

Original Article:

Adiponectin and leptin as discriminating biomarkers between pre-metabolic and metabolic syndromes in patients with type 2 diabetes* M.S.M. Al-Nimer¹, M.M. Mahmood², R.J. Abd-Alkaliq³**Abstract**

An imbalance in adipocytokines is reported in several diseases. The aim of the study was to assess the status of serum adipocytokines (notably leptin and adiponectin) in patients with type 2 diabetes presented with pre-metabolic or metabolic syndrome. This cross-sectional study was conducted in the Department of Pharmacology, College of Medicine in cooperation with department of Biology, College of Science at Al-Mustansiriya University in Baghdad, Iraq. A total of 78 patients with type 2 diabetes with pre-metabolic syndrome (n = 12) and metabolic syndrome (n = 66) of both genders were enrolled in the study. Data related to the components of metabolic syndrome including anthropometric measurements, blood pressure and fasting serum lipid profile were collected. Serum adiponectin and leptin were determined using the enzyme linked immunosorbent assay (ELISA). In patients with type 2 diabetes the levels of several components of metabolic syndrome were significantly higher than those with pre-metabolic syndrome. Although numerically higher levels of serum adiponectin and leptin levels were observed in subjects with metabolic syndrome (2.479 ± 0.321 pg/ml and 171.44 ± 29.57 pg/ml respectively) compared to pre-metabolic syndrome (2.096 ± 0.327 pg/ml and 148.23 ± 48.88 pg/ml respectively), the difference was not statistically significant. Multivariate analysis revealed non-significant correlation between serum levels of adipocytokines and all components of metabolic syndrome. Non-significant inverse correlations were observed between serum leptin and adiponectin in pre-metabolic ($r = -0.225$) and metabolic ($r = -0.148$) syndromes. Low serum adiponectin and leptin levels are associated with pre-metabolic syndrome compared to metabolic syndrome and these biomarkers are not good to discriminate between pre-metabolic and metabolic syndrome in patients with type 2 diabetes.

Key words: Adipocytokines, metabolic syndrome, type 2 diabetes

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Introduction

In humans, there are two major types of adipose tissue: brown and white. The former is responsible

for non-shivering thermogenesis and the later functions as an energy storage depot as well as an endocrine organ involved with metabolic function and several pathological states like diabetes. Adipose tissue produces adipocytokines, the actions of some of them are linked with eating disorders and diabetes (1). Some adipocytokines regulate the immune response by acting as proinflammatory mediator e.g. leptin and as anti-inflammatory mediator e.g. adiponectin (2-4). Leptin is a non-glycosylated polypeptide hormone acts centrally on the feeding centre and its serum level is correlated with body mass index and fat mass (5). Insulin hormone is one of many hormones that influence the secretion of leptin (6). Adiponectin in human exists in two forms; monomeric form in adipocytes and oligomeric complex which circulates in plasma. Adiponectin is secreted by adipocytes and circulates in blood stream as three oligomeric complexes, including trimer, hexamer and high molecular

weight multimers (7). Its levels are decreased in obesity and related pathologies. It is an independent risk factor for cardiovascular disease. High circulating adiponectin levels enhance the sensitivity of insulin while low levels were observed in patients with type 2 diabetes, hypertension and dyslipidemia (8). High circulating adiponectin level protects the individual from developing type 2 diabetes (9). Metabolic syndrome is a cluster of independent risk factors, central obesity, insulin resistance, elevated triglycerides, decreased high density lipoprotein, high blood pressure, that increase the likelihood of cardiovascular diseases.

The link between low adiponectin level and risk of metabolic syndrome was observed in individuals with mutation of adiponectin gene (10). Gender based effect was demonstrated between circulating adipokines levels and / or total regional adiposity in South Asians Living in America Study (SALAS). In addition, the study found that adiponectin was not associated with type 2 diabetes, hypertension, or metabolic syndrome in either women or men (11). On the other hand Vega and Grundy reported that the adiponectin/leptin ratio decreased progressively with the increase risk factors that related to metabolic syndrome (12). Hung et al., studied circulating adiponectin level in a community population sample of 1094 men and women and found that plasma adiponectin level significantly and inversely correlated with body mass index, waist-hip ratio, diastolic blood pressure, triglycerides, glucose and fasting insulin and positively correlated with high density lipoprotein-cholesterol (HDL-c) (13). Smits et al., reported the association between adiponectin and leptin with at least one of the risk factors of metabolic syndrome in patients without diabetes (14). This study aimed to look for the association between serum levels of adipocytokines (adiponectin and leptin) and the components of metabolic syndrome in patients with type 2 diabetes using multivariate analysis and taking into consideration their association with the cardio-metabolic risk factors.

Methods

This cross-sectional study was conducted in the department of Pharmacology, College of Medicine,

in cooperation with the department of Biology, College of Science at Al-Mustansiriya University in Baghdad, Iraq during 2011. The study was approved by the Institutional Review Committee and informed consent obtained from each patient prior to recruitment in the study. Patients with type 2 diabetes of both genders using oral glucose-lowering medication(s) alone or with once - or twice-daily insulin for short period were included in the study. Patients with history of hematological, neoplastic, renal, hepatic or thyroid diseases, acute or chronic infections, autoimmune diseases, or receiving treatment with anti-inflammatory drugs were excluded. A total of 78 patients (26 males and 52 females) with a median age of 58 years were recruited in the study.

Demographic data, medical history and treatment were obtained. Anthropometric measurements related to the cardio-metabolic risk factors were measured. These included height (m), weight (kg), waist circumference (cm), hip circumference (cm), mid-thigh circumference (cm) and neck circumference (cm). Body mass index (BMI), waist/hip ratio (W/H) and waist/height ratio (W/He) were calculated. According to the BMI values the patients were categorized into: normal (BMI < 25 kg/m²), over weight (BMI 25 - 29 kg/m²), and obese (BMI: ≥ 30 kg/m²). A value of W/H ratio > 0.9 (male) and 0.8 (female) indicated central obesity. Blood pressure measured in sitting position and the mean of the three readings recorded. Pulse and mean arterial pressures calculated using the following formula:

Pulse pressure (mm Hg) = Systolic blood pressure – diastolic blood pressure

Mean arterial blood pressure = Diastolic blood pressure + 1/3 Pulse pressure

Peripheral venous blood was drawn and the samples were centrifuged at 2500 rpm for 10 min and the sera were separated for determination of fasting serum glucose, lipid profile (total cholesterol, triglycerides, high density lipoprotein-cholesterol and therogetic index was calculated which is equal to the ratio

$$\frac{\text{Triglycerides}}{\text{High density lipoprotein}}$$

Table 1: Baseline characteristics of the studied population

	Pre-metabolic syndrome (n = 12) Mean \pm SE	Metabolic syndrome (n = 66) Mean \pm SE	Total (n = 78) Mean \pm SE
Gender (M:F)	2:10	24:42	26:52
Age (years)	60.1 \pm 2.46	57.9 \pm 1.19	58.20 \pm 1.08
Body weight (kg)	67.41 \pm 4.01	85.56 \pm 2.27*	82.77 \pm 2.14
Height (m)	1.58 \pm 0.01	1.62 \pm 0.011	1.61 \pm 0.00
Body mass index (kg/m ²)	26.91 \pm 1.81	32.51 \pm 0.74**	31.65 \pm 0.72
Waist circumference (cm)	85.41 \pm 4.58	104.37 \pm 1.91*	101.46 \pm 1.92
Hip circumference (cm)	93.42 \pm 3.01	109.10 \pm 1.63*	106.67 \pm 1.58
Mid-thigh circumference (cm)	50.75 \pm 2.85	49.06 \pm 1.51	49.32 \pm 1.34
Neck circumference (cm)	36.50 \pm 1.10	46.20 \pm 1.45*	44.71 \pm 1.30
Waist/hip ratio	0.91 \pm 0.04	0.95 \pm 0.01	0.95 \pm 0.01
Waist/height ratio	0.54 \pm 0.03	0.64 \pm 0.01**	0.62 \pm 0.01

Data presented as Mean \pm SE. * $p < 0.001$, ** $p < 0.01$ on comparison between pre-metabolic and metabolic syndrome

Table 2: Blood pressure measurements

	Pre-metabolic syndrome (n=12)	Metabolic syndrome (n=66)	Total (n=78)
Systolic blood pressure (mmHg)	132.9 \pm 4.46	150.7 \pm 2.65**	147.9 \pm 2.45
Diastolic blood pressure (mmHg)	82.5 \pm 2.5	94.9 \pm 1.79*	93.0 \pm 1.64
Pulse pressure (mmHg)	50.4 \pm 4.28	55.8 \pm 1.88	54.9 \pm 1.73
Mean blood pressure (mmHg)	99.3 \pm 2.6	113.5 \pm 1.92*	111.3 \pm 1.77

Data presented as Mean \pm SE. * $p < 0.001$, ** $p < 0.01$ on comparison between pre-metabolic and metabolic syndrome

Table 3: Serum fasting lipid profile

	Pre-metabolic syndrome (n=12)	Metabolic syndrome (n=66)	Total (n=78)
Cholesterol (mg/dl)	196.3 \pm 7.20	193.9 \pm 4.59	194.3 \pm 4.03
Triglycerides (mg/dl)	145.9 \pm 11.54	196 \pm 9.66*	188.3 \pm 8.60
High density lipoprotein (mg/dl)	45.3 \pm 1.94	44.1 \pm 1.73	44.3 \pm 1.03
Atherogenic index	3.2 \pm 0.21	4.8 \pm 0.39**	4.6 \pm 0.34

Data presented as Mean \pm SE. * $p < 0.001$, ** $p < 0.01$ on comparison between pre-metabolic and metabolic syndrome

Table 4 Serum levels of adipocytokines

	Pre-metabolic syndrome (n=12)	Metabolic syndrome (n=66)	Total (n=78)
Adiponectin (pg/ml)	2.0 ± 0.32	2.4 ± 0.32	2.4 ± 0.27
Leptin (pg/ml)	148.2 ± 48.88	171.4 ± 29.57	167.8 ± 26.04
Adiponectin/leptin ratio	0.0 ± 0.02	0.0 ± 0.02	0.08 ± 0.01
Leptin/adiponectin ratio	108.6 ± 48.04	218.8 ± 63.24	201.9 ± 54.12

Data presented as Mean ± SE.

Table 5 Multivariate correlation (correlation factor) between either serum adiponectin or serum leptin with any component of pre-metabolic and metabolic syndrome

	Adiponectin (pg/ml)	Leptin (pg/ml)
Waist circumference (cm)	-0.100	0.122
Hip circumference (cm)	-0.11	-0.054
Mid-thigh circumference (cm)	0.00	-0.071
Neck circumference (cm)	0.100	0.134
Body mass index (kg/m²)	0.00	-0.055
Waist/Hip ratio	0.164	0.122
Waist/Height ratio	-0.045	-0.105
Mean blood pressure (mmHg)	-0.1	0.00
Atherogenic index	0.173	0.00
Fasting serum triglycerides (mg/dl)	0.033	0.088
Fasting serum high density lipoprotein (mg/dl)	-0.119	0.167
Fasting blood glucose (mg/dl)	0.070	0.136

Adipocytokines; adiponectin and leptin levels were determined using the technique of enzyme linked immunosorbent assay (ELISA).

Patients were categorized according to the presence of metabolic syndrome components. According to the National Cholesterol Education Program (NCEP) definition metabolic syndrome was found to be present if a subject had three or more of the

following: high waist circumference value (> 102 cm), high serum triglyceride level (≥ 150 mg/dl), low serum HDL cholesterol level (< 40 mg/dl in male and < 50 mg/dl in female), elevated systolic blood pressure (≥ 130 mmHg) and diastolic blood pressure (≥ 85 mmHg), and elevated glucose level (≥ 110 mg/dl) (15). The presence of less than three components was considered as pre-metabolic syndrome (16).

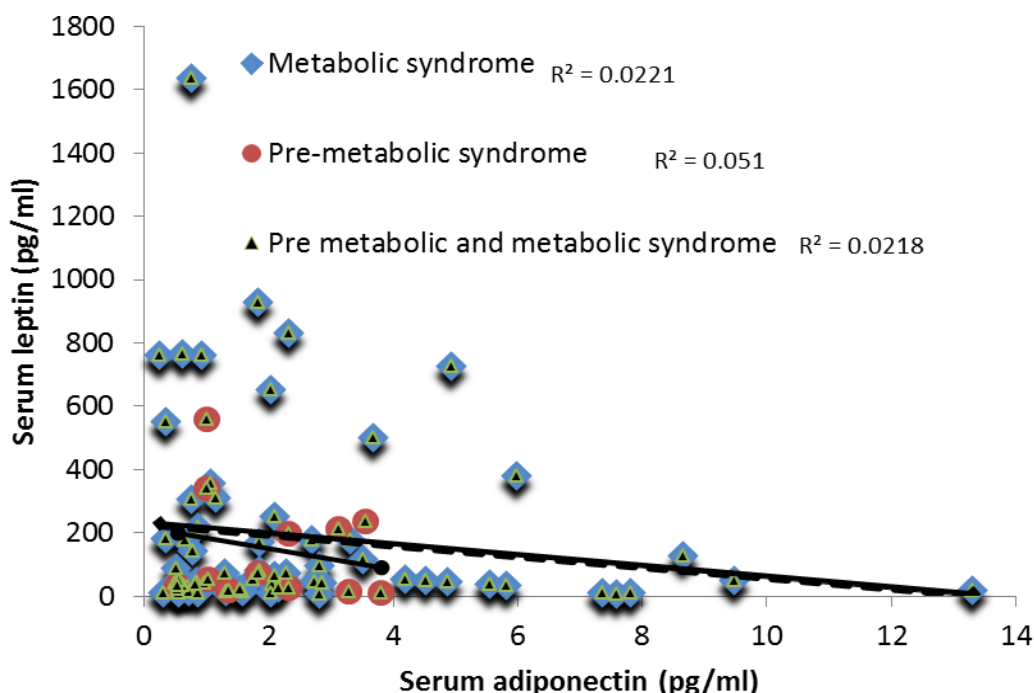


Figure 1 Non significant inverse correlation between serum adiponectin and leptin in patients with type 2 diabetes with pre-metabolic syndrome, metabolic syndrome and both.

Statistical analysis

Data presented as Means \pm SE. Unpaired Student's t-test and multivariate correlation test were used to evaluate differences between the two groups. For all tests, a two-tailed $p \leq 0.05$ was considered statistically significant. All calculations were made using Excel 2003 and statistical package for social sciences (SPSS) version 10 programs for Windows.

Results

A total of 78 patients (12 with pre-metabolic and 66 with metabolic syndrome) with a mean age of 58.2 years were enrolled in the study. Significant differences in anthropometric measurements (except height and mid-thigh circumference) between pre-metabolic and metabolic syndrome was observed (Table 1). Statistically significant high systolic, diastolic and mean blood pressures were noticed in metabolic syndrome compared to pre-metabolic syndrome (Table 2). Fasting serum lipid profile revealed significant high serum triglycerides and atherogenic index in patients with metabolic syndrome compared to pre-metabolic syndrome

(Table 3). Serum adipocytokines, leptin and adiponectin levels were non-significantly higher in metabolic syndrome than pre-metabolic syndrome (Table 4). The mean value of leptin to adiponectin ratio was twofold higher in patients with metabolic syndrome compared to corresponding ratio of pre-metabolic syndrome (Table 4). Multivariate analysis revealed non-significant correlation between either serum leptin or serum adiponectin in all component of pre-metabolic and metabolic syndrome (Table 5). Non-significant inverse correlation between serum adiponectin and leptin levels observed in patients presented with pre-metabolic and metabolic syndrome (Figure 1).

Discussion

The results of this study show that serum levels of adiponectin and leptin do not discriminate between pre-metabolic and metabolic syndrome. Also serum adipocytokines do not significantly correlate with any component of cardio-metabolic risk factors in patients with type 2 diabetes. Therefore, adiponectin and leptin are not good biomarkers to discriminate and predict the severity of metabolic syndrome in

patients with type 2 diabetes. Previous data showed that plasma adiponectin level is negatively associated with metabolic syndrome and body mass index or high blood pressure are considered as independent predictors for low plasma adiponectin level (17). Zhang et al., reported a decrease in serum adiponectin and an increase in serum leptin level as the number of metabolic syndrome components increased in women (18). The results of this study are not in agreement with other studies described above because previous studies did not refer to the pre-metabolic syndrome. Those studies determined serum levels of adiponectin and leptin in patients with metabolic syndrome and demonstrated non-significant correlations between components of metabolic syndrome and adipocytokines levels. Therefore, it is possible to consider serum levels of adipocytokines as associated rather than correlated biomarkers with cardio-metabolic risk factors. Previous studies suggested high serum level of leptin as a new component of metabolic syndrome because of its significant association with the key markers of metabolic syndrome (19, 20). Chubenko et al., reviewed the association between metabolic syndrome and serum leptin level and reached the conclusion that serum leptin level seems to be a predictor of acute cardiovascular event in metabolic syndrome (21). Others reviewed 25 articles and found significant inverse correlation between serum adiponectin level and triglyceride level and positive significant correlation with HDL-c (22). Such correlations were identified in this study but did not reach statistical significance perhaps due to small sample size which is considered as a limitation of the study. The other limitation of the study is the uneven distribution of cases with respect to gender. Hall et al., found that leptin/adiponectin ratio in patients with severe coronary heart disease was not a good marker to distinguish patients with and without metabolic syndrome (23). This study adds new information that the value of leptin/adiponectin ratio does not discriminate between pre-metabolic and metabolic syndrome. It concludes that low serum adiponectin and high serum leptin levels are associated with metabolic syndrome compared to pre-metabolic syndrome and these biomarkers are not good to discriminate between pre-metabolic and metabolic syndrome in patients with type 2 diabetes.

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