Original Paper

The Effects of Adiponectin and Adiponectin Receptor 1 Levels on Macrovascular Complications Among Patients with Type 2 Diabetes Mellitus

Na Su[a]   Nairui Zhao[a]   Guangya Wang[a]   Linxia Wang[a]   Yunna Zhang[a]   Ruijie Li[a]
Ying Liu[b]   Xinxin Yang[a]   Cuiliu Li[a]   Mingming Hou[c]

[a]Department of Second Endocrinology, Cangzhou Central Hospital, Cangzhou, China, [b]Department of Second Ultrasound, Cangzhou Central Hospital, Cangzhou, China, [c]Department of Medical Records, Cangzhou Central Hospital, Cangzhou, China

Key Words

Adiponectin • Adiponectin receptor 1 • Type 2 diabetes mellitus • Macrovascular complications

Abstract

Background/Aims: The present study aimed to investigate the serum levels of adiponectin (APN) and adiponectin receptor 1 (AdipoR1) in patients with type 2 diabetes mellitus (T2DM) combined with macrovascular complications (MVC), as well as their correlation with clinical parameters. ***Methods:*** A total of 60 T2DM patients were divided into 2 groups according to the presence of MVC: T2DM + MVC group (n=30) and T2DM group (n=30). Additionally, 30 healthy people were selected as control group (NC group). Clinical data and biological parameters were detected and recorded. T test was performed to compare the differences between two groups, and the results were corrected using Bonferroni method. Meanwhile, the correlation analysis and multiple stepwise regression analysis were used to analyze the association of APN and AdipoR1 with clinical factors. ***Results:*** The levels of APN and AdipoR1 were significantly decreased in T2DM group and T2DM + MVC group compared with NC group, with the lowest value in T2DM + MVC group (all $P < 0.01$). Serum APN levels were positively correlated with FINS and TG ($r = 0.412, 0.316$, respectively; both $P<0.05$), and negatively correlated with SBP, DBP and LDL-C ($r = -0.292, -0.383, -0.334$, respectively; all $P<0.05$). Serum levels of AdipoR1 were positively correlated with APN ($r = 0.726, P<0.01$), and negatively correlated with BMI, SBP, DBP, FBG, TC and LDL-C ($r = -0.440, -0.446, -0.374, -0.444, -0.344, -0.709$, respectively; all $P<0.01$) ***Conclusion:*** Serum levels of APN and AdipoR1 are significantly lower in T2DM group and T2DM + MVC group, showing lowest value in T2DM + MVC group. APN and AdipoR1 levels may influence glucose and lipid metabolism in T2DM patients.

© 2019 The Author(s). Published by
Cell Physiol Biochem Press GmbH&Co. KG

Na Su

Department of Second Endocrinology, Cangzhou Central Hospital
Cangzhou (China)
E-Mail sdourirr@163.com

Cellular Physiology and Biochemistry

Cell Physiol Biochem 2019;52:225-231

DOI: 10.33594/000000016
Published online: 28 February 2019

© 2019 The Author(s). Published by
Cell Physiol Biochem Press GmbH&Co. KG

226

Su et al.: APN and AdipoR1 in Macrovascular Complications Induced by T2DM

Introduction

Diabetes mellitus (DM) is a chronic disease, which significantly influences human health worldwide. The number of patients with DM is increasing rapidly along with the improvement of people's living standard, the aging of population and the change of life style. Type 2 diabetes mellitus (T2DM) is the major type of DM, accounting for more than 90% DM cases [1, 2]. According to the studies published in JAMA in 2013, the prevalence of T2DM in China has reached 11.6%, and the total number of the patients is up to 114 million, ranking the first position in the world. T2DM has become the third threat to people's health, after tumor and cardiovascular disease [3, 4].

Diabetic macrovascular disease (MVC) is a common complication of T2DM, and atherosclerosis (AS) represents the basic pathological change, which increases the risk of myocardial infarction, stroke, intermittent claudication and ischemic gangrene. To explore the potential risk factors for T2DM complicated with MVC is beneficial for the prevention and treatment in clinic.

Adiponectin (APN) is a cytokine secreted by adipose tissue, and highly expressed in white adipose tissue. APN can activate 5 '-AMP activated protein kinase AMPK pathway to improve glucose uptake and utilization and free fatty acid oxidation in skeletal muscle, liver and adipose tissue, thus regulating metabolism and insulin sensitivity [5-7]. APN can also inhibit the damage on vascular endothelial cells from inflammatory factors and protect the cardiovascular system [8, 9]. Recent studies have shown that APN is associated with obesity, insulin resistance, T2DM, AS, etc [10]. There are two APN receptors: adiponectin receptor 1 (AdipoR1) and adiponectin receptor 2 (AdipoR2). AdipoR1 and AdipoR2 express in a variety of tissues, but their distribution is different [11]. AdiPoR1 expression is mainly observed in vascular endothelial cells and macrophages *in vitro* [12], suggesting that AdiPoR1 may be involved in vascular physiology and pathology.

In this study, we investigated serum levels of APN and AdipoR1 in patients with T2DM complicated with MVC. Furthermore, we analyzed the correlation of APN with AdipoR1 in T2DM complicated with MVC.

Materials and Methods

Patients

All experiments in this study were approved by the ethics committee of Cangzhou Central Hospital. The patients and their families had signed written informed consents.

All T2DM patients were randomly selected from the Cangzhou Central Hospital medical examination center and Department of Endocrinology. T2DM cases were diagnosed based on the standard posed by WHO in 1999. All the patients received routine blood and urine tests, abdominal CT scan, B-ultrasonography and fundus examination. Additionally, the clinical information of the patients was also collected from the medical records. The clinical records and the laboratory measurement results were analyzed by two experienced clinicians. Based on the analysis results, the patients presenting any one of the following conditions would be excluded: 1) acute infectious diseases, 2) autoimmune diseases, 3) severe organ dysregulation, such as heart, liver and kidney dysregulation, 4) malignant tumors, 5) diabetic nephropathy, and 6) diabetic retinopathy. The patients were divided into two groups according to the presence of MVC: patients only with T2DM (T2DM group) and T2DM patients with MVC (T2DM + MVC group).

Patients would be confirmed with MVC when they met at least one of the following criteria: (1) the history of coronary heart disease (CHD) or clinical CHD diagnosed through electrocardiogram (ECG) and coronary angiography; (2) the history of cerebrovascular disease or cerebral CT-/magnetic resonance examination-confirmed cerebral infarction or cerebral hemorrhage; (3) with carotid plaques or stenosis shown in color Doppler ultrasound examination; (4) the history of arterial occlusive disease in lower extremity or lower extremity atherosclerosis, stenosis or occlusion demonstrated in color Doppler ultrasound examination. Meanwhile, 30 healthy persons were selected as control group (NC group).

Cellular Physiology and Biochemistry

Cell Physiol Biochem 2019;52:225-231

DOI: 10.33594/000000016
Published online: 28 February 2019

© 2019 The Author(s). Published by
Cell Physiol Biochem Press GmbH&Co. KG

227

Su et al.: APN and AdipoR1 in Macrovascular Complications Induced by T2DM

Collection of clinical data and specimens

On the day of admission, the basic parameters of the patients were measured, including height, weight, waist circumference, hip circumference, systolic blood pressure (SBP) and diastolic blood pressure (DBP). Body height was measured when the patients were in their socks, and the weight was detected with light clothing. Waist circumference was defined as the level perimeter between the lowest ribs and the hip bones with hands on the hips, and measured using a tape. Blood pressure was detected using an automatic upper arm oscillometric at the right brachial artery.

Waist hip rate (WHR) = waist circumference / hip circumference; body mass index (BMI) = weight / height^2 (kg/m^2).

Venous blood specimens was collected from forearm vein (empty stomach) to detect fasting blood glucose (FBG), glycosylated hemoglobin (HbAlc), triglyceride (TG), total cholesterol (TC), Low density Lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), fasting insulin (FINS) and APN. All test procedures were carried out using an autoanalyzer (Siemens Advia 1800, Siemens Healthcare GmbH, Henkestr, Germany) according to the manufacturer's instructions.

RNA extraction and quantitative real-time polymerase chain-reaction (qRT-PCR)

Total RNA was extracted from the serum samples using mirVana miRNA Kit (Ambion, Austin, TX, USA), and its concentration and quality were then detected using NanoDrop ND-1000 Spectrophotometer (Agilent, USA). The extracted RNA samples with an OD A260/A280 ratio of 1.9-2.0 were used to synthesize complementary DNA (cDNA). Subsequently, the relative expression of AdipoR1 was measured using the method of quantitative real-time PCR (qRT-PCR). The reaction was performed using SYBR Premix Ex Taq (Takara, Dalian, China) in the Applied Biosystems 7900 Fast Real-Time PCR system (Applied Biosystems, Foster City, California, USA). GAPDH served as an internal control, and the primer sequences were as follows: AdipoR1, forward: 5'-AAGTTGGATTATTCAGGAA-3', reverse: 5'-AATGGAGAGGTAGATGAG-3'; APN, forward: 5'-GCCTACCACATCACAGTC-3', reverse: 5'-GCATTACCTGAGATACGACT-3'; GAPDH, forward: 5'-TGGACTCCACGACGTACTCAG-3', reverse: 5'-CGGGAAGCTTGTCATCAATGGAA-3'. The relative expression of AdipoR1 was calculated via $2^{-\Delta\Delta Ct}$ method. Each experiment was repeated three times.

Statistical analysis

In this study, SPSS 21 statistical software was used to implement statistical analysis. All measurement data were summarized and expressed as mean ± standard deviation (SD). Kolmogorove-Smirnov test was performed to detect whether the data were in normal distribution. If not, the data should be log-transformed. T test was used to compare the differences between two groups. Correlation analysis and multiple stepwise regression analysis were used to analyze the relationship of APN and AdipoR1 with other factors. P < 0.05 indicated that the difference was statistically significant. In multiple analyses, *P* values were adjusted using by Bonferroni method.

Results

Comparison of clinical parameters between groups

A total of 30 patients with T2DM combined with MVC were included in this study. There were 30 patients only with T2DM, and 30 healthy volunteer composed the normal control group. No one was excluded from the study. And all data are shown in Table 1. The data in our study were normally distributed, so the comparisons between two groups were performed using student's t test. We found that the levels of BMI, WHR, SBP, DBP, FBG, FINS, HbAlc, TG and TC in T2DM and T2DM + MVC groups were significantly higher than those in NC group (all *P* < 0.01). Furthermore, the differences were still significant after corrected with Bonferroni method (all *P* < 0.025). The level of HDL-C was significantly decreased in T2DM (*P*=0.008) and T2DM + MVC groups when compared to control group (*P*=0.036). The difference between NC and T2DM groups was significant after correction, but not between T2DM + MVC and NC groups. In addition, the levels of DBP and LDL-C were observably

Cellular Physiology and Biochemistry

Cell Physiol Biochem 2019;52:225-231

DOI: 10.33594/000000016
Published online: 28 February 2019

© 2019 The Author(s). Published by
Cell Physiol Biochem Press GmbH&Co. KG

228

Su et al.: APN and AdipoR1 in Macrovascular Complications Induced by T2DM

higher in T2DM + MVC group than that in T2DM group ($P < 0.05$ for both). However, the difference was insignificant after correction using Bonferroni method ($P>0.025$). There was no significant difference in the other indexes (all $P > 0.05$) (Table 1).

The expression of APN and AdipoR1

QRT-PCR assay showed that the level of APN was significantly decreased in T2DM and T2DM + MVC groups when compared with NC group (all $P < 0.05$). Moreover, serum level of APN was obviously lower in T2DM + MVC group than that in T2DM group (Fig. 1).

The results for AdipoR1 expression were similar to the detection of APN. AdipoR1 expression level was observably down-regulated in T2DM and T2DM + MVC groups when compared to control group, with the lowest value in T2DM + MVC group (Fig. 2).

Correlation analyses between APN and other parameters in T2DM and T2DM + MVC groups

Correlation analyses showed that serum APN levels were positively correlated with FINS and TG ($r = 0.412, 0.316$, respectively; both $P<0.05$), and negatively correlated with SBP, DBP and LDL-C ($r = -0.292, -0.383, -0.334$, respectively; all $P<0.05$) (Table 2).

Correlation analyses between AdipoR1 and other parameters in T2DM and T2DM + MVC groups

Correlation analyses on serum AdipoR1 indicated that serum expression levels of AdipoR1 were positively correlated with APN ($r = 0.726, P<0.01$), and negatively correlated with BMI, SBP, DBP, FBG, TC and LDL-C ($r = -0.440, -0.446, -0.374, -0.444, -0.344, -0.709$, respectively; all $P<0.01$) (Table 3).

Table 1. Comparison on clinical parameters between groups. Notes: NC group: Normal healthy control group; T2DM group: Type 2 diabetes without macrovascular disease group; T2DM + MVC group: Type 2 diabetes mellitus complicated with macrovascular disease. *,P<0.05 when compared with the NC group, **, P<0.01 when compared with the NC group, α: compared with NC group after adjusting using Bonferroni method; #, P<0.05 when compared with the T2DM group, ##, P<0.01 when compared with the T2DM group, β: compared with T2DM group after corrected using Bonferroni method. No significant differences were detected in the mentioned parameters between T2DM and T2DM + MVC groups

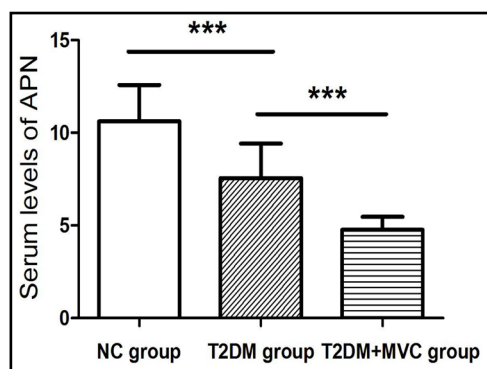| Parameters	NC group	T2DM group	T2DM + MVC group
Gender (M/F)	16/14	17/13	16/14
Age	54.83±6.05	54.97±5.54	54.80±5.34
BMI	21.69±1.39	24.23±0.85**,α	24.96±0.55**,α
WHR	0.81±0.06	0.95±0.14**,α	0.99±0.12**,α
SBP	117.47±7.12	122.87±6.80**,α	128.60±4.87**,α
DBP	72.17±6.60	77.50±7.28**,α	81.67±4.01**,α; #
FBG	4.64±0.32	9.81±0.31**,α	10.07±0.12**,α
FINS	5.89±0.63	6.75±0.71**,α	6.56±0.73**,α
HbA1c	5.50±0.31	9.49±0.33**,α	9.58±0.25**,α
TG	1.34±0.16	1.96±0.30**,α	1.93±0.28**,α
TC	4.29±0.29	5.42±0.23**,α	5.80±0.53**,α
LDL-C	2.77±0.31	2.80±0.28	3.27±0.16**,α; #
HDL-C	1.38±0.20	1.19±0.25**,α	1.11±0.22*

Fig. 1. Serum levels of APN in three groups. The results showed that the levels of APN was significantly reduced in T2DM and T2DM + MVC groups. ***: P<0.001.
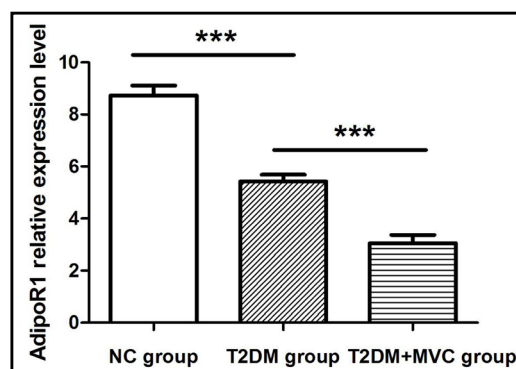

Fig. 2. The expression level of AdipoR1 in three groups. The results showed that the levels of AdipoR1 was significantly reduced in T2DM and T2DM + MVC groups. ***: P<0.001.

Cellular Physiology and Biochemistry

Cell Physiol Biochem 2019;52:225-231

DOI: 10.33594/000000016
Published online: 28 February 2019

© 2019 The Author(s). Published by
Cell Physiol Biochem Press GmbH&Co. KG

229

Su et al.: APN and AdipoR1 in Macrovascular Complications Induced by T2DM

Table 2. Correlation analyses between APN and other parameters in T2DM and T2DM + MVC groups

Variable	Age	BMI	WHR	SBP	DBP	FBG	FINS	HbAlc	TG	TC	LDL-C	HDL-C
r	0.138	-0.189	0.066	-0.292	-0.383	0.043	0.412	0.24	0.316	-0.165	-0.334	0.107
P	0.293	0.149	0.617	0.024	0.003	0.747	0.001	0.064	0.014	0.207	0.009	0.418

Table 3. Correlation analyses between AdipoR1 and other parameters in T2DM and T2DM + MVC groups

Variable	Age	BMI	WHR	SBP	DBP	FBG	FINS	HbAlc	TG	TC	LDL-C	HDL-C	APN
r	0.064	-0.44	-0.148	-0.446	-0.374	-0.444	0.115	-0.09	0.138	-0.344	-0.709	0.183	0.726
P	0.626	0.000	0.259	0.000	0.003	0.000	0.382	0.496	0.294	0.007	0.000	0.161	0.000

Discussion

APN gene is located in human chromosome 3q27 region, which has been found to be associated with T2DM and metabolic syndrome [13-15]. Its encoding protein contains 244 amino acids with a molecular weight of 30kDa. APN protein consists of four domains: the N-terminal signal peptide region, the non helical functional domain, the collagen domain and the carboxyl terminal globular domain [16]. Previous studies have shown that serum APN levels were significantly decreased in individuals with obesity, T2DM, metabolic syndrome, coronary heart disease and/or hypertension [17]. In this study, serum APN levels in T2DM and T2DM + MVC groups were lower than that in NC group. Compared with healthy controls, the T2DM cases with or without MVC exhibited significantly lower level of serum APN, which was consistent with findings from previous studies. Yamauchi T et al. found that APN could enhance muscle fatty acid oxidation and energy consumption, and reduce the level of triglyceride in liver and muscle, thereby reversing insulin resistance [13]. Maeda N et al. reported that APN could improve insulin sensitivity via increasing muscle fatty acid oxidation and inhibiting hepatic glucose output in APN gene-knockout mice [18]. APN can regulate glucose metabolism and improve insulin sensitivity. In our study, serum APN levels had highly positive correlation with FINS ($P < 0.01$), which was consistent with the previous studies [19]. In addition, we found that the T2DM patients combined with MVC showed obviously lower level of APN when compared to T2DM only patients. Nilsson PM et al. also got similar conclusion, and indicated that the serum level of APN was negatively correlated with carotid intima-media thickness, suggesting that APN was a biological marker for subclinical atherosclerosis and could predict long-term myocardial infarction and cerebral infarction [20].

The genome-wide analysis of human genome concluded that the chromosome 3q27 is a susceptible region for T2DM and metabolic syndrome, and coding genes for APN and its receptor are located in this region. Therefore, we hypothesized that AdipoR1 and AdipoR2 could affect individual susceptibility to T2DM and insulin. A family study demonstrated that people with a family history of T2DM had lower levels of AdipoR1/R2 than those without family history. Furthermore, the expression of AdipoR1/R2 showed close association with insulin sensitivity [21]. In our study, we detected the expression of AdipoR1 in three groups, and the results showed that AdipoR1 expression was significantly reduced in T2DM + MVC and T2DM groups, which was consistent with the conclusion by Civitarese et al. [21]. However, the study by Staiger et al. indicated that there was no correlation between AdipoR1/R2 mRNA and insulin sensitivity [22]. The divergences might be attributed to different populations and relative small sample size. Further studies should be performed to test those results.

In addition, we also analyzed the association of serum APN level and AdipoR1with clinical parameters among T2DM cases and T2DM patients combined with MVC. We found that serum APN and AdipoR1 levels were negatively correlated with SBP and DBP. Furthermore, the expressions of APN and AdipoR1 were also obviously correlated with FBG, TC, LDL-C HDL-C. And APN defect might promote diabetic progression and increase the risk

Cellular Physiology and Biochemistry

Cell Physiol Biochem 2019;52:225-231

DOI: 10.33594/000000016
Published online: 28 February 2019

© 2019 The Author(s). Published by
Cell Physiol Biochem Press GmbH&Co. KG

230

Su et al.: APN and AdipoR1 in Macrovascular Complications Induced by T2DM

of hypertension and cardiovascular disease. Hussain et al. suggested that genetic alterations in the coding gene for APN could significantly influence disease development and serum lipid concentrations in T2DM patients [23]. Jiang et al. reported that serum APN was negatively correlated with peripheral arterial disease onset in T2DM patients [24]. However, there were still some shortcomings in current study. Firstly, the sample size was relatively small, which reduced the statistical power of our findings. Secondly, the molecular mechanism of AdipoR1 and APN in regulating glucose and lipid metabolism in T2DM remained to be further studied.

Conclusion

The serum levels of APN and AdipoR1 are significantly lower in T2DM and T2DM + MVC groups, and exhibit the lowest level in T2DM + MVC group. Low APN and AdipoR1 levels are associated with T2DM and MVC.

Disclosure Statement

The authors declare to have no competing interests.

References

1   American Diabetes Association: Diagnosis and classification of diabetes mellitus: Diabetes Care 2012;35:S64-71.
2   Sun G, Ma M, Ye N, Wang J, Chen Y, Dai D, Sun Y: Diabetes mellitus is an independent risk factor for atrial fibrillation in a general Chinese population. J Diabetes Investig 2016;7:791-796.
3   Xu Y, Wang L, He J, Bi Y, Li M, Wang T, Jiang Y, Dai M, Lu J, Xu M, Li Y, Hu N, Li J, Mi S, Chen CS, Li G, Mu Y, Zhao J, Kong L, Chen J, et al.: Prevalence and control of diabetes in Chinese adults. JAMA 2013;310:948-959.
4   Ren Y, Zhang M, Zhao J, Wang C, Luo X, Zhang J, Zhu T, Li X, Yin L, Pang C, Feng T, Wang B, Zhang L, Li L, Yang X, Zhang H, Hu D: Association of the hypertriglyceridemic waist phenotype and type 2 diabetes mellitus among adults in China. J Diabetes Investig 2016;7:689-694.
5   Achari AE, Jain SK: Adiponectin, a Therapeutic Target for Obesity, Diabetes, and Endothelial Dysfunction. Int J Mol Sci 2017;18:pii:E1321.
6   Yamauchi T, Kadowaki T: Physiological and pathophysiological roles of adiponectin and adiponectin receptors in the integrated regulation of metabolic and cardiovascular diseases. Int J Obes (Lond) 2008;32:S13-18.
7   Liu M, Liu F: Regulation of adiponectin multimerization, signaling and function. Best Pract Res Clin Endocrinol Metab 2014;28:25-31.
8   Ohashi K, Ouchi N, Matsuzawa Y: Anti-inflammatory and anti-atherogenic properties of adiponectin. Biochimie 2012;94:2137-2142.
9   Letra L, Sena C: Cerebrovascular Disease: Consequences of Obesity-Induced Endothelial Dysfunction. Adv Neurobiol 2017;19:163-189.
10   van Andel M, Heijboer AC, Drent ML: Adiponectin and Its Isoforms in Pathophysiology. Adv Clin Chem 2018;85:115-147.
11   Yamauchi T, Iwabu M, Okada-Iwabu M, Kadowaki T: Adiponectin receptors: a review of their structure, function and how they work. Best Pract Res Clin Endocrinol Metab 2014;28:15-23.
12   Parker-Duffen JL, Walsh K: Cardiometabolic effects of adiponectin. Best Pract Res Clin Endocrinol Metab 2014;28:81-91.
13   Yamauchi T, Kamon J, Waki H, Terauchi Y, Kubota N, Hara K, Mori Y, Ide T, Murakami K, Tsuboyama-Kasaoka N, Ezaki O, Akanuma Y, Gavrilova O, Vinson C, Reitman ML, Kagechika H, Shudo K, Yoda M, Nakano Y, Tobe K, et al.: The fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. Nat Med 2001;7:941-946.

14   Lindberg S, Jensen JS, Pedersen SH, Galatius S, Frystyk J, Flyvbjerg A, Bjerre M, Mogelvang R: Low adiponectin levels and increased risk of type 2 diabetes in patients with myocardial infarction. Diabetes Care 2014;37:3003-3008.

15   Wang G, Gao S, Su N, Xu J, Fu D: Plasma Adiponectin Levels Inversely Correlate to Clinical Parameters in Type 2 Diabetes Mellitus Patients with Macrovascular Diseases. Ann Clin Lab Sci 2015;45:287-291.

16   Scherer PE, Williams S, Fogliano M, Baldini G, Lodish HF: A novel serum protein similar to C1q, produced exclusively in adipocytes. J Biol Chem 1995;270:26746-26749.

17   Arita Y, Kihara S, Ouchi N, Takahashi M, Maeda K, Miyagawa J, Hotta K, Shimomura I, Nakamura T, Miyaoka K, Kuriyama H, Nishida M, Yamashita S, Okubo K, Matsubara K, Muraguchi M, Ohmoto Y, Funahashi T, Matsuzawa Y: Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. 1999. Biochem Biophys Res Commun 2012;425:560-564.

18   Maeda N, Shimomura I, Kishida K, Nishizawa H, Matsuda M, Nagaretani H, Furuyama N, Kondo H, Takahashi M, Arita Y, Komuro R, Ouchi N, Kihara S, Tochino Y, Okutomi K, Horie M, Takeda S, Aoyama T, Funahashi T, Matsuzawa Y: Diet-induced insulin resistance in mice lacking adiponectin/ACRP30. Nat Med 2002;8:731-737.

19   Kim MJ, Yoo KH, Park HS, Chung SM, Jin CJ, Lee Y, Shin YG, Chung CH: Plasma adiponectin and insulin resistance in Korean type 2 diabetes mellitus. Yonsei Med J 2005;46:42-50.

20   Nilsson PM, Engstrom G, Hedblad B, Frystyk J, Persson MM, Berglund G, Flyvbjerg A: Plasma adiponectin levels in relation to carotid intima media thickness and markers of insulin resistance. Arterioscler Thromb Vasc Biol 2006;26:2758-2762.

21   Civitarese AE, Jenkinson CP, Richardson D, Bajaj M, Cusi K, Kashyap S, Berria R, Belfort R, DeFronzo RA, Mandarino LJ, Ravussin E: Adiponectin receptors gene expression and insulin sensitivity in non-diabetic Mexican Americans with or without a family history of Type 2 diabetes. Diabetologia 2004;47:816-820.

22   Staiger H, Kaltenbach S, Staiger K, Stefan N, Fritsche A, Guirguis A, Peterfi C, Weisser M, Machicao F, Stumvoll M, Haring HU: Expression of adiponectin receptor mRNA in human skeletal muscle cells is related to *in vivo* parameters of glucose and lipid metabolism. Diabetes 2004;53:2195-2201.

23   Hussain MK, Deli FA, Algenabi AHA, Abdul-Rudha KH: Adiponectin gene polymorphisms as a predictor for development of type 2 diabetes mellitus in Iraqi population. Gene 2018;662:118-122.

24   Jiang W, Tang Q, Zhang L, Chao Y, Hong B: Association of serum C1q tumour necrosis factor-related protein 9 with the severity of lower extremity peripheral arterial disease in type 2 diabetes patients. Diab Vasc Dis Res 2018;15:270-273.