

Adiponectin and pro-inflammatory cytokines are modulated in Vietnamese patients with type 2 diabetes mellitus

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Keywords

Cytokines, Overweight, Type 2 diabetes mellitus

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J Diabetes Investig 2017; 8: 295–305

doi: 10.1111/jdi.12579

ABSTRACT

Aims/Introduction: Adipose tissue-derived hormones are associated with metabolic disorders including type 2 diabetes mellitus. The present study investigated the levels of adiponectin and pro-inflammatory cytokines including tumor necrosis factor- α (TNF- α), interleukin-1 beta (IL-1 β) and IL-10 in Vietnamese patients with type 2 diabetes mellitus, and their correlations with clinical parameters of overweight and type 2 diabetes mellitus.

Materials and Methods: Based on body mass index, 73 patients with type 2 diabetes mellitus were categorized either as overweight or non-overweight. As healthy controls, 57 overweight and non-overweight individuals without type 2 diabetes mellitus were included. The adiponectin, TNF- α , IL-1 β and IL-10 levels were measured in the sera samples in all study participants by enzyme-linked immunosorbent assay and were correlated with clinical parameters.

Results: The adiponectin levels were lower in patients with type 2 diabetes mellitus ($2.5 \pm 1.5 \mu\text{g/mL}$) compared with controls ($16 \pm 18.6 \mu\text{g/mL}$; $P < 0.0001$), and were decreased in overweight individuals compared with those who were not overweight. The TNF- α and IL-1 β levels were increased, whereas the IL-10 levels were decreased in patients with type 2 diabetes mellitus and in overweight controls compared with non-overweight controls ($P < 0.0001$). The adiponectin levels were correlated with the TNF- α , IL-1 β , IL-10 levels, and the clinical parameters of overweight and type 2 diabetes mellitus. The quantitative insulin sensitivity check index and homeostasis model assessment insulin resistance indexes were correlated with the relative ratios of adiponectin/TNF- α , adiponectin/IL-1 β , adiponectin/IL-10, TNF- α /IL-10 and IL-1 β /IL-10.

Conclusions: Adiponectin and pro-inflammatory cytokines are associated with type 2 diabetes mellitus, and might serve as a prognostic marker and a therapeutic intervention for overweight-related type 2 diabetes mellitus.

INTRODUCTION

Type 2 diabetes mellitus is a chronic metabolic disorder with an exponential increase in developing countries. The international diabetes federation reported 382 million diabetes cases in 2013, with a prediction of 592 million cases by 2035, and 80% of these cases are in developing countries^{1,2}. Diabetes accounts

for 5.1 million deaths worldwide every year^{1,2}. The prevalence of type 2 diabetes mellitus in developed countries is 1.2%, whereas in developing countries the prevalence is assumed to be fourfold higher^{2,3}. An exponential increase of obesity and type 2 diabetes mellitus in developing countries is well recognized by an increased consumption of energy-rich food, sedentary lifestyle and urbanization⁴. In Vietnam, the number of patients with type 2 diabetes mellitus is growing, with estimated 3.3 million diabetes cases reported in 2014. The prevalence of

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Received 19 June 2016; revised 2 September 2016; accepted 26 September 2016

diabetes in the age group of 30–69 years is estimated to be 5.7% across Vietnam and 7% in urban areas⁵.

Type 2 diabetes mellitus constitutes up to 95% of all diabetes, and is characterized by chronic hyperglycemia resulting from defects in insulin secretion and/or insulin action and metabolic disorders of protein and lipids^{6,7}. The pathogenesis of type 2 diabetes mellitus consists of two major abnormalities including insulin resistance and dysfunction of insulin production, which lead to the inability to regulate blood glucose level⁸. The damage to pancreatic β -cells resulting in insufficient production of insulin and adiponectin, as well as an increased production of pro-inflammatory cytokines as a result of obesity are the major contributing factors to type 2 diabetes mellitus^{6,9,10}. Insulin resistance appeared years before the clinical manifestation of type 2 diabetes mellitus and is significantly associated with obesity, especially with abdominal and visceral obesity with an abnormally increased waist-to-hip ratio, dyslipidemia, hypertension and other metabolic disorders¹¹. Therefore, obesity largely contributes to insulin resistance in patients with type 2 diabetes mellitus¹².

Adipose tissue has recently been recognized as an organ for the metabolism of sexual steroids and the production of adipin, which significantly contributes to loss of body-weight^{13,14}. Adipose tissue-derived proteins regulate metabolic functionalities including hormone activities¹⁵. Adipose tissue is also known as an endocrine organ that can produce various peptides with bioactivities, namely adipocytokine (or adipokines)^{16,17}. In addition, adipocytes carry many receptors for hormones of the endocrine and central nervous system; therefore, adipocytes are involved in various biological processes, such as energy metabolism, neuroendocrine function and immune response¹⁴.

Increased adipose tissue as a result of obesity, especially deposition of visceral fat, is associated with insulin resistance, increased blood glucose levels, lipid metabolic disorders, hypertension and inflammation¹⁵. Those metabolic syndromes eventually lead to obesity-related type 2 diabetes mellitus. The adipose tissue-derived hormones, such as adiponectin, leptin, adipin and resistin, have been shown to be associated with metabolic disorders, and are risk factors for type 2 diabetes mellitus and cardiovascular diseases^{18–20}. The present study aimed to investigate the levels of adiponectin and different pro-inflammatory cytokines, such as tumor necrosis factor- α (TNF- α), interleukin-1 beta (IL-1 β) and interleukin-10 (IL-10), as well as their correlations with insulin resistance and clinical progressions of overweight and type 2 diabetes mellitus in a Vietnamese study group.

MATERIALS AND METHODS

Patients and controls

A total of 73 Vietnamese patients with type 2 diabetes mellitus and 57 control individuals were included in the study. The patients were newly diagnosed to have type 2 diabetes mellitus, and some patients (such as the patients who had high levels of

glucose) were immediately treated with the diabetes drug, Diamicon MR (gliclazide MR), and/or a low dose of insulin injection. Patients were classified into two subgroups based on their body mass index (BMI) and type 2 diabetes mellitus status. The first subgroup included the patients who were both overweight (BMI ≥ 25) and had type 2 diabetes mellitus (patients with overweight type 2 diabetes mellitus, $n = 20$). The second subgroup included patients with type 2 diabetes mellitus, but who were not overweight (BMI < 25 ; patients with non-overweight type 2 diabetes mellitus, $n = 53$). At the time of sampling, most of the recruited patients had not been treated with any antidiabetic drugs.

The patients were diagnosed for type 2 diabetes mellitus based on the standard criteria reported by the World Health Organization in 1998 and by the International Diabetes Federation in 2005^{21,22}. The anthropometric indicators, such as height, weight, and waist and hip circumference were measured for all study participants. BMI and waist-to-hip ratio were calculated based on their anthropometric indicators. Blood pressure and electrocardiogram were measured and recorded.

The exclusion criteria for the patient group included diabetes complications or more severe complications, acute brain stroke, paroxysmal hypertensive crises, patients with liver cirrhosis, heart and kidney failure or other infectious diseases, such as bacterial infections, hepatitis and tuberculosis. Patients taking certain medications, which might affect the biochemical test results, such as glucocorticoid, drugs for treatment of dyslipidemia, thiazide diuretics, antihypertensives enzyme inhibitors and angiotensin II receptor blockers, must discontinue taking the medications at least a week before sampling (Table 1).

All the individuals in the control group were clinically examined and were considered healthy during sampling. None of them had any chronic infectious diseases or conditions such as hepatitis, liver cirrhosis, obstructive pulmonary disease, gout and/or any infection. The control group was also further divided into two subgroups. The first control subgroup included normal healthy individuals, (non-overweight control individuals, $n = 33$), with fasting venous blood glucose test < 5.6 mmol/L, blood pressure < 130 mmHg and < 85 mmHg, electrocardiogram in the normal limits, other tests in the normal range, and BMI 18.5–25. The second control subgroup was healthy individuals with BMI ≥ 25 (overweight control individuals, $n = 24$), fasting venous blood glucose test < 5.6 mmol/L, blood pressure < 130 mmHg and < 85 mmHg, and electrocardiogram in the normal limits (Table 1). In addition, a history of type 2 diabetes mellitus, such as diabetes, symptoms associated with diabetes, and personal and family history of diabetes; family history of premature cardiovascular diseases; smoking; alcohol use; and lifestyle were obtained from all participants using the standard study questionnaires.

Table 1 | Characteristics of patients with type 2 diabetes mellitus and controls

Characteristics	Type 2 diabetes mellitus		Without type 2 diabetes mellitus		P-value†
	Overweight T2DM (n = 20)	Non-overweight T2DM (n = 53)	Overweight individuals (n = 24)	Non-overweight individuals (n = 33)	
Age, years (range)	55 (41–76)	53 (41–83)	48 (40–72)	47 (38–61)	<0.0001
Sex (male/female)	11/9	29/24	4/20	8/25	NS
Smoking (yes/no)	4/16	27/26	0/24	1/32	<0.0001
Alcohol use (yes/no)	7/13	26/27	0/24	1/32	<0.0001
BMI (range)	25.3 (25–28.3)	19.4 (13.6–21.7)	25.5 (25–32.9)	18.7 (15.8–24.9)	NS
WHR (range)	0.92 (0.85–0.96)	0.85 (0.74–0.98)	0.87 (0.81–0.93)	0.83 (0.73–0.92)	<0.0001
Fasting glucose, mmol/L (range)	16.5 (8.2–25)	15 (7.1–29.8)	5.1 (3.8–5.5)	5 (3.8–5.5)	<0.0001
Blood urea, mmol/L (range)	5.5 (3.8–8.6)	5.2 (2.5–9.2)	4.9 (2.5–5.1)	5.2 (2.1–7.4)	NS
Blood creatinine, µmol/L (range)	82 (5.7–127)	84 (55–121)	75 (49–110)	68 (45–110)	NS
Triglycerides, mmol/L (range)	3.9 (1.5–12)	2.3 (0.5–11.8)	1.5 (0.6–2.7)	1.8 (0.7–6.9)	NS
Total cholesterol, mmol/L (range)	6.6 (4.6–8.7)	5.7 (3.4–8.1)	4 (2.6–7.4)	4.2 (2.6–6.8)	<0.0001
HDL-C, mmol/L (range)	0.9 (0.7–1.1)	0.97 (0.5–1.9)	1.7 (0.8–2.3)	1.2 (0.6–2.2)	<0.0001
LDL-C, mmol/L (range)	3.2 (0.9–5.3)	3.1 (1.4–5.4)	2.3 (1–3.6)	2.4 (1.1–3.6)	<0.0001
Total bilirubin, µmol/L (range)	13 (6.9–20)	12.3 (2.1–21)	12.7 (5.7–18)	14.8 (4.4–21.5)	NS
GOT (AST), U/L (range)	19.5 (8–35)	19 (10–39)	25.5 (10–39)	22 (11–36)	NS
GPT (ALT), U/L (range)	25 (10–39)	23 (10–37)	30 (6–39)	27 (10–39)	NS
Glycosylated hemoglobin (%)	11 (6.8–13.2)	10.6 (6.6–17)	NA	NA	NA
Insulin, pmol/L (range)	70 (14–133.7)	40 (6.9–160)	84 (18–118)	12 (8–88)	0.05
HOMA-RI (range)	45.8 (6.3–119.3)	23 (4.7–125)	19.7 (3.8–29.5)	2.6 (2–2.9)	<0.0001
QUICKI (range)	0.5 (0.5–0.7)	0.6 (0.4–0.7)	0.5 (0.5–0.7)	0.8 (0.5–0.9)	<0.0001
HOMA-β (range)	124.2 (19–365.5)	75.8 (8.8–650)	1147.6 (237.5–4000)	157.1 (64–1563.6)	<0.0001

†Comparison between type 2 diabetes mellitus and non-diabetes mellitus. BMI, body mass index; GOT, glutamic-oxaloacetic transaminase (aspartate transaminase [AST]); GPT, glutamic-pyruvic transaminase (aspartate transaminase [ALT]); HDL-C, high-density lipoprotein; LDL-C, low-density lipoprotein; HOMA-β, homeostatic model assessment β-cell function; HOMA-IR, homeostasis model assessment -insulin resistance; NA, not applicable; NS, not significant; QUICKI, quantitative insulin sensitivity check index; T2DM, type 2 diabetes mellitus; WHR, waist-to-hip ratio.

Ethics statement

Informed written consent was received from all studied participants. The study was approved by the institutional review board of the Vietnam Military Medical University.

Measurement of biochemical parameters

The levels of lipid components including cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were measured by using an automatic biochemical Olympus AU400 Chemistry Analyzer (Olympus, Shinjuku, Japan). Fasting blood glucose levels were measured by measurement of ultraviolet with hexokinase. Insulin levels were measured by the electrochemical immune fluorescence method using the ELESYS-2010 system (Roche Diagnostics Ltd, Basel, Switzerland). Blood fasting glycosylated hemoglobin levels were quantified by the ion-exchange method using high-performance liquid chromatography. The insulin resistance index was evaluated according to the homeostasis model assessment insulin resistance (HOMA-IR) and the quantitative insulin sensitivity check insulin resistance index (QUICKI). In addition, the β -cell function (HOMA- β) and the insulin secretion of β -cells were evaluated according to Matthews' method¹⁰.

Measurement of TNF- α , IL-1 β and IL-10 levels

The levels of TNF- α , IL-1 β and IL-10 were measured in the respective serum samples of the study participants by using commercially available enzyme-linked immunosorbent assay kits including AviBion Human TNF- α , AviBion Human IL-1 β and AviBion Human IL-10, respectively according to the manufacturer's instruction (Orgenium, Helsinki, Finland).

Measurement of adiponectin levels

The adiponectin levels were measured in the respective serum samples of the study participants by using a commercial enzyme-linked immunosorbent assay kit following the manufacturer's instructions (AviBion Human Adiponectin; Orgenium). The detection limit of the kit is 0.185 ng/mL.

Statistical analysis

Clinical and demographic data are presented as median values with a range for continuous variables. Student's *t*-tests or one-way ANOVA were used for comparing the mean of two or more groups, respectively. The χ^2 -test or Fisher's exact test were used to compare categorical variables. The Kruskal–Wallis or Mann–Whitney *U*-test were used to analyze the serum levels of adiponectin, TNF- α , IL-1 β and IL-10 in the patients with type 2 diabetes mellitus and in controls wherever appropriate. Spearman's rank correlation coefficient was used to analyze the correlation of adiponectin serum levels with clinical parameters of overweight and type 2 diabetes mellitus, as well as the correlation of insulin sensitivity/resistance indexes with the relative ratios of studied adiponectin and cytokine levels. All statistical

analyses were carried out using IBM Statistics Spss version 19 (IBM Corp, Armonk, New York, USA), and the level of significance was set at a *P*-value of <0.05.

RESULTS

Demographic, clinical and biochemical characteristics of the study participants

The demographic, clinical and biochemical characteristics of the patients with type 2 diabetes mellitus and controls are summarized in Table 1. The mean age of the patients with type 2 diabetes mellitus (55.7 ± 11.5 years) was higher than control individuals (without type 2 diabetes mellitus, 48.5 ± 6.8 years; $P < 0.001$), whereas there was no difference of the mean age between patients with overweight type 2 diabetes mellitus and non-overweight type 2 diabetes mellitus. The proportion of men among patients with type 2 diabetes mellitus was higher than the control group ($P = 0.002$). In addition, the proportions of smokers and alcohol users among patients with type 2 diabetes mellitus were significantly higher compared with the control group ($P < 0.0001$ for both smoking and alcohol use). These results imply that smoking and alcohol use could significantly contribute towards increased risk of type 2 diabetes mellitus.

The levels of fasting glucose, creatinine, triglycerides and total cholesterol were significantly higher in the patients with type 2 diabetes mellitus compared with the control individuals ($P < 0.0001$). The level of HDL-C was lower, while the level of LDL-C was higher in the patients with type 2 diabetes mellitus compared with the control individuals ($P < 0.0001$ for both HDL-C and LDL-C). For the patients with type 2 diabetes mellitus, the levels of triglycerides and total cholesterol were significantly higher, whereas the HDL-C level was decreased in the patients with overweight type 2 diabetes mellitus compared with those with non-overweight type 2 diabetes mellitus ($P = 0.01$ for triglycerides, $P = 0.0018$ for total cholesterol and $P = 0.016$ for HDL-C). The levels of insulin, HOMA-IR, QUICKI and HOMA- β were significantly higher in the patients with type 2 diabetes mellitus compared with the control individuals ($P = 0.05$ for insulin, and $P < 0.0001$ for HOMA-IR, QUICKI and HOMA- β). Among the patients with type 2 diabetes mellitus, the levels of insulin and HOMA-IR were increased, whereas the level of the QUICKI was decreased in the patients with overweight type 2 diabetes mellitus compared with those with non-overweight type 2 diabetes mellitus ($P = 0.04$, 0.028 and 0.016 , respectively). Similarly, among the control individuals, the levels of insulin, HOMA-IR and HOMA- β were increased, whereas the QUICKI level was decreased in the overweight controls compared with the non-overweight individuals ($P < 0.0001$). In addition, no difference in liver enzyme levels (aspartate transaminase, alanine transaminase and total bilirubin) was observed between the patients with type 2 diabetes mellitus and the controls ($P > 0.05$; Table 1).

Adiponectin and cytokine levels in patients with type 2 diabetes mellitus and in controls

The levels of adiponectin, TNF- α , IL-1 β and IL-10 were measured in the sera of the patients with type 2 diabetes mellitus and controls, and were compared among subgroups. We observed that the adiponectin levels were significantly lower in the patients with type 2 diabetes mellitus ($2.5 \pm 1.5 \mu\text{g/mL}$) compared with the controls ($16 \pm 18.6 \mu\text{g/mL}$; $P < 0.0001$; Figure 1a). Among the patients with type 2 diabetes mellitus, the adiponectin levels were lower in the patients with overweight type 2 diabetes mellitus ($2.1 \pm 0.9 \mu\text{g/mL}$) compared with those with non-overweight type 2 diabetes mellitus ($2.7 \pm 1.7 \mu\text{g/mL}$). However, the difference did not reach

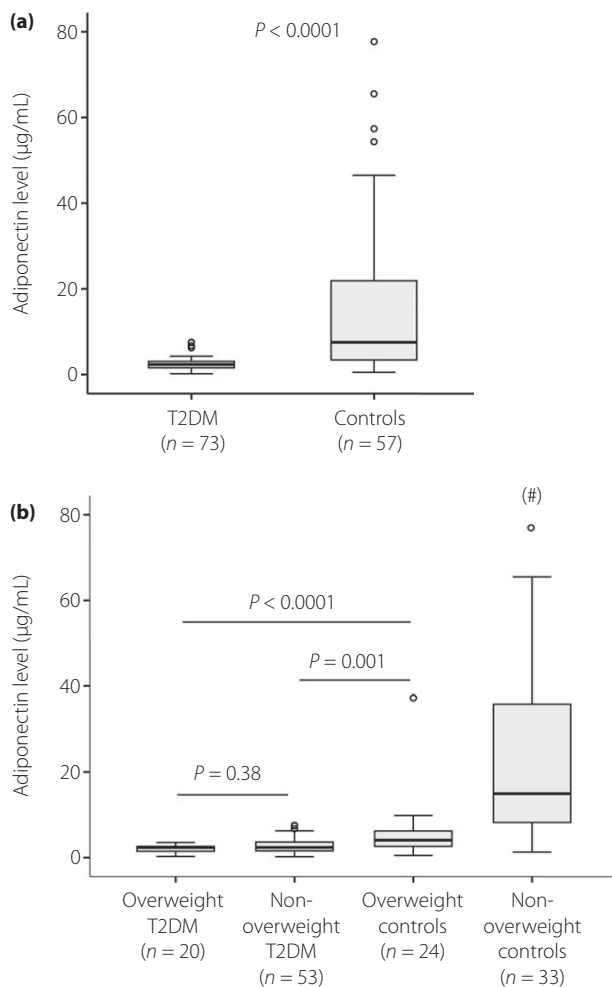


Figure 1 | Adiponectin level in type 2 diabetes mellitus (T2DM) patients and controls. Adiponectin level was measured in the sera of patients with type 2 diabetes mellitus and controls, and was compared between groups. (a) Adiponectin levels in patients with type 2 diabetes mellitus and controls. (b) Adiponectin levels in patients with overweight type 2 diabetes mellitus and those with non-overweight type 2 diabetes mellitus, and in overweight and non-overweight control individuals. P -values were calculated by using the Mann–Whitney U -test. # $P < 0.0001$ when compared with other groups.

statistical significance ($P = 0.38$). Among the control group, adiponectin levels were significantly decreased in the overweight individuals ($5.7 \pm 7.3 \mu\text{g/mL}$) compared with the non-overweight individuals ($23.5 \pm 20.7 \mu\text{g/mL}$; $P < 0.0001$; Figure 1b).

The TNF- α and IL-1 β levels were significantly increased, whereas the IL-10 levels were significantly decreased in the patients with type 2 diabetes mellitus and in the overweight individuals compared with the non-overweight control individuals ($P < 0.0001$; Figure 2a–c). Among the patients with type 2 diabetes mellitus, the TNF- α , IL-1 β and IL-10 levels were not significantly different between the patients with overweight and those with non-overweight type 2 diabetes mellitus. However, within the control group, the TNF- α and IL-1 β levels were higher, whereas the IL-10 levels were lower in the overweight individuals compared with the non-overweight individuals ($P < 0.0001$; Figure 2a–c). In addition, we analyzed the correlations of the adiponectin levels with the TNF- α , IL-1 β and IL-10 levels. The adiponectin levels were negatively correlated with the TNF- α levels (Spearman’s rho = -0.56 , $P < 0.0001$) and the IL-1 β levels (Spearman’s rho = -0.51 , $P < 0.0001$), whereas they were positively correlated with IL-10 levels (Spearman’s rho = 0.23 , $P = 0.013$; Figure 3a–c).

Correlation of adiponectin levels with clinical parameters

We analyzed the correlation of the adiponectin levels with the clinical parameters of overweight and type 2 diabetes mellitus, and observed that the adiponectin levels were negatively correlated with the levels of blood glucose, triglycerides and total cholesterol (Spearman’s rho = -0.49 , -0.34 and -0.32 , respectively). Regarding HDL-C and LDL-C, the adiponectin levels were positively correlated with HDL-C, whereas they were negatively correlated with LDL-C (Spearman’s rho = 0.39 and -0.23 , respectively). Regarding the correlation of adiponectin levels with insulin resistance and β -cell function, adiponectin levels were negatively correlated with insulin levels and HOMA-IR (Spearman’s rho = -0.4 and -0.51 , respectively), whereas they were positively correlated with the QUICKI and HOMA- β (Spearman’s rho = 0.43 and 0.19 , respectively; Figure 4). However, we did not observe any significant correlation of adiponectin levels with levels of urea, creatinine and liver enzymes (aspartate transaminase, alanine transaminase and total bilirubin).

Correlation of adiponectin-to-cytokine ratios with insulin resistance index

An index based on the relative proportion of adiponectin-to-resistin has been proposed to have diagnostic potential for insulin resistance²³. Because of the strong correlation of adiponectin levels with the levels of other studied cytokines (TNF- α , IL-1 β and IL-10), and the association of these cytokines with insulin resistance and type 2 diabetes mellitus²⁴, the relative proportion of these studied cytokines might also have a potential for the diagnosis of insulin resistance. We calculated the relative ratios of adiponectin/TNF- α , adiponectin/IL-1 β , adiponectin/IL-10,

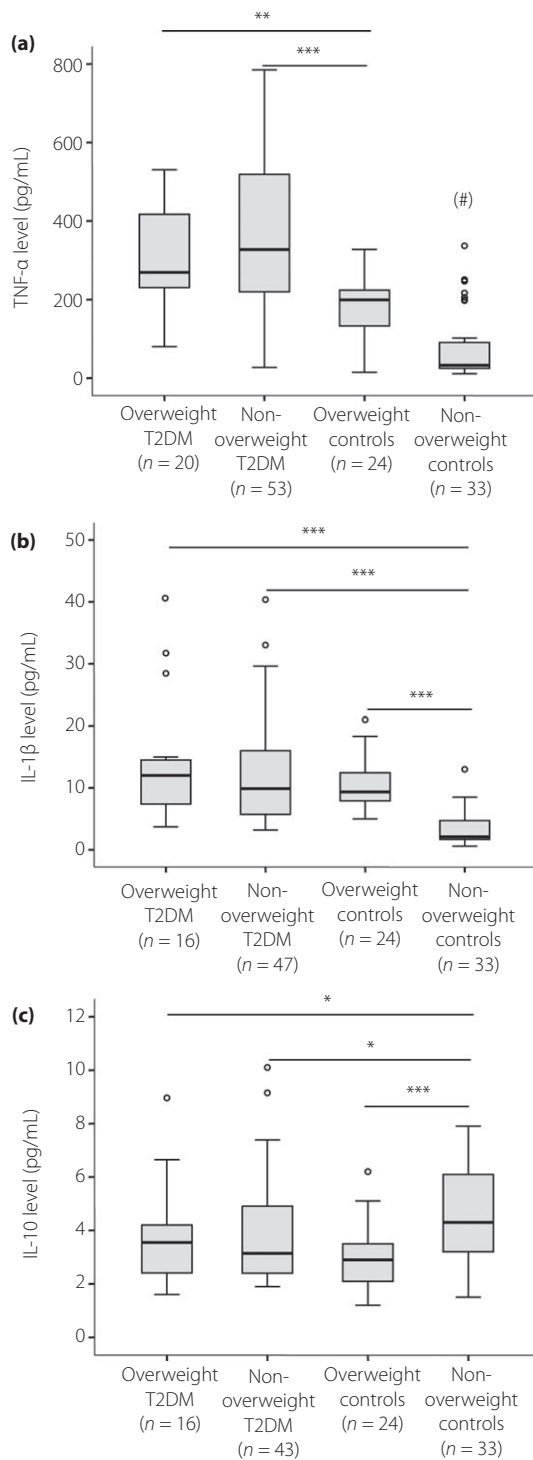


Figure 2 | Cytokine levels in type 2 diabetes mellitus (T2DM) patients and controls. The levels of (a) tumor necrosis factor (TNF)- α , (b) interleukin (IL)-1 β and (c) IL-10 were measured in the sera of patients with overweight type 2 diabetes mellitus and non-overweight type 2 diabetes mellitus, and of overweight and non-overweight control individuals. *P*-values were calculated by using the Mann–Whitney *U*-test. #*P* < 0.0001 when compared with other groups; **P* < 0.05; ***P* < 0.005; ****P* < 0.0005.

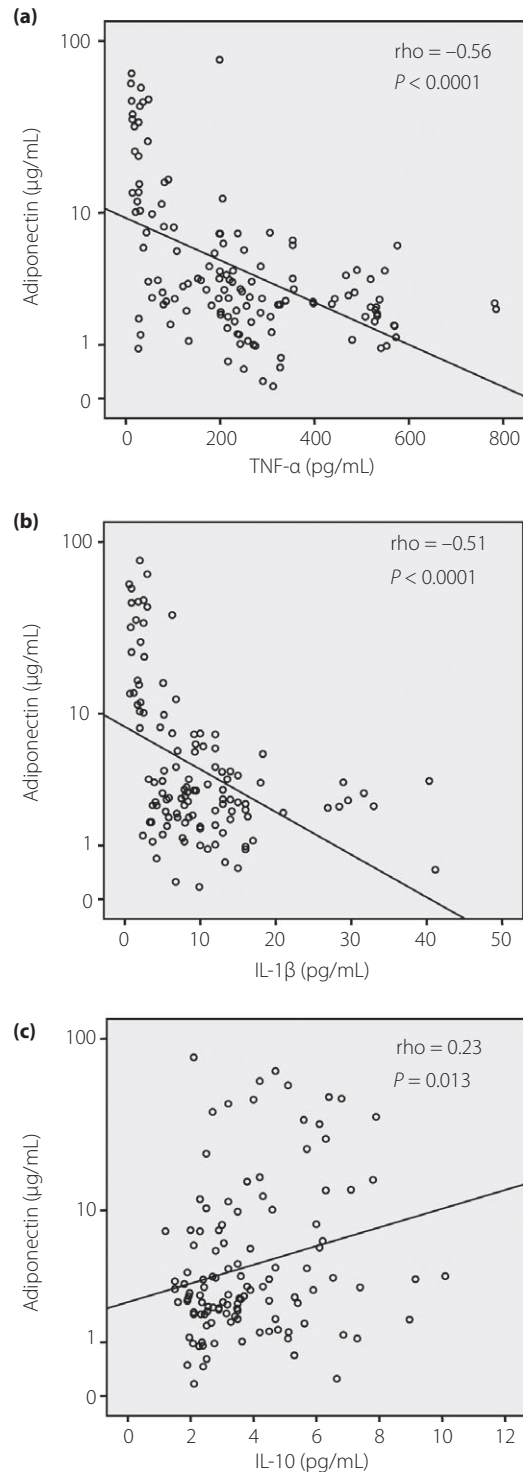


Figure 3 | Correlation between adiponectin and cytokine levels. The correlations between adiponectin levels and the levels of (a) tumor necrosis factor (TNF)- α , (b) interleukin (IL)-1 β and (c) IL-10 were calculated by using the Spearman's rank correlation coefficient. Spearman's rho and *P*-values are presented.

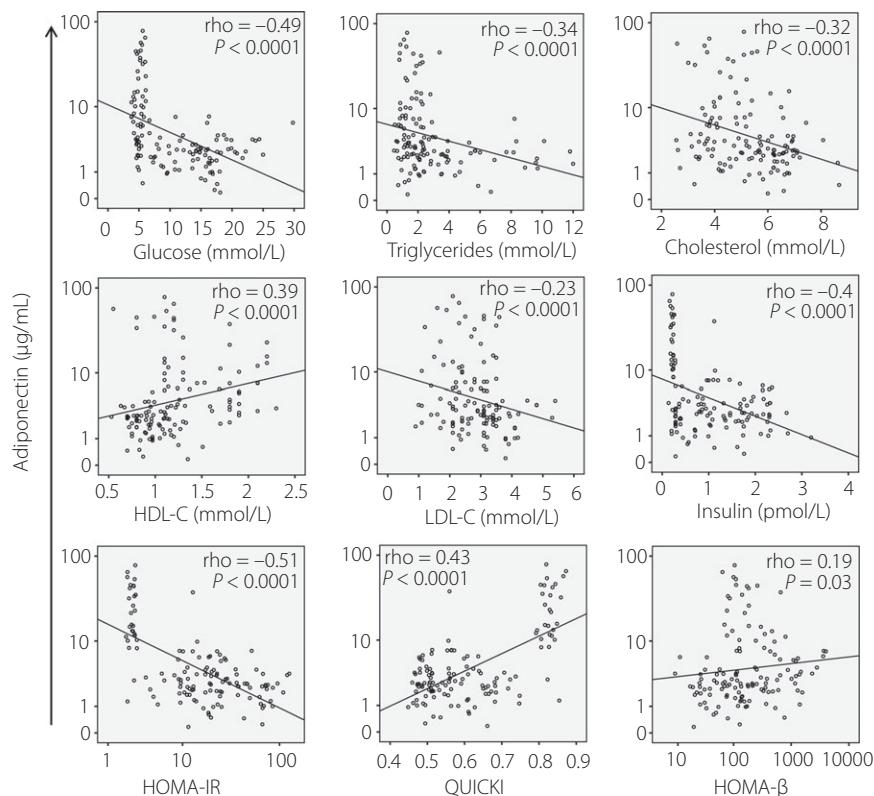


Figure 4 | Correlation between adiponectin level and clinical parameters of obesity and type 2 diabetes mellitus. The correlations between adiponectin and numbers of clinical parameters of overweight and type 2 diabetes mellitus were calculated by using Spearman's rank correlation coefficient. Spearman's ρ and P -values are presented. HDL-C, high-density lipoprotein cholesterol; HOMA- β , homeostasis model assessment β -cell resistance; HOMA-IR, homeostasis model assessment insulin resistance; LDL-C, low-density lipoprotein cholesterol; QUICKI, quantitative insulin sensitivity check insulin resistance index.

TNF- α /IL-1 β , TNF- α /IL-10 and IL-1 β /IL-10, and analyzed their correlations with the QUICKI and HOMA-IR indexes. We observed that the QUICKI was positively correlated with the relative ratios of adiponectin/TNF- α , adiponectin/IL-1 β and adiponectin/IL-10, whereas it was negatively correlated with the ratios of TNF- α /IL-10 and IL-1 β /IL-10 (Figure 5). Similarly, the HOMA-IR index was negatively correlated with the relative ratios of adiponectin/TNF- α , adiponectin/IL-1 β and adiponectin/IL-10, whereas it was positively correlated with the ratios of TNF- α /IL-10 and IL-1 β /IL-10 (Figure 6). These results show that the relative ratios of adiponectin to studied cytokines (TNF- α , IL-1 β and IL-10) might also serve as a potential indicator for insulin resistance.

DISCUSSION

Adiponectin plays an important role in the pathogenesis of obesity, type 2 diabetes mellitus, atherosclerosis and inflammation²⁵. The present results showed that adiponectin levels were significantly decreased in patients with type 2 diabetes mellitus and in overweight individuals compared with patients with non-overweight type 2 diabetes mellitus and healthy individuals, respectively. In addition, our investigations showed that

adiponectin levels were correlated with several pro-inflammatory cytokines, and with clinical markers of overweight and type 2 diabetes mellitus. These results indicate that adiponectin and adipose-derived cytokines could potentially influence the development of overweight and type 2 diabetes mellitus.

Most of the adipokines are able to regulate metabolism and inflammation, thus they play a vital role in the pathogenesis of metabolic disorders and type 2 diabetes mellitus^{26,27}. Studies have shown that adipokines are involved in the pathogenesis of obesity, atherosclerosis, inflammation and type 2 diabetes mellitus¹⁵. Of those adipokines, adiponectin is considered as a potential marker for the treatment of obesity, type 2 diabetes mellitus, atherosclerosis and inflammation²⁶⁻²⁸. In the normal condition, the circulating adiponectin serum levels are high in contrast to the low level of other adipocytokines²⁹. In line with other studies³⁰⁻³⁵, the present study also showed that adiponectin levels were significantly decreased in patients with type 2 diabetes mellitus compared with individuals without type 2 diabetes mellitus. In addition, decreased adiponectin levels observed in overweight individuals supports the fact that adiponectin serum levels were inversely correlated with obesity, especially with the accumulation of visceral fat³³. Furthermore,

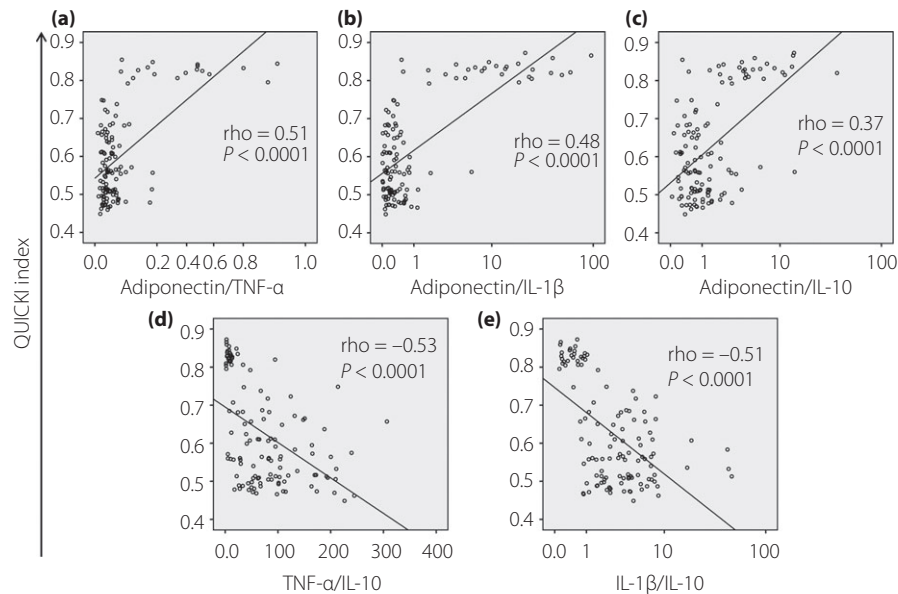


Figure 5 | Correlation between the quantitative insulin sensitivity check index (QUICKI) and the relative ratios of studied cytokines. Relative ratios of (a) adiponectin/tumor necrosis factor (TNF)- α , (b) adiponectin/interleukin (IL)-1 β , (c) adiponectin/IL-10, (d) TNF- α /IL-10 and (e) IL-1 β /IL-10 were calculated and correlated with the QUICKI. The correlations were calculated by using Spearman's rank correlation coefficient. Spearman's rho and *P*-values are presented.

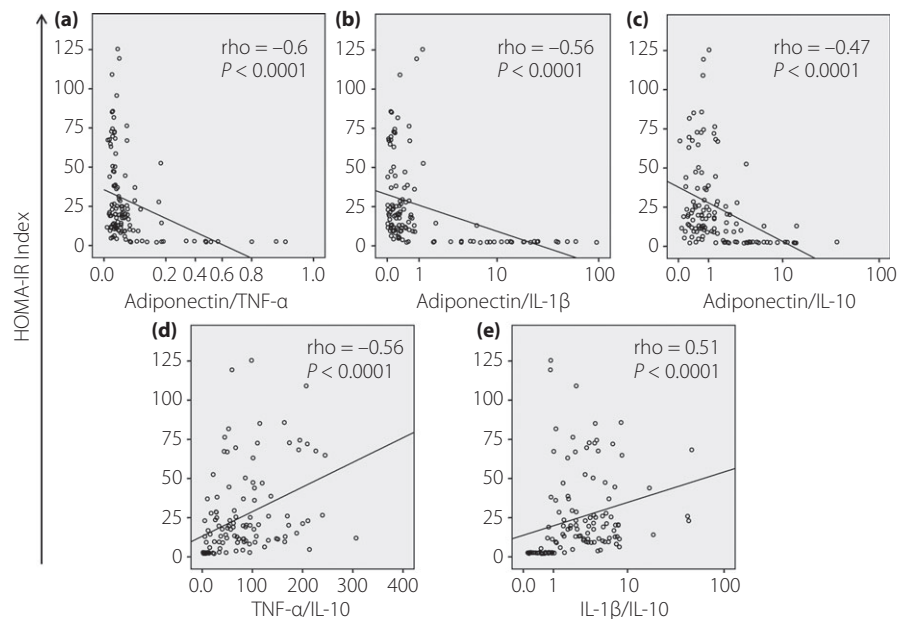


Figure 6 | Correlation between the homeostasis model assessment insulin resistance (HOMA-IR) index and the relative ratios of studied cytokines. Relative ratios of (a) adiponectin/tumor necrosis factor (TNF)- α , (b) adiponectin/interleukin (IL)-1 β , (c) adiponectin/IL-10, (d) TNF- α /IL-10 and (e) IL-1 β /IL-10 were calculated and correlated with the HOMA-IR index. The correlations were calculated by using Spearman's rank correlation coefficient. Spearman's rho and *P*-values are presented.

decreased adiponectin levels were also associated with the pathogenesis of many other diseases, such as lipid metabolic disorders and cancer^{36–38}. More directly, our data show that

adiponectin levels are significantly correlated with numerous clinical parameters of obesity, such as triglycerides, cholesterol, HDL-C and LDL-C. The adiponectin level was also strongly

related to type 2 diabetes mellitus clinical parameters, including insulin level and index of insulin sensitivity/resistance (HOMA-RI and QUICKI) and β -cell function (HOMA- β). Those observations indicate that adiponectin levels are involved in the development of type 2 diabetes mellitus.

TNF- α and IL-6 are pro-inflammatory cytokines not only produced mainly by immune cells, but also by adipocytes, and therefore the levels of TNF- α and IL-6 are associated with obesity and type 2 diabetes mellitus³⁹. The present study showed that the TNF- α and IL-1 β levels were increased, whereas the IL-10 level was decreased in patients with type 2 diabetes mellitus and in overweight individuals compared with non-overweight individuals. The TNF- α , IL-1 β and IL-10 levels were significantly correlated with the adiponectin levels. These results show that pro-inflammatory cytokines, such as TNF- α , IL-1 β , IL-6 and IL-10, contribute significantly to the pathogenesis of type 2 diabetes mellitus. A previous study has shown that increased TNF- α , IL-1 β and IL-6 levels lead to increased insulin resistance in overweight individuals, and subsequently results in type 2 diabetes mellitus²⁴. Adiponectin was shown to induce the expression and secretion of IL-10⁴⁰, hence IL-10 levels were more decreased in patients with type 2 diabetes mellitus than in healthy individuals^{41,42}. The present results along with previous observations suggest that IL-10 contributes to influencing the development of type 2 diabetes mellitus, and such an association was further supported by a meta-analysis showing that the polymorphisms in the promoter region of the *IL-10* gene are associated with type 2 diabetes mellitus risk⁴³. Also, it is likely that decreased adiponectin impairs IL-10 production, and thus predisposes to metabolic syndrome and type 2 diabetes mellitus.

IL-1 carries out many functional activities in the regulation of inflammatory responses and metabolism, and can regulate insulin secretion and induce β -cell apoptosis that can subsequently lead to type 2 diabetes mellitus^{44,45}. High IL-1 β levels in patients with type 2 diabetes mellitus and in overweight individuals, and their converse correlation with adiponectin levels observed in the present study suggest that IL-1 β might play a key role in the modulation of β -cell function. Also, high cytokine secretion over a long period of time resulted in impairment of β -cell function⁴⁶. Furthermore, IL-18, another interleukin recently classified as a member of the IL-1 cytokine super-family, was associated with obesity and insulin resistance, hypertension, and atherosclerotic disease^{47–49}.

In contrast to adiponectin, increased insulin sensitivity is improved by reducing bodyweight or treatment with insulin sensitivity affecting drugs. Adiponectin is a hormone derived from adipose tissue that is effective against diabetes and inflammation^{50–52}. Low adiponectin levels caused by the deficiency of its production were associated with a higher risk of overweight-related type 2 diabetes mellitus, as observed in the present study. Therefore, adiponectin might be considered as a risk marker for incident prediabetes^{53,54}, and a promising therapeutic intervention for type 2 diabetes mellitus in overweight individuals^{28,55,56}. Increasing the adiponectin serum levels could be

one of the strategies for treatment of overweight-related type 2 diabetes mellitus by reducing the adipose tissue. In addition, a previous study has proposed a novel index based on the relative proportion of adiponectin-to-resistin to diagnose of insulin resistance²³. The present study also showed that the insulin sensitivity/resistance indexes (QUICKI and HOMA-IR) have a strong correlation with the relative ratios of adiponectin/TNF- α , adiponectin/IL-1 β , adiponectin/IL-10, TNF- α /IL-10 and IL-1 β /IL-10. Therefore, an index based on the levels of adipokines and adipose-derive cytokines could be useful for diagnosis of insulin resistance. However, more studies are required to propose a new index and verify the diagnostic accuracy in clinical practice, and to establish a cut-off value and reference range of insulin sensitivity for specific populations²³.

In conclusion, the present study showed that the levels of adiponectin, TNF- α , IL-1 β and IL-10 are significantly modulated during the development of overweight and type 2 diabetes mellitus. The adiponectin levels were significantly correlated with the TNF- α , IL-1 β and IL-10 levels, and with clinical parameters of obesity and type 2 diabetes mellitus. Adipokines, together with pro-inflammatory cytokines, could possibly modulate the pathogenesis of overweight and type 2 diabetes mellitus, and might serve as a prognostic marker and a therapeutic intervention for overweight-related type 2 diabetes mellitus.

ACKNOWLEDGMENTS

We thank all the study participants. This research is funded by the Vietnam National Foundation for Science and Technology Development (NAFOSTED) under grant number 106-YS.02-2014.36.

DISCLOSURE

The authors declare no conflict of interest.

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