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Circulating Levels of C1q/TNF-Related Protein-12 (CTRP-12) in Patients with Type 2 Diabetes: A Case-control Study

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Abstract

Background: The C1q complement/TNF-related protein (CTRP) superfamily is a newly diagnosed adipokine with anti-inflammatory, insulin sensitivity, and glucose lowering effects. This study aimed to assess the plasma circulating levels of CTRP12 in type 2 diabetic (T2D) patients and healthy subjects.

Methods: In this case-control study, plasma concentration of CTRP12 was measured by ELISA in 60 subjects (30 T2D and 30 healthy participants). The systolic blood pressure (SBP), waist circumference (WC), waist-to-hip ratio (WHR), body mass index (BMI), fasting blood sugar (FBS), glycated hemoglobin (HbA1c), insulin, and homeostasis model assessment of insulin resistance (HOMA-IR) were measured.

Results: In contrast to the SBP, WC, WHR, BMI, FBS, HbA1c, insulin, and HOMA-IR, the levels of CTRP12 were significantly lower in T2D patients. There was significant negative correlation between CTRP12, FBS, and HbA1c. The regression analysis showed that when subjected to stepwise multiple regression analysis, HbA1c (β = -2.21; $P < 0.004$) was predictive of plasma levels of CTRP12.

Conclusion: Finally, HbA1c was predictive of CTRP12 levels. It seems that CTRP12 could be considered as a novel biomarker for the prediction of T2D.

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Introduction

Obesity-induced type 2 diabetes (T2D) is one of the most serious problems in the world. According to the WHO statistics, 422 million people worldwide suffered from diabetes in 2014 and it is estimated that there will be at least 629 million people living with diabetes by 2045 (1). Adipose tissue cytokines which are collectively called adipocytokines or adipokines are the main culprits leading to insulin resistance and beta cell dysfunction characterizing T2D (2). Among adipokines, adiponectin is one of the most potent molecules with respect to its anti-inflammatory and insulin sensitizing activity (2). A growing body of evidence indicates that adiponectin level is reduced in T2D (3-5). Despite its anti-inflammatory and insulin sensitizing properties, adiponectin gene knockout mice show surprisingly mild phenotypes (6-8). These findings suggest that there may be other factors that

could compensate the absence of adiponectin. Recent studies shed light on C1q TNF-related protein (CTRP) superfamily as a new adipokine resolving these discrepancies (9,10). The CTRP superfamily is a paralogue of adiponectin that consists of 15 members (11-13). Among CTRPs, CTRP12 (adipolin) is an adipokine which exerts beneficial effects on insulin sensitivity, inflammation and glucose metabolism (14-18). Lower circulating concentration of CTRP12 are found in rodent models of obesity-induced T2D (19). With the aforementioned in mind, circulating CTRP12 concentrations were measured in patients with T2D and in healthy subjects.

Material and Methods

Participants

Thirty T2D patients and 30 healthy subjects aged between 40 and 60 years participated in this case-control study. Participants were recruited from endocrine and metabolism research institute – Tehran University of Medical Sciences. The study protocol was approved by Ethics Committee of AJA University of Medical Sciences in accordance with the declaration of Helsinki and informed consents were obtained from all subjects prior to the study. T2D diagnosis was based on American Diabetes Association criteria; fasting blood sugar (FBS) ≥126 mg/dl, two hours plasma glucose after a standard oral glucose tolerance test (OGTT) ≥ 200 mg/dl or glycated hemoglobin (HbA1c) ≥ 6.5 % (20). Insulin resistance was determined by homeostasis model assessment of insulin resistance (HOMA-IR) using the following formula: HOMA-IR = [FBS (mg/dl) × insulin (µU/ml)]/405 (21). Exclusion criteria included a history of acute or chronic inflammatory diseases, congestive heart failure, liver or kidney diseases, endocrine disorders, malignancy, pregnancy, treatment with anti-inflammatory drugs such as aspirin and insulin therapy.

Anthropometric and clinical assessment

Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured by a standard sphygmomanometer. Participants were asked to rest for 5 minutes in sitting position before blood pressure measurement. Height and weight were measured using a stadiometer and standard weighing balance, respectively. Body mass index (BMI) was calculated as weight in kilograms divided by the square of height in meters. Waist circumference (WC) was measured at the narrowest level over light clothing by a plastic tape meter to the nearest 0.1 cm. Hip was measured at the maximum circumference of the buttocks. Waist-to-hip ratio (WHR) was calculated as waist circumference in centimeters divided by hip circumference in centimeters. Blood samples were taken from all participants after an overnight fasting. Biochemical parameters including fasting blood sugar (FBS), triacylglycerol (TG), total cholesterol, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) were measured by enzymatic methods (Pars Azmoon kits, Iran) using automated BIOLIS 24i Premium autoanalyser (Tokyo Boeki Machinery Ltd., Japan). Glycated hemoglobin (HbA1c) was measured with HPLC method by Tosoh G8 instrument (South San Francisco, CA). Insulin was measured by Insulin AccuBind ELISA Kit (Monobind Inc, California, USA).

Measurement of plasma CTRP12

Plasma levels of CTRP12 were measured using a commercially available ELISA kit (Aviscera, Santa Clara, USA; Catalog number: SK00392-06) according to manufacturer's protocol, with an intra-assay and inter-assay coefficient of variation of less than 8% and 12%, respectively.

Statistical analysis

Qualitative data are shown as proportions. Quantitative data are expressed as the mean value ± standard error of the mean (SEM) or as the median and the 25th to 75th interquartile range (IQR). Assumptions of normality and equal variances were checked to perform the appropriate statistical test. Variables with skewed distribution were logarithm transformed prior to analysis. Normally distributed data were compared using independent t- test and Mann-Whitney U test was used for nonparametric distributions. Partial (age-adjusted) correlation was used for calculation of associations between variables. Subsequently, if individual bivariate correlations achieved statistical significance, variables were entered into a linear regression model and stepwise multiple regression analysis was performed. Statistical analysis was performed using SPSS version 22.0 (SPSS Inc., Chicago, USA). The accepted level of significance was $P < 0.05$.

Results

Anthropometric and clinical characteristics of all participants have been shown in Table 1. WC, SBP, WHR, BMI, FBS, HbA1c, insulin, and HOMA-IR were significantly higher in T2D patients. In contrast, the levels of CTRP12 were significantly lower in T2D compared to healthy participants. There were no significant differences with respect to DBP, weight, hip, and lipid profiles between two groups.

Table 1. Anthropometric and laboratory characteristics of participants

Variable	Control	T2D	P-value
Age (years)	46.44 ± 1.25	55.95 ± 1.97	<0.001
SBP (mmHg)	122.47 ± 1.71	130.65 ± 3.31	0.037
DBP (mmHg)	83.50 (79.50 , 88.125)	80 (70.025 , 82.50)	0.089
Weight (kg)	75.66 ± (3.77)	85.74 ± 3.30	0.051
BMI (kg/m^2)	26.45 ± 1.0	30.88 ± 1.02	0.004
WC (cm)	90.22 ± 4.20	107.05 ± 3.0	0.003
Hip (cm)	111.61 ± 4.66	112.80 ± 2.67	0.827
WHR	0.68 ± 0.02	0.92 ± 0.01	<0.001
FBS (mg/dl)	83.50 (79.50 , 88)	144.50 (133.75 , 186.50)	<0.001
HbA1c (%)	5.39 ± 0.11	7.81 ± 0.32	<0.001
Insulin (µU/ml)	5.70 (4.07 , 16.87)	13.85 (7.30 , 28.02)	0.013
HOMA-IR	1.13 (0.81 , 3.64)	6.39 (2.92 , 10.22)	<0.001
TG (mg/dl)	145.61 ± 22.21	152.05 ± 11.06	0.357
Total cholesterol (mg/dl)	163.38 ± 8.31	157.30 ± 7.94	0.537
HDL-C (mg/dl)	45.50 ± 1.55	43.80 ± 1.59	0.452
LDL-C (mg/dl)	79.83 ± 4.66	86.50 ± 4.42	0.307
CTRP12 (pg/ml)	391.96 ± 0.091	167.36 ± 0.033	0.009

Values are mean ± SEM for data with normal distribution and median (interquartile ranges) for data not normally distributed. SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; WC, waist circumference; WHR, waist to hip ratio; FBS, fasting blood sugar; HbA1c, glycated hemoglobin; HOMA-IR, homeostasis model assessment of insulin resistance; TG, triacylglycerol; HDL-C, high-density lipoprotein; LDL-C, low-density lipoprotein; CTRP12, C1q complement/TNF-related protein 12.

Correlation of CTRP12 with biochemical and metabolic parameters

In all participants, partial correlation analysis showed that plasma levels of CTRP12 were negatively and significantly correlated with FBS and HbA1c (Table 2). When subjected to stepwise multiple regression analysis, HbA1c ($\beta = -2.21$; $P < 0.004$) was predictive of plasma levels of CTRP12 (Table 2).

Table 2. Linear regression analysis of variables associated with CTRP12.

	Simple		Multiple	
Variable	**r**	**P**	**β**	**P**
SBP (mmHg)	0.062	0754		
DBP (mmHg)	0.162	0.406		
Weight (kg)	0.024	0.904		
BMI (kg/m^2)	0.004	0.984		
WC (cm)	-0.002	0.990		
Hip (cm)	0.061	0.757		
WHR	-0.237	0.224		
FBS (mg/dl)	-0.457	0.015		
HbA1c (%)	-0.510	0.008	-2.21	0.004
Insulin (µU/ml)	-0.195	0.319		
HOMA-IR	-0.334	0.083		
TG (mg/dl)	0.106	0.593		
Total cholesterol (mg/dl)	0.022	0.912		
HDL-C (mg/dl)	-0.129	0.512		
LDL-C (mg/dl)	-0.105	0.594		

Partial (age-adjusted) correlation was used for calculation of associations between variables. If individual bivariate correlations achieved statistical significance, stepwise multiple regression analysis with CTRP12 was performed to test the joint effect of these parameters on CTRP12. SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index; WC, waist circumference; WHR, waist to hip ratio; FBS, fasting blood sugar; HbA1c, glycated hemoglobin; HOMA-IR, homeostasis model assessment of insulin resistance; TG, triacylglycerol; HDL-C, high-density lipoprotein; LDL-C, low-density lipoprotein.

Discussion

The importance of CTRP superfamily as a new adipokine in the pathogenesis of T2D is still emerging (22). CTRP12 is one of the members of this superfamily with anti-inflammatory, insulin sensitivity and glucose lowering effects (14-18). Despite ample evidence from in vitro and animal models, clinical evidences are still limited. In this context, the current study adds new knowledge in the area being studied. The main findings of this case-control study were; a) circulating levels of CTRP12 were lower in T2D patients compared to healthy subjects b) Plasma levels of CTRP12 were negatively and significantly correlated with FBS and HbA1c. Using regression analysis, HbA1c was found to be predictive of plasma levels of CTRP12.

The findings regarding plasma levels of CTRP12 in diabetic patients were consistent with a recent report (22) in which levels of CTRP12 were lower in diabetic patients compared to age and BMI matched control group.

Obesity, as is shown in our data, is characterized by a chronic inflammatory state contributing to the development of insulin resistance. Enomoto et al. (23) showed that obesity is linked with the reduced expression of plasma CTRP12. Thus, it is plausible that obesity enhances the development of insulin resistance via suppression of insulin sensitizing effect of CTRP12.

An in-depth study of polycystic ovary syndrome (PCOS), a pro- inflammatory state associated with obesity and diabetes, revealed that serum and subcutaneous adipose tissue CTRP12 mRNA expression and protein concentrations were significantly lower in women with PCOS compared with control subjects (16).

Based on linear regression analysis, it was found that CTRP12 was significantly and negatively correlated with FBS, and HbA1c. This fact can be explained by the observations in the study by Bai *et al*. (22) showing that glucose as OGTT significantly decreased levels of CTRP12 in T2D patients. Tan et al. showed that serum CTRP12 concentrations were significantly and negatively correlated with glucose (16). Moreover, glucose profoundly reduced and metformin significantly increased CTRP12 protein production in human adipose tissue explants respectively (16). In another study by Tan et al. on healthy subjects, it was revealed that hyperinsulinaemic induction in healthy lean human subjects significantly increased circulating levels of CTRP12 (18). Furthermore, in subcutaneous adipose tissue explants, insulin significantly increased CTRP12 protein expression and secretion (18). In another study, it was shown that metformin treatment substantially increased serum CTRP12 concentrations in women with PCOS (17). Serum CTRP12 decreased significantly in response to the OGTT in PCOS and control subjects (17). Moreover, it is noteworthy to mention that increasing CTRP12 levels in animal models of obesity and diabetes lowers blood glucose concentrations (24). Taken together, these explanations illustrate that there is a negative correlation between glucose parameters and levels of CTRP12 in line with the results of the present study.

In a recent study by Mehrdadi et al, it was demonstrated that supplementation with coenzyme Q10 reduced the HbA1c significantly in patients with T2D, although interestingly adipolin levels declined considerably at the same time. They concluded that the depressing effect of coenzyme Q10 on adipolin levels might be due to improved glucose homeostasis, which was expected to be mediated by increasing adipolin concentration. As a result, the adipolin requirement, its secretion to blood, and its serum levels declined (25).

The current study had a few limitations including a relatively small sample size. Hence, we advise caution with regard to the findings; further studies with a larger number of subjects are needed to confirm the findings of the present study. Moreover, since metformin affects the CTRP12 levels, it is needed to perform a study in newly diagnosed T2D to preclude the effects of drugs on circulating levels of CTRP12. Finally, the present study only involved the Iranian population. Thus, findings should be generalized cautiously to other populations.

Conclusion

The present study revealed that the plasma levels of CTRP12 are lower in T2D patients compared to those of healthy controls. In addition, the results showed that CTRP12 levels could predict as well as diagnose T2D. Further studies are required to investigate the mechanistic action of CTRP12 in T2D patients.

Ethics approval and consent to participate

The study protocol was approved by Ethics Committee of AJA University of Medical Sciences in accordance with the declaration of Helsinki and informed consents were obtained from all subjects prior to study.

Competing interests

The authors declare that they have no competing interests.

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References

1. Organization WH. Classification of diabetes mellitus. 2019.
2. Ouchi N, Parker JL, Lugus JJ, Walsh K. Adipokines in inflammation and metabolic disease. Nat Rev Immunol 2011; 11(2):85-97.
3. Khodabandehloo H, Gorgani-Firuzjaee S, Panahi G, Meshkani R. Molecular and cellular mechanisms linking inflammation to insulin resistance and β-cell dysfunction. Transl Res 2016; 167(1):228-56.
4. Lindberg S, Jensen JS, Pedersen SH, Galatius S, Frystyk J, Flyvbjerg A, et al. Low adiponectin levels and increased risk of type 2 diabetes in patients with myocardial infarction. Diabetes Care 2014; 37(11):3003-8.
5. Wang Y, Meng RW, Kunutsor SK, Chowdhury R, Yuan JM, Koh WP, et al. Plasma adiponectin levels and type 2 diabetes risk: a nested case-control study in a Chinese population and an updated meta-analysis. Sci Rep 2018; 8(1):406.
6. Kubota N, Terauchi Y, Yamauchi T, Kubota T, Moroi M, Matsui J, et al. Disruption of adiponectin causes insulin resistance and neointimal formation. J Biol Chem 2002; 277(29):25863-6.
7. Maeda N, Shimomura I, Kishida K, Nishizawa H, Matsuda M, Nagaretani H, et al. Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. Nat Med 2002; 8(7):731-7.
8. Nawrocki AR, Rajala MW, Tomas E, Pajvani UB, Saha AK, Trumbauer ME, et al. Mice lacking adiponectin show decreased hepatic insulin

sensitivity and reduced responsiveness to peroxisome proliferator-activated receptor γ agonists. J Biol Chem 2006; 281(5):2654-60.

9. Davis KE, Scherer PE. Adiponectin: no longer the lone soul in the fight against insulin resistance? Biochem J 2008; 416(2):e7-9.

10. Wong GW, Krawczyk SA, Kitidis-Mitrokostas C, Revett T, Gimeno R, Lodish HF. Molecular, biochemical and functional characterizations of C1q/TNF family members: adipose-tissue-selective expression patterns, regulation by PPAR-gamma agonist, cysteine-mediated oligomerizations, combinatorial associations and metabolic functions. Biochem J 2008; 416(2):161-77.

11. Flehmig G, Scholz M, Kloting N, Fasshauer M, Tonjes A, Stumvoll M, et al. Identification of adipokine clusters related to parameters of fat mass, insulin sensitivity and inflammation. PLoS One 2014; 9(6):e99785.

12. Schaffler A, Buechler C. CTRP family: linking immunity to metabolism. Trends Endocrinol Metab 2012; 23(4):194-204.

13. Wong GW, Wang J, Hug C, Tsao TS, Lodish HF. A family of Acrp30/adiponectin structural and functional paralogs. Proc Natl Acad Sci U S A 2004; 101(28):10302-7.

14. Bell-Anderson KS, Funnell AP, Williams H, Jusoh HM, Scully T, Lim WF, et al. Loss of Krüppel-like factor 3 (KLF3/BKLF) leads to upregulation of the insulin-sensitizing factor adipolin (FAM132A/CTRP12/C1qdc2). Diabetes 2013; 62(8):2728-37.

15. Enomoto T, Ohashi K, Shibata R, Higuchi A, Maruyama S, Izumiya Y, et al. Adipolin/C1qdc2/CTRP12 protein functions as an adipokine that improves glucose metabolism. J Biol Chem 2011; 286(40):34552-8.

16. Tan BK, Chen J, Adya R, Ramanjaneya M, Patel V, Randeva HS. Metformin increases the novel adipokine adipolin/CTRP12: role of the AMPK pathway. J Endocrinol 2013; 219(2):101-8.

17. Tan BK, Chen J, Hu J, Amar O, Mattu HS, Ramanjaneya M, et al. Circulatory changes of the novel adipokine adipolin/CTRP12 in response to metformin treatment and an oral glucose challenge in humans. Clin Endocrinol (Oxf) 2014; 81(6):841-6.

18. Tan BK, Lewandowski KC, O'Hare JP, Randeva HS. Insulin regulates the novel adipokine adipolin/CTRP12: in vivo and ex vivo effects. J Endocrinol 2014; 221(1):111-9.

19. Wei Z, Peterson JM, Lei X, Cebotaru L, Wolfgang MJ, Baldeviano GC, et al. C1q/TNF-related protein-12 (CTRP12), a novel adipokine that improves insulin sensitivity and glycemic control in mouse models of obesity and diabetes. J Biol Chem 2012; 287(13):10301-15.

20. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2010; 33(Suppl 1):S62–9.

21. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28(7):412-9.

22. Bai B, Ban B, Liu Z, Zhang MM, Tan BK, Chen J. Circulating C1q complement/TNF-related protein (CTRP) 1, CTRP9, CTRP12 and CTRP13 concentrations in Type 2 diabetes mellitus: In vivo regulation by glucose. PloS One 2017; 12(2):e0172271.

23. Enomoto T, Shibata R, Ohashi K, Kambara T, Kataoka Y, Uemura Y, et al. Regulation of

adipolin/CTRP12 cleavage by obesity. Biochem Biophys Res Commun 2012; 428(1):155-9.

24. Wei Z, Peterson JM, Lei X, Cebotaru L, Wolfgang MJ, Baldeviano GC, et al. C1q/TNF-related protein-12 (CTRP12), a novel adipokine that improves insulin sensitivity and glycemic control in mouse models of obesity and diabetes. J Biol Chem 2012; 287(13):10301-15.

25. Mehrdadi P, Mohammadi RK, Alipoor E, Eshraghian MR, Esteghamati A, Hosseinzadeh-Attar M. The effect of coenzyme q10 supplementation on circulating levels of novel adipokine adipolin/CTRP12 in overweight and obese patients with type 2 diabetes. Exp Clin Endocrinol Diabetes 2017; 125(03):156-62.