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ADIPONECTIN, NON-ESTERIFIED FATTY ACIDS AND ANTIPHOSPHOLIPID ANTIBODIES IN TYPE II DIABETES MELLITUS

ADIPONEKTIN, NEESTERIFIKOVANE MASNE KISELINE I ANTIFOSFOLIPIDNA ANTITELA U DIJABETESU TIP II

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Summary: The importance of the association of antiphospholipid antibodies (aPL Abs) with the features of type II diabetes mellitus has not yet been elucidated. The aim of this work was to investigate the association of aPL Abs with adiponectin and non-esterified fatty acids (NEFA) in type II diabetes mellitus patients without micro and/or macrovascular complications, and to analyze the differences between the male and female patients with regard to the abovementioned parameters. Male patients with type II diabetes mellitus showed a positive correlation between NEFA concentrations and anti-oxLDL antibodies (r=0.334, p=0.019). A weak, but statistically significant correlation between adiponectin concentrations and the IgM isotype of anti-annexin A5 antibodies was found in type II diabetes mellitus patients (r=0.285, p=0.011). The presence of a positive correlation between NEFA and anti-oxLDL antibodies might be useful in the detection of patients with premature atherosclerosis in type II diabetes mellitus patients without any micro and/or macrovascular complications among type II diabetes mellitus patients.

Keywords: antiphospholipid antibodies, adiponectin, diabetes mellitus type II

Kratak sadržaj: Značaj povezanosti antifosfolipidnih antitela (aPL Abs) sa odlikama dijabetesa tip II još nije razjašnjen. Cilj rada bilo je ispitivanje povezanosti aPL Abs sa adiponektinom i neesterifikovanim masnim kiselinama (NEFA) kod pacijenata sa dijabetesom tip II bez mikro i/ili makrovaskularnih komplikacija i analiziranje razlika u navedenim parametrima između muškaraca i žena sa dijabetesom. Kod muškaraca sa dijabetesom tip II pokazana je pozitivna korelacija između koncentracija NEFA i anti-oxLDL antitela (r=0,334, p=0,019). Slaba ali statistički značajna korelacija između koncentracija adiponektina i IgM izotipa anti-aneksin A5 antitela pronađena je kod pacijenata sa dijabetesom tip II (r=0,285, p=0,011). Pozitivna korelacija između NEFA i anti-oxLDL antitela mogla bi da bude korisna u detekciji pacijenata sa prevremenom aterosklerozom u dijabetesu tip II bez mikro i/ili makrovaskularnih komplikacija.

Ključne reči: antifosfolipidna antitela, adiponektin, diabetes mellitus tip II

Introduction

The existence of a procoagulant state has been found in type II diabetes mellitus patients (1). Antiphospholipid antibodies (aPL) may cause and/or promote thrombosis (2–5). Previously it was reported that antiphospholipid monoclonal antibodies reduced the anticoagulant effect of annexin A5, and those antibodies were involved in the stimulation of thrombin generation (6). Various mechanisms that explain the roles of antiphospholipid antibodies in the pathogenesis of the antiphospholipid syndrome have been

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proposed, but the importance of the association of aPL Abs with the features of type II diabetes mellitus has not yet been elucidated.

Adiponectin is a protein secreted by adipocytes that exerts antiinflammatory and antiatherogenic characteristics. Moreover, adiponectin has a role in the relationship between obesity and insulin resistance (7). Previous reports have suggested that inflammation might be considered as the link between insulin resistance, obesity and diabetes mellitus (8).

Impaired non-esterified fatty acids (NEFA) metabolism in adipose tissue is associated with some of the features of the metabolic syndrome (9, 10). Dysregulation of NEFA metabolism is atherogenic (11).

Recently it has been suggested that an association between aPL Abs and TNF-alpha might be a predictor of a severe atherogenic profile in type II diabetes mellitus patients without vascular complications (12). In the present study we investigated the association of aPL Abs with adiponectin and NEFA in a well-formed group of type II diabetes mellitus patients without micro and/or macrovascular complications. Furthermore, we analyzed the differences between male and female patients with regard to the above-mentioned parameters.

Patients and Methods

Patients

The study was approved by the local Ethical Committee and all participants gave their written informed consent. Well formed group of 78 consecutive patients with type II diabetes mellitus without micro and/or macrovascular complications was studied. Patients were assessed for the presence of diabetic complications, i.e. retinopathy, nephropathy, history of myocardial infarction and the presence of angina pectoris. The body mass index was calculated as the weight (kg)/height (m^2). Cut-off values for waist circumference (WC) and the waist-hip ratio (WHR) were set as recommended (13, 14).

Methods

Apolipoproteins AI, AII, B, E were measured by immunonephelometry using a Behring Nephelometer Analyzer II, Marburg, Germany. Cholesterol, triglycerides, HDL-cholesterol and HbA1c were measured on an AU2700® Chemistry analyzer (Beckman Coulter, Clinical Diagnostics). Cut-off values for all the above-mentioned parameters were set in accordance with the manufacturer's recommendations. Measurement of the concentrations of oxLDL (Mercodia, Sweden) was done by ELISA. Concentrations of analyzed antibodies were measured by ELISA using the commercial reagents of Imtec Immunodiagnostika, Germany, for the detection of anti-oxLDL (aoxLDL) antibodies, and Orgentec Diagnostika GmbH, Germany, for the detection of anti-cardiolipin (aCL), anti-β2glycoprotein I (aβ2gpI) and anti-annexin A5 (anxA5) antibodies. Cut-off values for all the analyzed antibodies were set as the manufacturer recommended.

Statistical analysis

Kolmogorov-Smirnov test was used to determine whether the analyzed variables followed a normal distribution. Continuous variables were expressed as median (25^{th}–75^{th} percentiles). The association between the presence of antiphospholipid antibodies (aCL, aβ2gpI, anxA5, aoxLDL) and the anthropomorphic features of patients was examined by χ^2-test, Mann-Whitney or t-test, when appropriate. The correlation between two quantitative variables was determined with the correlation tests (Spearman's, Pearson's, when appropriate). Analyses were conducted in SPSS 10 (SPSS, Inc, Chicago, IL, USA).

Results

Differences in the investigated parameters between the female and male patients with type II diabetes mellitus are shown in *Tables I* and *II*. Waist-hip ratio, NEFA, glucose concentrations and HbA1c were significantly different between the female and male type II diabetes mellitus patients. Concentrations of the analyzed aPL Abs did not reach statistically significant difference between the investigated female and male subjects.

Adiponectin concentrations were in a positive correlation with HDL ($r=0.423$, $p=0.000$) and apo AI concentrations ($r=0.333$, $p=0.004$) and in an inverse correlation with triglyceride concentrations ($r=-0.234$, $p=0.039$) and with WHR ($r=-0.324$, $p=0.004$). No significant association between adiponectin and aCL, aβ2gpI and aoxLDL Abs was found. A weak, but statistically significant correlation between adiponectin concentrations and the IgM isotype of anti-annexin A5 antibodies was found in type II diabetes mellitus patients ($r=0.285$, $p=0.011$). However, logistic regression analysis failed to confirm the strength of this association.

Concentrations of NEFA and adiponectin were in a negative correlation ($r=-0.235$, $p=0.047$). A positive correlation was found for NEFA concentrations and BMI ($r=0.244$, $p=0.039$), HbA1C ($r=0.248$, $p=0.037$), glucose ($r=0.281$, $p=0.017$), apo E ($r=0.327$, $p=0.006$) and triglyceride concentrations ($r=0.237$, $p=0.045$). No significant association between NEFA and the analyzed aPL Abs was found.

In the male patients with type II diabetes mellitus NEFA concentrations showed positive correlations with apoAI ($r=0.361$, $p=0.013$) and anti-oxLDL antibodies ($r=0.334$, $p=0.019$).

Table I Differences in analyzed parameters (adiponectin, NEFA, apolipoproteins) between female and male patients with type II diabetes mellitus.

Parameters Median (25^{th}–75^{th})	Female	Male	P value
Number	25	53	
Age (years)	57 (50–62.50)	54 (47–59)	n.s.
WC (cm)	99 (96.5–121.0)	108 (99–115)	n.s.
WHR	0.91 (0.89–0.92)	0.93 (0.90–0.97)	0.005
Glucose (mmol/L)	6.55 (6.02–8.75)	8.20 (7.05–9.45)	0.011
HbA1c (%)	6.50 (6.10–7.17)	7.10 (6.70–7.90)	0.005
Cholesterol (mmol/L)	5.52 (4.49–6.63)	5.63 (4.72–6.59)	n.s.
LDL (mmol/L)	3.02 (2.72–3.99)	3.39 (2.69–4.45)	n.s.
HDL (mmol/L)	1.12 (0.95–1.29)	0.97 (0.83–1.19)	n.s.
Triglycerides (mmol/L)	2.19 (1.44–3.58)	2.17 (1.45–3.50)	n.s.
Apolipoprotein AI (g/L)	1.94 (1.69–2.29)	1.73 (1.47–1.97)	n.s.
Apolipoprotein AII (mg/L)	349 (339–378)	321 (279–373)	n.s.
Apolipoprotein B (g/L)	1.13 (0.97–1.41)	1.21 (1.05–1.57)	n.s.
Apolipoprotein E (mg/L)	50.85 (40.42–59.77)	45.30 (34.55–57.90)	n.s.
oxLDL (mU/L)	8.83 (7.98–11.65)	10.09 (8.45–11.51)	n.s.
Adiponectin (μg/mL)	56.88 (36.76–83.54)	59.19 (27.51–79.49)	n.s.
NEFA (mmol/L)	0.60 (0.42–0.92)	0.48 (0.36–0.66)	0.039

n.s. not significant

Table II Differences in analyzed antibodies between female and male patients with type II diabetes mellitus.

Analyzed antibodies Median (25^{th}–75^{th})	Female	Male	P value
Number	25	53	
aCL IgG (U/mL)	5.78 (4.07–8.06)	5.64 (3.34–7.76)	n.s.
aCL IgM (U/mL)	4.97 (4.03–6.19)	4.34 (2.96–6.02)	n.s.
ab2gpI IgG (U/mL)	2.74 (1.12–4.80)	3.16 (0.00–5.57)	n.s.
ab2gpI IgM (U/mL)	2.46 (1.49–3.43)	2.28 (1.18–3.35)	n.s.
aanxA5 IgG (U/mL)	2.05 (1.05–2.57)	2.12 (1.46–2.68)	n.s.
aanxA5 IgM (U/mL)	1.38 (0.99–2.16)	1.98 (1.14–2.51)	n.s.
aoxLDL (IgG + IgM) (U/mL)	15.30 (12.85–20.95)	18.57 (16.49–23.75)	n.s.

aanxA5, anti-annexin A5 antibodies; ab2gpI, anti-β2gpI antibodies; aCL, anticardiolipin antibodies; aoxLDL, anti-oxLDL antibodies; n.s. not significant

In the female patients with type II diabetes mellitus a negative correlation between adiponectin concentrations and HbA1c ($r=-0.481$, $p=0.017$) was found. Also, NEFA and HDL concentrations were in an inverse correlation ($r=-0.442$, $p=0.035$) in the investigated female subjects. Menopause was present in 21 out of 25 (84%) female patients with type II diabetes mellitus. Two out of 25 (8%) female patients were on hormone substitution therapy. Statistically non-significantly elevated NEFA levels were present in five out of 19 (26.31%) menopause patients ($\chi^2=0.003$, $p=0.957$). In patients receiving hormone substitution therapy a significant association with elevated levels of apoAI was observed ($\chi^2=6.545$, $p=0.011$). Also, these patients showed a significant association with the presence of the IgG isotype of aCL antibodies ($\chi^2=5.210$, $p=0.022$).

Discussion

Plasma adiponectin exists in three isoforms, and high molecular weight adiponectin (HMWA) levels were higher in women (in comparison to the age and BMI-matched men) because testosterone regulates the secretion of HMWA from adipocytes, whereas middle and low molecular weight adiponectin levels are comparable between both genders (15).

Differences between men and women are thought to be a direct effect of androgens on adiponectin synthesis (16). Testosterone inhibits adiponectin secretion from adipocytes (17), and testosterone replacement therapy caused a decrease in adiponectin levels in hypogonadal patients (15). Androgens decrease adiponectin levels, and androgen-induced hypoadiponectinemia may be related to the high risk of insulin resistance and atherosclerosis in men (17). The difference in adiponectin concentrations between men and women vanished in patients older than 80 years, obese persons and in patients with type II diabetes mellitus. Serum adiponectin concentrations did not differ between patients with and without type II diabetes mellitus (18). It was reported that postmenopausal women had significantly higher levels of adiponectin than premenopausal women (19). However, in our study almost all of the analyzed menopausal patients (85.71%) had elevated adiponectin levels, and this is the explanation for the discrepancy in the results.

Adiponectin levels are lower in those with obesity (20) and type II diabetes mellitus, and the levels increase with weight reduction (21). Adiponectin levels were in a negative correlation with BMI (22–24), WC (25), WHR (26).

No significant difference in age, WHR and adiponectin levels between the female and male type II diabetes patients was found.

Adiponectin is negatively correlated with the features of metabolic syndrome and other associated features of insulin resistance and conventional cardiovascular risk factors. These include serum insulin, total cholesterol, LDL, apoB100, triglycerides, glucose, HbA1c, lower HDL and smaller LDL particle size (27–29). Our findings are in concordance with the abovementioned results in regard to the negative correlation between adiponectin and WHR, triglyceride concentrations and HbA1c (only in the female subgroup of patients).

Increased NEFA has an important role in the development of insulin resistance and type II diabetes mellitus (30, 31). Diabetes mellitus is associated with an increased risk of CVD, and NEFA might be directly related to coronary events (32–34). Impaired NEFA metabolism in adipose tissue is linked to some components of the metabolic syndrome (impaired glucose disposal, hypertriglyceridemia, low HDL, small LDL (9, 10). In our study, NEFA concentrations were in a negative correlation with adiponectin levels, and in a positive correlation with glucose, triglycerides, HbA1c, apoE.

It was reported that oxidized LDL are the product of oxidation of their polyunsaturated fatty acid component, and that the uptake of oxLDL by macrophages activates the immune system. Increased immunogenicity of the oxLDL molecule elicits the formation of anti-oxLDL antibodies. The presence of anti-oxLDL antibodies is associated with atherosclerosis (35). In our study, in the male patients with type II diabetes mellitus a positive correlation between the NEFA concentrations and anti-oxLDL antibodies titers was shown.

It was suggested that adiponectin should serve as a marker for the progression of atherosclerosis (36) and may be helpful in preventing the development of atherosclerotic vascular disease or its complications (37). Based on our results, the presence of a positive correlation between NEFA and anti-oxLDL antibodies might be useful in the detection of patients with premature atherosclerosis among type II diabetes mellitus patients without any micro and/or macrovascular complications.

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Conflict of interest statement

The authors declare having no conflict of interest related to the publication of this manuscript.

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