

Anti-beta2-glycoprotein I Autoantibodies and Metabolic Syndrome

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Abstract

Background: The metabolic syndrome (MetS) is a proatherogenic entity. Autoantibodies to phospholipid cofactors such as beta2-glycoprotein I (beta2-gpl) can influence atheroma appearance. Previous studies confirmed an association of IgA anti-beta2-gpl antibodies with cerebral ischemia, myocardial infarction, peripheral artery disease and carotid disease.

Objective: This case-control study evaluates a possible association of anti-beta2-gpl and anticardiolipin (aCL) antibodies with non-complicated MetS.

Methods: Cases comprised patients with MetS without history of vascular events; controls included individuals from the Orthopedic Infirmary admitted due to musculoskeletal disorders. Age, sex, race, history of hypertension, smoking, hypercholesterolemia and diabetes mellitus were evaluated as risk factors in both groups. IgG, IgM, and IgA anti-beta2-gpl and aCL antibodies were detected by enzymatic immunoassay.

Results: Sixty-eight patients with MetS and 82 controls were studied. Patients with MetS showed mean age higher than controls ($p = 0.001$), while males ($p = 0.003$; OR 0.31; 95%CI 0.15-0.16) and Caucasian ethnicity ($p = 0.004$; OR 0.25; 95%CI 0.10-0.60) predominated in controls. History of hypertension, hypercholesterolemia and diabetes mellitus were more prevalent in cases than in controls ($p < 0.05$). The frequency of aCL antibodies (all isotypes) and of IgG and IgM anti-beta2 gpl did not significantly differ in cases and controls. IgA anti-beta2-gpl antibodies were significantly more frequent in MetS patients (42.2%) than controls (10.9%) ($p < 0.001$). The adjusted OR for IgA anti-beta2-gpl antibodies was 3.60 (95%CI 1.55-8.37; $p = 0.003$).

Conclusion: The current study shows that elevated levels of IgA autoantibodies to β 2-gpl might be independently associated to MetS. (Arq Bras Cardiol 2011;96(4):272-276)

Keywords: Autoantibodies; metabolic syndrome; antibodies, anticardiolipin; atherosclerosis.

Introduction

The metabolic syndrome (MetS) is a cluster of metabolic abnormalities, including insulin resistance, dyslipidemia and systemic hypertension, in which visceral obesity is prominent¹. Initially identified sixty years ago, MetS is currently an issue of major interest². It is well accepted that MetS predicts diabetes mellitus (DM)³ and cardiovascular diseases³⁻⁴.

In MetS, low-density lipoproteins (LDL) particles are prone to oxidation⁵. Persistent hyperglycemia accelerates the generation of advanced glycation end products, yet another trigger to arterial inflammation⁶. Adipocyte accumulation, in turn, promotes tumor necrosis factor (TNF) secretion and reduces levels of adiponectin, the latter an anti-atherogenic adipokine⁷.

Atherosclerosis is currently considered an immunoinflammatory condition. Endothelial activation,

a primary event in atherogenesis, can be a consequence of typical MetS manifestations or TNF effect. The activated endothelial cell is characterized by low thrombomodulin production and a permissive phenotype to hypercoagulability⁸.

Autoimmunity, particularly antibodies against cardiolipin or phospholipid co-factors such as beta2-glycoprotein I (β 2-gpl), can influence atheroma development⁹. Anticardiolipin (aCL) antibodies are classically detected in the so-called antiphospholipid syndrome (APS), a characteristic thrombotic diathesis of young adults¹⁰. The etiopathogenic role of aCL antibodies in MetS syndrome is yet unknown.

β 2-gpl is a phospholipid co-factor with 50 kilodaltons and 5 domains. It is a natural anticoagulant. In APS patients, the inhibitory effects of β 2-gpl on coagulation pathways are apparently dysregulated by antiphospholipid antibodies¹¹.

β 2-gpl is found in atherosclerotic plaque and can be immunogenic. Atherosclerosis induction in low-density lipoprotein receptor-(LDLR) deficient mice immunized with β 2-gpl has been reported¹². The oral administration of human β 2-gpl in mice prevent atheroma formation¹³. It is well known that β 2-gpl binds to oxidized LDL particles; the complex can

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be internalized only by antibodies occupying Fc receptors on macrophage surface¹⁴.

A possible association of anti-β2-gpl autoantibodies with non-complicated MetS has not been evaluated so far. This study verifies the frequency of aCL and anti-β2-gpl in patients with MetS; it also assesses whether these autoantibodies are associated to the occurrence of non-complicated MetS.

Methods

Subjects

This case-control study of incident cases included patients with MetS from our Outpatient Clinic of Cardiometabolic Risk, not selected by sex or ethnicity. MetS patients included in this study did not have history of ischemic vascular events. The diagnosis of MetS was based on the classical criteria of the National Cholesterol Educational Program (NCEP): abdominal circumference > 102 cm for men or > 88 cm for women; triglycerides serum levels > 150 mg/dl; blood pressure ≥ 130/85 mmHg; fasting glucose > 110 mg/dl; at least 3 criteria should be present for the diagnosis¹⁵. The exclusion criteria were as follows: 1) history of acute myocardial infarction, ischemic stroke or acute arterial occlusion of lower limbs; 2) infective endocarditis; 3) neoplasms (current or past); 4) infection by human immunodeficiency virus or *treponema pallidum*; 5) presence of known inherited causes of thrombosis, such as homocystinuria or factor V (Leiden) mutation; 5) APS or other connective tissue disorder (CTD).

The control group comprised patients admitted to the Orthopedic Ward due to fractures or muscle-ligament disorders and with no previous diagnosis of MetS. The exclusion criteria were: 1) osteonecrosis; 2) current infections, neoplasias, hereditary disorders, APS, or CTD; 3) history of acute myocardial infarction, ischemic stroke or acute arterial occlusion of lower limbs.

Clinical and demographic data were obtained from a chart review and interviews with patients and their families after informed consent, considering the following features: 1) age, sex, race/ethnicity; 2) history of high blood pressure¹⁶; 3) current cigarette smoking¹⁷; 4) DM, according to clinical history or current treatment with insulin and/or oral antidiabetic drugs¹⁸; 5) history of hypercholesterolemia¹⁹.

Specimens

Serum specimens were centrifuged and frozen within 2 hours of collection and subsequently, stored at -70° C until laboratory testing by ELISA (enzyme-linked immunosorbent assays). IgG, IgM and IgA aCL (INOVA Quantalite cardioliipin kits, INOVA diagnostics, Inc., San Diego, USA)²⁰ and anti-β2-gpl antibodies (INOVA Quantalite β2-gpl kits, INOVA diagnostics, Inc., San Diego, USA)²¹ were tested. The assays were evaluated by spectrophotometry, measuring and comparing the color intensity that developed in the patient wells with the color in the control wells. Moderate to high titers of aCL and anti-β2-gpl antibodies (above 20 units for all isotypes) were considered positive result^{20,21}. Low aCL or anti-β2-gpl titers (10-20 units) were not considered. The study was approved by the local ethics committee.

Data analysis

Odds ratios (OR) with 95% confidence intervals (95%CI) were used for univariate analysis. Logistic regression with 95%CI was performed to adjust for the effects of age, sex, ethnicity and current smoking. The Hopkins scale for OR was used, whereby an OR 1-1.5 was considered trivial; between 1.5-3.5, small; between 3.5-9, moderate; between 9-32, strong; and above 32, very strong²². Fisher's exact test and Chi-square analysis were used for comparison of categorical variables and the Student's *t* test was used for comparison of continuous variables. A level of 5% ($p < 0.05$) was considered significant. All analyses were carried out using SPSS for Windows, version 11.5, Chicago, IL.

Results

A total of 68 patients in the MetS group and 82 controls were enrolled for the study. Patients with MetS were more likely to be older, while males and Caucasians predominated in the control group. Hypertension, hypercholesterolemia and DM were significantly more prevalent in MetS patients than in controls. Cigarette smoking was more prevalent in the control group. The demographic and clinical characteristics of cases and controls are shown in Table 1.

Table 2 categorizes cases and controls according to aCL and anti-β2 gpl profiles. The frequency of aCL antibodies (all isotypes) and of IgG and IgM anti-β2 gpl did not significantly differ between cases and controls. IgA anti-β2-gpl antibodies were significantly more frequent in MetS patients than in controls.

The OR for aCL and anti-β2-gpl antibodies adjusted for risk factors (age, sex, ethnicity and current smoking) seen in Table 3. IgA anti-β2-gpl, but not other autoantibodies, was independently associated with MetS.

Discussion

The study evaluates, for the first time, the frequency of both aCL and anti-β2-gpl antibodies in patients with MetS. Cases were selected from a Tertiary Clinic specialized in cardiometabolic risk. Patients with MetS had a higher mean

Table 1 - Clinical and demographic characteristics of patients with metabolic syndrome (cases) and controls

	Cases (n = 68)	Controls (n = 82)	P
Mean age, years (SD†)	58.7 ± 9.2	51.1 ± 17	0.001‡
Males	14 (20.6%)	37 (45.1%)	0.003*
White race	46 (67.7%)	73 (89%)	0.004*
Hypertension	65 (95.6%)	22 (26.8%)	<0.001*
Current smoking	7 (10.3%)	28 (34.1%)	0.001*
Hypercholesterolemia	55 (80.9%)	16 (19.5%)	<0.001*
DM	42 (61.8%)	5 (6.1%)	<0.001*

N - sample number; *, odds ratios with 95% confidence interval; †: SD - standard deviation ; ‡ Student's *t* test; * Chi-square test; DM: diabetes mellitus.

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Table 2 - Frequency of aCL and anti-β2-gpl antibodies in patients with metabolic syndrome (cases) and controls

	Cases (n = 68)	Controls (n = 82)	OR (95%CI)*	P**
Positive IgG aCL	3 (4.4%)	1 (1.2%)	3.73 (0.38-36.79)	0.32
Positive IgM aCL	0	3 (3.7%)	1.86 (1.60-2.16)	0.25
Positive IgA aCL	0	0	NC	NC
Positive IgG anti-β2-gpl	2 (2.4%)	4 (4.9%)	0.59 (0.10-3.32)	0.68
Positive IgM anti-β2-gpl	12 (14.6%)	8 (9.8%)	0.89 (0.29-2.71)	0.34
Positive IgA anti-β2-gpl	30 (42.2%)	9 (10.9%)	3.84 (1.75-8.39)	<0.001

n - sample number; *odds ratio with 95% confidence interval, non-adjusted for risk factors; **Chi-square test; NC - not calculate

Table 3 - OR for different aCL and anti-β2-gpl isotypes adjusted for metabolic syndrome risk factors

	OR*	95%CI**	P***
IgG aCL	3.79	0.35-41.21	0.273
IgM aCL	NC	NC	NC
IgA aCL	NC	NC	NC
IgG anti-β2-gpl	0.62	0.10-3.99	0.618
IgM anti-β2-gpl	0.90	0.28-2.93	0.864
IgA anti-β2-gpl	3.60	1.55-8.37	0.003

Data adjusted for age, sex, ethnicity and smoking; *odds ratio adjusted for risk factors; **95% confidence interval; ***chi-square test; NC - not calculated.

age as compared to controls, while males and the Caucasian ethnicity predominated in the control group. These differences were corrected by logistic regression, nonetheless. Classical risk factors for atherosclerosis were evaluated in both populations. Some variables (hypertension, DM) are criteria for MetS and, predictably, were more frequent in cases.

The non-adjusted data showed that only IgA anti-β2-gpl antibodies differed in the two groups, being significantly more elevated in MetS patients. Of note, the cutoff used for both aCL and anti-β2-gpl assays followed international recommendations, with only moderate to high titers (above 20 units) being considered relevant^{20,21}. aCL and anti-β2-gpl antibody levels were not stratified, as the clinical implications for moderate or high levels do not differ¹⁰. Nevertheless, the IgM and IgA anti-β2-gpl cutoff yielded positive results in approximately 10% of controls, probably demanding adjustments in the future.

After the adjustment for sex, age, ethnicity and smoking, the OR (3.60) indicated an independent association of IgA anti-β2-gpl antibodies with MetS, with convincing statistical significance (p = 0.003).

A possible association of IgG aCL with MetS (adjusted OR 3.79) could not be confirmed (p = 0.273). An eventual protective effect of IgG anti-β2-gpl for MetS (adjusted OR 0.62) was also not confirmed (p = 0.618). We emphasize that the variables hypertension, hypercholesterolemia and DM were not used for adjustments, as they are diagnostic criteria for MetS.

The meaning of the association of IgA anti-β2-gpl antibodies with MetS remains unknown. IgA autoantibodies to β2-gpl, according to previous studies from our group, were

independently associated with ischemic cerebral disease²³, acute myocardial infarction²⁴, symptomatic peripheral artery disease²⁵ and carotid disease²⁶.

Recently, a relevant association of IgA anti-β2-gpl and IgG anti-phosphatidylserine antibodies with ischemic stroke was reported²⁷. Of interest, IgM anti-β2-gpl antibodies were linked to thrombotic disorders in young women without autoimmune disease, particularly when classical risk factors or MetS were absent; the IgA isotype was not tested in this study²⁸. These data suggest that the IgM anti-β2-gpl autoantibody could behave differently from the IgA isotype as regard to MetS.

aCL antibody testing resulted negative in the majority of these studies²³⁻²⁸ and also in our patients with MetS. Altogether, these findings did not show a relevant role for aCL antibodies in patients with atherosclerotic disease or MetS.

The fine specificity of anti-β2-gpl antibodies in patients with atherosclerosis and APS appears to differ²⁹. Anti-β2-gpl antibodies from APS patients target the domain 1 of the molecule³⁰. In recent years, it has been shown that IgA antibodies from patients with atherosclerosis recognize specifically domain 4 of β2-gpl³¹. Thus, a distinct subgroup of anti-β2-gpl antibodies in patients with atherosclerotic disease could be postulated.

It is known that the β2-gpl/oxidized LDL oxidized complex is internalized to macrophages by IgG occupation of Fc receptors^{14,32}. Alternatively, we could hypothesize that IgA anti-β2-gpl antibodies (that might bind to the whole complex) also play this role on the macrophage surface. In fact, Fc receptors for IgA are found on macrophage membranes³³. The complex so internalized could generate foamy cells.

Our data on patients with established atherosclerotic disease²³⁻²⁶ and MetS look reproducible as far as the frequency of the IgA anti-β2-gpl autoantibody is concerned. Data on MetS, not previously reported, suggest that circulating IgA anti-β2-gpl autoantibodies could precede an ischemic event.

The reason why IgA isotype is involved in this immunologic response is not well defined, but chronic infection can at least be postulated. Of interest, molecular mimicry of viral and bacterial products with β2-gpl has been reported³⁴.

Our study shows limitations which must be mentioned. The case-control design with a small sample size and a limited multivariate analysis cannot define the issue or control for all risk factors; it is rather exploratory and warrants larger studies.

In summary, IgA anti-β2-gpl antibodies were more frequent in patients with MetS than in controls. This

association occurred independently from other risk factors for atherosclerotic disease. The clinical implications which arise from these findings should be detailed in the future.

Potential Conflict of Interest

No potential conflict of interest relevant to this article was reported.

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