate than did those in the control group (all $P < .05$). Higher ALT, AST, serum uric acid, FBG, HbA1c, FINS, HOMA-IR indices, CRP, IL-6 and TG were identified in the NAFLD group than control group (all $P < .05$). However, serum CTRP3 levels were significantly lower in the NAFLD group than the control group ($P = .002$). Furthermore, HDL-cholesterol levels were markedly decreased in NAFLD group relative to the control group ($P < .001$). There was no significant difference in age, gender, smoking status, alcohol consumption, SBP, DBP, serum creatinine, TC and LDL-cholesterol were identified between the two groups ($P > .05$).

3.2. The correlation between serum CTRP3 level and other variables

The correlation coefficients between serum CTRP3 level and other variables were calculated using *Spearmen* correlation analysis. As shown in Table 2, serum CTRP3 level was negatively correlated with BMI ($r = -0.271$, $P = .001$), waist circumference ($r = -0.330$, $P < .001$), visceral fat area ($r = -0.285$, $P < .001$), body fat mass ($r = -0.212$, $P = .009$), body fat rate ($r = -0.229$, $P = .005$), ALT ($r = -0.208$, $P = .006$), FBG ($r = -0.160$, $P = .037$), HbA1c ($r = -0.270$, $P < .001$), FINS level ($r = -0.289$, $P = .001$), HOMA-IR indices ($r = -0.334$, $P < .001$), CRP ($r = -0.222$, $P = .010$), IL-6 ($r = -0.212$, $P = .008$), TG ($r = -0.267$, $P < .001$). Serum CTRP3 level was positively correlated with serum creatinine ($r = 0.192$, $P = .012$). However, serum CTRP3 level was found not to be associated with age, gender, smoking status, alcohol consumption, SBP, DBP, AST, serum uric acid, TC HDL-cholesterol, and LDL-cholesterol (all $P > .05$).

3.3. The effect of serum CTRP3 on the incidence of NAFLD

Further, the effect of serum CTRP3 on the incidence of NAFLD was assessed using the logistic regression analysis methods. As shown in Table 3, it was demonstrated that the unadjusted OR for the incidence of NAFLD was 5.19 (95% CI, 2.35–11.47) for the middle tertile and 6.37

Table 1
Baseline characteristics of type 2 diabetic patients with or without NAFLD.

Characteristics	Control (n = 82)	NAFLD (n = 93)	*P* value
Age (years)	53.99 ± 9.88	51.02 ± 11.05	.082
Male/Female (n/n)	45/37	53/40	.879
Smoking [n (%)]	31 (37.80%)	33 (35.48%)	.756
Alcohol [n (%)]	23 (28.05%)	29 (31.18%)	.741
Body mass index (kg/m^2)	23.65 ± 3.02	24.86 ± 2.51	.008
Waist circumference (cm)	83.09 ± 8.42	86.46 ± 7.61	.011
Visceral fat area (cm^2)	94.79 ± 37.76	108.94 ± 31.49	.013
Body fat mass (kg)	16.79 ± 4.87	19.08 ± 4.14	.002
Body fat rate (%)	25.40 ± 5.58	27.48 ± 5.15	.018
Systolic blood pressure (mmHg)	120.91 ± 14.59	122.68 ± 16.47	.460
Diastolic blood pressure (mmHg)	75.13 ± 9.64	77.22 ± 11.31	.197
ALT (U/L)	18.93 (12.00–21.75)	25.40 (15.00–29.00)	.003
AST (U/L)	17.58 (13.00–19.75)	20.68 (15.00–22.00)	.032
Serum creatinine	64.00 ± 15.46	59.32 ± 15.87	.053
Uric acid (μmol/L)	300.29 ± 89.01	331.92 ± 96.05	.027
Fasting glucose (mmol/L)	7.41 ± 2.46	8.50 ± 2.39	.004
Glycosylated hemoglobin A1c (%)	8.54 ± 2.71	9.55 ± 1.94	.005
Fasting insulin (mIU/L)	44.76 ± 29.77	61.02 ± 35.49	.005
HOMA-IR	19.04 ± 15.22	22.89 ± 15.05	< .001
CRP (mg/L)	1.81 (0.61–2.47)	2.47 (1.21–2.88)	.048
IL-6 (pg/mL)	89.28 (14.49–129.29)	128.85 (49.63–171.01)	.018
Total cholesterol (mmol/L)	4.93 ± 0.99	4.92 ± 1.12	.968
Triglycerides (mmol/L)	1.68 (0.86–1.85)	2.57 (1.36–3.33)	.001
HDL-cholesterol (mmol/L)	1.25 ± 0.31	1.08 ± 0.24	< .001
LDL-cholesterol (mmol/L)	2.47 ± 0.69	2.42 ± 0.81	.714
CTRP3 (ng/mL)	14.23 (12.81–17.83)	5.25 (3.31–9.56)	.002

Data are mean ± standard deviation, median (inter-quartile range) or percentage of participants.
ALT alanine aminotransferase, *AST* aspartate aminotransferase, *CRP* C-reactive protein, *HDL* high-density lipoprotein, *CTRP3* C1q/TNF-related protein-3, *HOMA-IR* homeostasis model assessment index-insulin resistance, *IL-6*, interleukin-6, *LDL* low-density lipoprotein, *NAFLD* nonalcoholic fatty liver disease.

Table 2
Correlation analysis between serum CRP3 levels and other variables.

Variables	Correlation coefficient	*P* value
Age (years)	−0.011	.884
Sex	0.001	.987
Smoking	0.007	.929
Alcohol	−0.055	.470
Body mass index (kg/m^2)	−0.271	.001
Waist circumference (cm)	−0.330	< .001
Visceral fat area (cm^2)	−0.285	< .001
Body fat mass (kg)	−0.212	.009
Body fat rate (%)	−0.229	.005
Systolic blood pressure (mmHg)	−0.127	.095
Diastolic blood pressure (mmHg)	−0.086	.258
ALT (U/L)	−0.208	.006
AST (U/L)	−0.130	.091
Serum creatinine	0.192	.012
Uric acid (μmol/L)	−0.018	.819
Fasting glucose (mmol/L)	−0.160	.037
Glycosylated hemoglobin A1c (%)	−0.270	< .001
Fasting insulin (mIU/L)	−0.289	.001
HOMA-IR	−0.334	< .001
CRP (mg/L)	−0.222	.010
IL-6 (pg/mL)	−0.212	.008
Total cholesterol (mmol/L)	0.016	.834
Triglycerides (mmol/L)	−0.267	< .001
HDL-cholesterol (mmol/L)	0.126	.101
LDL-cholesterol (mmol/L)	0.018	.818

ALT aspartate aminotransferase, *AST* alanine aminotransferase, *CRP* C-reactive protein, *HDL* high-density lipoprotein, *CTRP3* C1q/TNF-related protein-3, *HOMA-IR* homeostasis model assessment index-insulin resistance, *IL-6* interleukin-6, *LDL* low-density lipoprotein.

Table 3
ORs (95% CI) for NAFLD in type 2 diabetes according to serum CTRP3 tertiles.

	ORs (95% CI)			*P value*
	T1	T2	T3	
Model 1	6.37 (2.84–14.31)	5.19 (2.35–11.47)	Reference	< .001
Model 2	6.67 (2.93–15.16)	5.28 (2.35–11.83)	Reference	< .001
Model 3	8.77 (3.36–22.89)	5.42 (2.24–13.08)	Reference	< .001
Model 4	5.80 (1.60–21.02)	4.54 (1.53–13.47)	Reference	.006

Model 1: no variables adjustment.
Model 2: adjustment for age, gender, smoking and alcohol consumption.
Model 3: further adjustment for BMI.
Model 4: further adjustment for glycosylated hemoglobin A1c, triglycerides, IL-6 and HOMA-IR.

(95% CI, 2.84–14.31) for the lowest tertile when compared with the highest one. With the highest tertile as the conference, after correction for age, gender, smoking, alcohol consumption, BMI, HbA1c, TG, IL-6, and HOMA-IR, the adjusted OR of the middle tertile was 4.54 (95% CI, 1.53–13.47) and 5.80 (95% CI, 1.60–21.02) for the lowest tertile. Moreover, it was found that OR for the incidence of NAFLD decreased gradually according to CTRP3 tertiles. These findings demonstrated that lower serum CTRP3 level might play a role in the higher incidence of NAFLD even after correction for confounding factors.

4. Discussion

In the present study, serum CTRP3 levels were found to be significantly lower in newly diagnosed T2DM with NAFLD. The participants in the lowest CTRP3 tertile had a significantly increased risk for NAFLD after adjusting for potential cofounders. Furthermore, serum CTRP3 concentration was negatively correlated with various risk factors of cardiovascular diseases. Our findings may provide people novel insight into the potential role of low CTRP3 level in the prevalence of NAFLD for T2DM.

In recent years, there is growing evidence that CTRP3 may play a potential beneficial role in NAFLD. By contrast, low CTRP3 level is involved in the occurrence and development of NAFLD. Wong GM' team found that CTRP3 overexpression mice fed with high-fat diet (HFD) had improved hepatic steatosis and decreased hepatic triglyceride levels compared with its wild-type (WT) controls [18]. This interesting phenomenon was verified by a short-term CTRP3 protein administration to obese mice induced by HFD, which showed dramatically decreased hepatic triglyceride expression despite no change in serum triglyceride levels [18]. Their further work reported that, compared to their WT controls, CTRP3 knockout (KO) mice fed with HFD showed higher hepatic triglyceride levels, larger visceral fat area and the upregulated expression of inflammatory factors in spite of smaller liver size [19].

Data from animal models suggest a potential role of CTRP3 in fatty liver, however, it remains unknown whether the results are similar in human study. In the present study, it was found that CTRP3 levels were significantly lower in diabetic patients with NAFLD than those without NAFLD. Multivariate logistic regression analyses showed that, compared with the highest CTRP3 tertile, the incidence of NAFLD was increased by 4.54-fold for the middle tertile, and 5.80-fold for the lowest tertile, respectively. Here, for the first time, we demonstrated that low CTRP3 level may be implicated in the incidence of NAFLD of type 2 diabetic patients. Although the mechanisms by which CTRP3 improves NAFLD have remained largely unclear, some potential explanations

have been proposed. CTRP3 can lead to an increase in hepatic lipid oxidation and promote triglyceride to take apart in forming VLDL-C particles [18]. Additionally, CTRP3 can reduce hepatic triglyceride synthesis by inhibiting several key enzymes' activity [18].

Obesity is one of the most important risk factors for NAFLD, which is characterized by ectopic fat accumulation in the liver. In our study, it also showed that, when compared with diabetic patients without NAFLD, diabetic patients with NAFLD had higher BMI, waist circumference, visceral fat area, body fat mass, and body fat rate. Multiple studies have demonstrated that low CTRP3 level may be associated with obesity state. Serum CTRP3 levels were found to be decreased in HFD feeding obese rats with or without diabetes [14,20]. Several clinical surveys have reported that, compared with healthy controls, serum CTRP3 levels are lower both in obesity and other obesity-related diseases, such as T2DM, obesity hypertension and polycystic ovarian syndrome [15,21–23]. Consistently, our study found that serum CTRP3 level was reversely associated with BMI, waist circumference, visceral fat area, body fat mass, and body fat rate.

Our results revealed that NAFLD group had higher FBG, HbA1c, TG, FINS, HOMA-IR indices, CRP, and IL-6 compared to control group, indicating a more severe state of abnormal glucose-lipid metabolism, insulin resistance, and chronic low-grade inflammation. It has been well documented that CTRP3 level may be associated with these disorders. Peterson et al. [20] reported that recombinant CTRP3 protein could lead to a decrease in blood glucose levels in obese or diabetic animal models. Wolf et al. [22] found that serum CTRP3 level negatively correlated with triglycerides and positively correlated with HDL-cholesterol in obese patients. Another study conducted by Deng et al. [23] suggested that serum CTRP3 level was negatively associated with HOMA-IR indices in obesity hypertension. It has been suggested that some hypoglycemic agents improve insulin resistance via upregulation the expression of CTRP3. Metformin was reported to raise serum CTRP3 levels in women with polycystic ovary syndrome [21]. Exendin-4, a GLP-1 receptor agonist, also could cause an increase in the gene and protein expression of CTRP3 in 3 T3-L1 adipocytes and adipose tissue of diabetic rats [14,24]. Additionally, in some circumstance, CTRP3 has an anti-inflammatory effect resembling adiponectin. In vitro study, CTRP3 was found to ameliorate insulin sensitivity via inhibiting the expression of tumor necrosis factor-α (TNF-α) and IL-6 in 3 T3-L1 adipocytes [25]. Another in vivo study in human reported that serum CTRP3 was negatively correlated with CRP levels [15]. In line with these findings, in the present study, we observed that serum CTRP3 level was significantly correlated with FBG, HbA1c, TG, HDL-cholesterol, FINS, HOMA-IR indices, CRP, and IL-6.

The main limitation of our study is the cross-sectional design. Thus, it is difficult to verify a causal relation between lower CTRP3 levels and NAFLD in type 2 diabetic patients. It is urgent to have some prospective and randomized clinical trials to investigate this association. Another limitation is that only Chinese population were included in the present study, suggesting that it is uncertain whether our conclusions could be generalizable to subjects of other ethnicities. However, our preliminary data might provide a pathophysiological correlation between lower CTRP3 and NAFLD in type 2 diabetic patients.

Taken together, our findings suggested that serum CTRP3 levels were significantly lower in type 2 diabetic patients with NAFLD compared to those without NAFLD. We showed here for the first time that low CTRP3 levels were strongly predictive of new onset of NAFLD in T2DM. From a clinical point of view, it could be speculated that the supplementation of CTRP3 recombinant protein may be one of effective treatment strategies to protect type 2 diabetic patients from NAFLD. Prospective clinical trials are needed to prove the real role of CTRP3 in the occurrence and development of NAFLD in T2DM.

Acknowledgments

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Conflict of interest

We declare that we have no conflicts of interest.

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