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Levels of Adipokines–Adiponectin and Resistin in Patients with Metabolic Syndrome and Newly Diagnosed Diabetes Mellitus Type 2

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Abstract

It has been recently proven, that the adipose tissue is not just a passive energy depot, but rather active endocrine organ – adipocytes are expressing on their surface multiple receptors and are releasing adipokines, hormones, cytokines, growth factors. Aim of the present study was to examine the serum levels of adipokines – adiponectin and resistin in patients with metabolic syndrome (MS) and newly diagnosed diabetes mellitus type 2 (NDDMt2). A prospective, comparative, observational study was performed. In 45 subjects with MS and NDDMt2 (n_1=45) and 35 clinical health subjects (n_2=35) were measured and compared the serum levels of adipokines – adiponectin and resistin. Two homoeostasis models assessment of insulin resistance (HOMA-IR) and of β-cell function (HOMA-%B)

were calculated. A significantly lower level of adiponectin was detected in patients with MS and NDDMt2 in comparison whit the control group (n_1=0.97±0.47 vs. n_2=1.96±0.73 μg/ml; p=0.005). A significantly higher level of resistin was found in patients with MS and NDDMt2 in comparison whit controls (n_1=1.85±0.70 vs. n_2=0.71±0.31 ng/ml; p=0.025). We have found negative correlations between concentration of adiponectin and serum levels of resistin, BMI and waist circumference in MS and NDDMt2 patients. The resistin was positively correlated with BMI and waist circumference, and was negatively correlated with HDL-cholesterol in MS and NDDMt2 patients.Patients with MS and NDDMt2 exhibited significantly changes in levels of adiponectin and resistin. Indicated adipokines may have predictive value of progression from normal to pathological carbohydrate metabolism.
***Key words**:* metabolic syndrome, newly diagnosed diabetes mellitus type 2, adiponectin, resistin.

Introduction

The ongoing global epidemic of diabetes mellitus type 2 (DMt2) is propelled by a concurrent rise in the prevalence of visceral obesity, insulin resistance (IR) and MS. Adipose tissue is a known endocrine organ secreting several soluble factors, known as adipocytokines and adipokines [1]. In normal healthy adults, adipokines regulate utilization and storage of lipids and help coordinate their distribution throughout the body. Their actions can be endocrine, paracrine, and autocrine as well [2]. Many of these adipokines have a physiological role in metabolic functions, lipid and glucose metabolism. Adiponectin and resistin are adipokines that can partly explain the link between obesity, IR, MS and beta-cell dysfunction in NDDMt2 [3].

Adiponectin (also known as AcrP30, adipocyte complement-related protein 30kDa or AdipoQ) is a 247-amino acid peptide secreted primary from adipose tissue. It was identified as an adipocyte-derived hormone almost simultaneously by four teams in the 1990s of the 20th century, but remained in obscurity until the early twenty-first century. Adiponectin binds to its receptor AdipoR1 and AdipoR2 in muscles, liver and adipose tissue. Accumulation of visceral fat results in hypoadiponectinemia because adiponectin expression is down regulated in the

adipose tissue. This inhibits the insulin action in the muscles, liver and other peripheral tissue [4,5]. The mechanisms whereby adiponectin reduces IR in MS and DMt2 are not fully understood. It has been suggested that these mechanisms lower serum free fatty acids (FFA) levels by stimulating there oxidation in the skeletal muscles. This reduces the concentration of triglycerides in the muscles with the result that insulin sensitivity is improved. The FFA influx in the liver (uptake and/or the FFA oxidation) also decreases because of the action of adiponectin. Adiponectin also directly stimulates glucose uptake in adipocytes and muscles by activating AMP- activated protein kinase [6,7,8]. There is a reverse correlation between serum adiponectin levels and the degree of obesity, IR, MS, NDDMt2, dyslipidemia and atherosclerosis [9,10]. In case-control studies low plasma levels of adiponectin were shown to be a risk factor for future development of NDDMt2, but not of obesity [11]. In addition to its effects adiponectin may have anti-inflammatory properties. It inhibits myelomonocytic and phagocytic activity, and Tumor necrotic factor-alfa (TNF-α) production by macrophages [12].

Because of its effects on insulin sensitivity and inflammation process, adiponectin is regarded as an anti-atherogenic factor. It has been shown that lower adiponectin in knockout mice have high levels of TNF-α, increased IR and susceptibility to atherosclerosis. Lower adiponectin is also associated with increased production of pro-inflammatory proteins-interleukin-6 (IL-6) and C-reactive protein (CRP). The low levels of adiponectin have been found to be correlated with coronary artery disease [13,14].

Resistin was first described in 2001 as a biologically active molecule secreted mainly by adipocytes and peripheral-blood mononuclear cells, and expressed by the macrophages embedded in fat tissue. It is a member of a class of cysteine-rich proteins collectively termed resistin-like molecules (REML). The resistin receptor in mice appears to be a fragment of decortin, lacking the glycosaminoglycan-binding site. It also binds to Toll-like receptor (TLR)-4, activating proinflammatory pathways in the hypothalamus. The main target organs of resistin action are the liver, muscles and adipose tissue [15]. Resistin has be associated with development of chronic low-grade inflammatory process. Secretion of this adipokin is beneficially affected by the inflammatory cytokines- TNF-α and interleukin (IL)-6 and has been found to induce the expression of TNF-α and IL-6 in white adipose tissue and peripheral

mononuclear cells [16]. Up to date, no precise and clear mechanisms of resistin action have been described. Because of its relationship with adipose tissue and IR, this adipokine has been inflected to be a link between obesity, MS and diabetes mellitus type 2. As a support of this hypothesis, a lot of data showing higher levels of resistin in patients with MS compared to those in clinically healthy individuals have been published [17,18]. Contradictory results concerning the relationship between resistin and IR in MS and NDDMt2 have been recently presented [19,20].

The aim of the study was to investigate the serum levels of adipokines- adiponectin and resistin in patients with metabolic syndrome and newly diagnosed diabetes mellitus type 2.

Material and Methods

A prospective, comparative, observational study was performed among 45 subjects with MS and NDDMt2 (n_1=45). All patients were involved in the study according to specific inclusion and exclusion criteria. Patients with anamnesis for DMt2, impaired fasting glucose (IFG) or impaired glucose tolerance (IGT) had been excluded. The carbohydrate tolerance was assessed by a standard oral glucose-tolerance test (OGTT) with 75 g glucose. The results were compared with a control group of 35 clinical health subjects with normal glucose tolerance (n_2=35).The diagnose of MS was evaluated by the IDF criteria from 2010 [21]. The diagnose of DMt2 was established according to the WHO diagnostic criteria, from 2011 [22]. The study protocol was approved by the local medical ethic committee. Informed consent was obtained from all participants.

Anthropometric data were taken including height (meters) and body weight (kilograms) for the determination of body mass index (BMI). The waist circumference (cm) was measured on a horizontal plain located in the middle between the lower edge of the 12th rib and the upper edge of the iliac bone, with accuracy of up to 0.5 cm. The blood pressure (mmHg) was measured in a sitting position in standard conditions, after a 5-minute rest, with an interval of 5 minutes between two successive measurements. Existing arterial hypertension

was alleged for values ≤ 130 mm Hg of the systolic and/ or ≤ 85 mm Hg of the diastolic arterial pressure or administration of anti-hypertensive medications in the patients with proven arterial hypertension.

All blood samples were of venous blood taken after an 8-h overnight fast. Blood glucose was measured in venous plasma at the 0, 60 and 120 minute of the OGTT by applying the glucose oxidising method (Glucose Analyzer Beckman, USA). The insulin level was monitored at 0, 60 and 120 minute of the OGTT by the immune-radio-metric method (IRMA) based on reference values 4.0-16.0 mIIU/l. The total cholesterol, HDL- cholesterol and triglycerides were assessed by an enzyme colorimetric method (GPO- PAP; Biocon® Diagnostik). LDL-cholesterol was calculated by the Friedewald formula (LDL-cholesterol = total cholesterol - HDL-cholesterol - triglycerides/2.2).

Two homoeostasis assessment models were used: HOMA-IR to assess the insulin resistance and HOMA-%B to assess the β-cell function. HOMA-IR was calculated using the folowing formula: fasting plasma glucose (mmol/l) x fasting serum insulin (mIU/l) / 22,5; HOMA-%B was calculated using the folowing formula: 20 x fasting serum insulin (mIU/l) / fasting plasma glucose (mmol/l)-3,5 [23].

Adipokines- adiponectin and resistin were determined by enzyme-linked immunosorbent assay (ELISA) method which is a competitive analysis for quantitative measurement of human adiponectin and resistin in serum or plasma, intended for in *vitro* research studies, BioVendor Laboratory Medicin, Inc.,Czech Republic.). Adiponectin sensivity was 0,2 μg/ml; the intra- and interassay coefficients of variation (CV) were ≤ 7,0% and ≤ 8,2%, repectivly. Resistin sensivity was 0,012 ng/ml; the intra- and interassay CV were ≤ 6,6% and ≤ 8,1%, respectively.

Statistical analyzes

All analyses were performed using STATGRAPHICS Centurion XV.I. Data were presented as their mean value and their standards deviations (means ± SD) or as individual data and median values. Comparisons between groups were done using: Independent sample

t-test for parametric comparison of the two means, Kolmogorov Smirnov for a non-parametric comparison and Mann-Withey tests for the test median of two groups. Two-sided P values < 0.05 were considered to indicate statistical significant differences. The Pearson (r) correlation for measurement the strengths of association between two variables were also done.

Results

The basal clinical characteristics of the patients and the control group are shown on Table 1. The mean age of the study participant was 42.65 ±13.0 years, and the mean age of the control group was similar- 43.26 ± 12.0 years, P>0.05. Patients with MS and NDDMt2 met clinical (waist circumference, blood pressure) and biochemical (fasting plasma glucose, HDL-cholesterol, triglycerides) IDF criteria for MS.

When comparing the MS and NDDMt2 patients with the controls statistically significant differences in BMI (n_1=34.83 ± 5.9 vs. n_2= 21.64 ± 2.33 kg/m^2; P<0.05), waist circumference (n_1=112.64 ± 12.92 vs. n_2= 76.42 ± 3.56 cm; P<0.05), systolic blood pressure (n_1=131.22 ± 15.23 vs. n_2= 116.32 ± 7.61 mmHg; P<0.05), fasting plasma glucose (n_1=8.82 ± 1.50 vs. n_2= 4.89 ± 0.45 mmol/l; P<0.05) and serum triglycerides (n_1=2.52 ± 0.40 vs. n_2=1.00 ± 0.15 mmol/l; P<0.05) were observed.

Patients with MS and NDDMt2 had significant higher level of basal insulin in comparison with the control group (n_1=19.22±5.87 vs. n_2=7.93±3.41; P=0.001). The significant differences of HOMA-IR (n_1=7.01±1.86 vs. n_2=1.72±0.36; P=0.001) and HOMA-%B (n_1=88.67±34.96 vs. n_2=131.45±44.0; P=0.049) were observed (Table 2).
A significant reduction in the serum concentration of adiponectin was detected in cases of MS with NDDMt2 in comparison with the control group (n_1= 0.97±0.47 vs. n_2= 1.96±0.73 μg/ml; P=0.005). The higher resistin level was observed in MS and NDDMt2 patients (n_1=1.85±0.70 ng/ml) and the differences with the control group (n_2=0.71±0.31 ng/ml) were significant, P=0.025. (Table 3).

In the present study negative correlations between serum concentration of adiponectin with level of resistin (r = -0.40), BMI (r = -0.36) and waist circumference (r = -0.37) was

Table.1 Basal clinical characteristics of patients

Parameter	Control group (n=35)	Patients with MS and NDDMt2 (n= 45)	Significance P<0.05
Average age (years)	43.26 ± 12.0	42.65 ± 13.0	NS
Body mass index (kg/m^2)	21.64 ± 2.33	34.83 ± 5.92	P*
Waist circumference (cm)	76.42 ± 3.56	112.64 ± 12.92	P*
Systolic blood pressure (mmHg)	116.32 ± 7.61	131.22 ± 15.23	P*
Diastolic blood pressure (mmHg)	77.37 ± 6.42	83.56 ± 10.42	NS
Fasting blood glucose (mmol/l)	4.89 ± 0.45	8.82 ± 1.50	P*
Total cholesterol (mmol/l)	5.44 ± 1.2	5.40 ± 1.21	NS
HDL-cholesterol (mmol/l)	1.45 ± 0.39	1.06 ± 0.35	NS
LDL-cholesterol (mmol/l)	3.47± 1.05	3.21 ± 1.01	NS
Triglycerides (mmol/l)	1.00 ± 0.15	2.52 ± 0.40	P*

P*<0.05

NS (non significant)

Table.2 Levels of basal Insulin, HOMA-IR and HOMA-%B

Parameters	Control group (n=35)	Patients with MS and NDDMt2 (n=45)	Significance P<0.05
Basal Insulin (mIU/l)	7.93±3.41	19.22±5.87	P=0.001
HOMA-IR	1.72±0.36	7.01±1.86	P=0.001
HOMA-%B	131.45±44.0	88.67±34.96	P=0.049

Table. 3 Serum levels of adiponectin and resistin

Parameters	Control group (n=35)	Patients with MS and NDDMt2 (n=45)	Significance P<0.05
Adiponectin (µg/ml)	1.96±0.73	0.97± 0.47	P=0.005
Resistin (ng/ml)	0.71±0.31	1.85±0.70	P=0.025

observed in MS and NDDMt2 patients. There were positive correlations between level of resistin, BMI (r = 0.45) waist circumference (r = 0.36) and negative correlations between resistin and HDL-cholesterol (r = -0.42) in MS and NDDMt2 patients.

Discussion

Our results showed lower level of adiponectn and higher level of resistin in insulin resistant patients with MS and NDDMt2 compared to healthy controls. The data from the current research correspond to the information in the literature confirming the close link between the hypoadiponectinemia and higher level of resistin with the IR observed in cases of

NDDMt2 [24,25]. The previous study carried out by our team had identified more pronounced IR in patients with MS and NDDMt2 as compared to the MS patients with normal glucose tolerance, IFG, IGT and control group.

In the same study no significant differences in the serum adiponectin and resistin levels comparing MS patients with normal glucose tolerance, IFG, IGT, and healthy controls were observed [26]. Our data showed significant lower level of HOMA-%B in subject with MS and NDDMt2 as compared to the control group. The other others also discussed about the role of adipokines in progress of β-cell failure in of type 2 diabetes [27].

The results obtained by us supported conclusions made the other authors- serum adiponectin concentrations like a predictor for the developments of type 2 diabetes and the metabolic syndrome with NDDMt2 in elderly patients [28]. Jaime Lee et al. conducted a case-control study that proved the presence of significantly lower adiponectin level in 60 patients with MS and type 2 diabetes mellitus compared with lean control subject. The investigators were found that adiponectin serum level was negatively correlated with systolic blood pressure, triglycerides, fasting plasma glucose, BMI and waist circumference, and positively correlated with HDL-cholesterol [29]. Zaidi and co-workers have found higher resistin level in obese patient with type 2 diabetes mellitus compared to metabolically healthy obese individuals without type 2 diabetes mellitus. The authors were found positively correlated of resistin with BMI and HOMA-IR in the type 2 diabetes mellitus [30]. Data in the literature regarding the changes in serum concentration of resistin in patients with MS and NDMt2 are not consistent. Some authors reported an increase serum resistin levels in cases of visceral obesity, MS, IR, and DMt2 [31]. De Luis et al. did not find association between resistin with metabolic syndrome criteria in obese [32]. Other research team pointed out the significantly higher serum level of resistin in women with polycystic ovary syndrome and IR without DMt2 and significant correlation between resistin and insulin levels at 0 and 120 minute (during OGTT), HOMA-IR, Matsuda and QUICKI indexes [33].

Conclusion

Subjects with metabolic syndrome and newly diagnosed diabetes mellitus type 2 exhibited significant changes in serum levels of adiponectin and resistin. The results suggested a possible pathophysiological role of these adipokines in the insulin resistance development in metabolic syndrome and newly diagnosed diabetes mellitus type 2.

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