RESEARCH ARTICLE

Correlation between hsCRP and Anti-β2GPI Antibody in Metabolic Syndrome

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Abstract

BACKGROUND: Several researches reported that inflammatory and immunological mechanism such as autoantibody to β2-glycoprotein I (anti β2GPI) appear as related factors in initiation and progress of atherosclerosis lesion in patient with autoimmune disease. Antibody to β2GPI titers are correlated with atherosclerosis and *in vitro* studies showed that they enhance oxidized low density lipoprotein (ox-LDL) uptake by macrophages. Immunization with auto-antigen β2GPI elicits an immune response to influence lesion progression that mostly happens in autoimmune subjects. The metabolic syndrome (MetS) is combination of several metabolic disorders such as obesity, dyslipidemia, Diabetes Mellitus (DM) and conditions due to inflammation and stress oxidative. The Correlation between inflammatory markers such as High sensitivity C-Reactive Protein (hsCRP) and anti-β2GPI antibody in MetS needs to be further investigated.

METHODS: This was an observational study with cross sectional design on subject with MetS as determined by the International Diabetes Federation (IDF) 2005's criteria.

RESULTS: There was a positive and significant correlation between hsCRP and anti-β2GPI antibody in MetS group ($r = 0.406$; $p = < 0.05$) as compared to non-MetS group. We found that there was elevated level of anti-β2GPI antibody in hsCRP of 3-10 mg/L.

CONCLUSIONS: Anti-β2GPI antibody may be elevated in subjects with MetS who have low grade of inflammation as shown by hsCRP.

KEYWORDS: Metabolic syndrome, inflammation, autoantigen, atherosclerosis, obesity.

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Introduction

The prevalence of obesity has risen dramatically, leading to a marked increase of MetS which is characterized by atherosclerotic cardiovascular disease risk factors, including visceral adiposity, insulin resistance, low HDL cholesterol (HDL-C), and systemic inflammatory state (1).

Based on WHO report in 2003, vascular disease is the leading cause of death in developed countries with a death rate reaching 46%. Reports of *Survei Kesehatan Rumah Tangga Nasional* in 2001 showed that blood vessel and coronary heart diseases are the leading cause of death in Indonesia (2). In the last decade, it is found that not just traditional factors, such as hyperlipidemia, hypertension, smoking are the risk factors in atherogenesis, but there are other risk factors such as inflammation and immune mechanism that can influence the progress of atherosclerotic lesions.

The state of inflammation and oxidative stress that occurs in MetS will cause continuous activation of endothelial cells that further leads to endothelial dysfunction (3). Some researches have reported that in the state of endothelial dysfunction, the level of C-Reactive Protein (CRP) is elevated. This suggests that CRP is not only as a marker of inflammation, but also a marker of endothelial dysfunction (4,5). Presumably concentration of hsCRP is a predictor of atherosclerosis and vascular death as well as a predictor of cardiovascular events, hence it can provide a prognostic value (6).

There are a lot of immune cells in a lesion of atherosclerosis, particularly macrophages and T cells. This disorder is associated with systemic immune response and signs of inflammation. When a macrophage shows a surface complex that contain an antigenic peptide bound to neighbouring T cell, the adaptive immune reactions *(ie.* Antigen specific) are initiated. This will result in secretion of cytokine, production of antibody, activation of T-cell and other components of an immune reaction (7).

The candidate auto-antigen that is involved includes modified form of low density lipoprotein cholesterol (LDL-C), heat shock proteins (HSP), and β2GPI (8). Modified form of LDL-C or ox-LDL is the prime candidate for auto-antigen. It is incriminated in foam cell generation through uptake by the unregulated scavenger receptors on macrophages (9). Following the study of induced immune responses to ox-LDL, Palinski *et al.* reported on the effects of homologous ox-LDL immunization showing that ox-LDL immunized rabbit developed anti ox-LDL antibodies and experienced reduction of atherosclerosis (10).

Another auto-antigen β2GPI is a normal glycoprotein synthetized by the liver that behaves as anticoagulant and anti-atherogenic agent. Binding of β2GPI to ox-LDL reduces uptake of ox-LDL by scavenger receptors on macrophages (9). The titers of antibody to β2GPI are correlated with atherosclerosis, and *in vitro* studies showed they enhance ox-LDL uptake by macrophages. Immunization with auto-antigen β2GPI elicits an immune response to influence lesion progression that mostly occurs in autoimmune subjects (13).

Methods

STUDY DESIGN & SUBJECT RECRUITMENT

This was an observational study with a cross-sectional design conducted on 60 obese subjects. Our study protocol was approved by the Health Research Ethics Committee, Faculty of Medicine, Hasanuddin University (no.UH08120125). All necessary written informed consents were obtained. All subjects were recruited from Prodia Clinical laboratory, Pekanbaru, Indonesia. For gathering the baseline data, each subject completed a self questionnaire covering medical history, exercise, medicine intake for any inflammatory history, and smoking habit. Apparently healthy subjects, aged 30-55 years, with central obesity (Waist circumference > 90 cm), were screened based on IDF 2005's criteria. Subjects with fever and acute inflammation were excluded.

All subjects were examined after an overnight fasting for 10-12 hours. Measurement of anthropometric parameter (waist circumference and blood pressure) and biochemical variables (fasting blood glucose, HDL-C, Triglyceride, hsCRP, ox-LDL, Apolipoprotein B (Apo B), Anti-β2GPI antibody) were done on all subjects. Fasting serum samples were obtained and kept at -20ºC.

ASSAY OF BIOCHEMICAL MARKERS

Hexokinase method was used to measure fasting blood glucose, while Glycerol-3-Phosphate Oxidase-Phenol Amino Phenazone (GPO-PAP) method was used to determine triglyceride level (both methods used Dialine-manufactured reagents). Homogenous method was used to measure HDL-C (reagents manufactured by Daichi), concentration of CRP was determined using a high sensitivity chemiluminescence assay (reagents manufactured by Siemens). ox-LDL was measured using an enzyme-linked immunosorbent assay (ELISA) kit from Mercodia. ApoB was determined by Immunoturbudimetry method (reagents manufactured by Daichi). Anti-β2GPI antibody was determined by ELISA kit from Euroimun. All assays were performed according to each manufacturer's instruction at Prodia Clinical laboratory located in Pekanbaru and Jakarta, Indonesia. The controls were included for each run of the assays, which showed that all results were within acceptable ranges.

STATISTICAL ANALYSIS

Statistical analysis was performed with the SPSS version of 13,0 software for windows. Normal distribution of variables was assessed using the Kolmogorov-Smirnov. Associations between variables were analyzed using Pearson and Spearman's correlation analysis, and differences were considered significant at $p < 0.05$.

Results

Sixty central-obesity subjects in this study were subdivided into 30 MetS (16 males & 14 females) and 30 non-MetS (8 males & 22 females). General description of the subjects' characteristics are shown in Table 1. Table 2 shows significant positive correlation between hsCRP with Anti-β2GPI antibody (r = 0.406; P = 0.026) in MetS subject than in non MetS subject.

Figure 1 shows the concentration of anti-β2GPI antibody in three categories of hsCRP, 1 = low risk factor to athereosclerosis category; 2 = mild risk factor to atherosclerosis category; 3 = high risk factor to atherosclerosis category. It shows that there is correlation between the hsCRP in high risk factor to atherosclerosis and concentration of anti-β2GPI antibody.

Table 1. Characteristics of the study subjects

Variables	Non Mets Mean ± SD	Mets Mean ± SD
Age (years)	39.1 ± 6.8	42.8 ± 6.3
Clinical Variables		
Waist circumference (cm)	93.6 ± 7.9	97.8 ± 8.7
Biochemical Variables		
Fasting glucose (mg/dL)	88.1 ± 7.7	121 ± 58.6
HDL-C (mg/dL)	48.2 ± 10.1	46 ± 23.5
Triglyceride (mg/dL)	110.7 ± 50.9	166.7 ± 82.9
hsCRP (mg/L)	2.3 ± 1.7	3.4 ± 0.3
Ox LDL (mg/dL)	176.5 ± 23.3	183.8 ± 30.8
Apo B (mg/dL)	84.3 ± 20.8	99.1 ± 24.2
Anti ß2GP1 (RU/mL)	1.77 ± 1.1	1.35 ± 0.57

HDL= High Density Lipoprotein; hsCRP = High Sensitivity C- Reactive Protein; Apo B = Apolipoprotein B; ox-LDL = oxidized Low Density Lipoprotein; Anti β2GPI = Anti β2-Glycoprotein I.

Table 2. Correlation between hsCRP and each variable

Variables	Mets		Non Mets	
	r	p	r	p
WC (cm)	0.248	0.186	-0.018	0.924
Fasting Glucose (mg/dL)	0.235	0.210	0.124	0.514
HDL-C (mg/dL)	-0.084	0.657	0.121	0.524
Anti ß2GP1 (RU/mL)	0.406*	0.026	-0.248	0.187

WC = Waist Circumference; HDL-C= High Density Lipoprotein Cholesterol; Anti-β2GPI = Anti-β2-Glycoprotein I; RU = R Unit.
* = < 0.05.

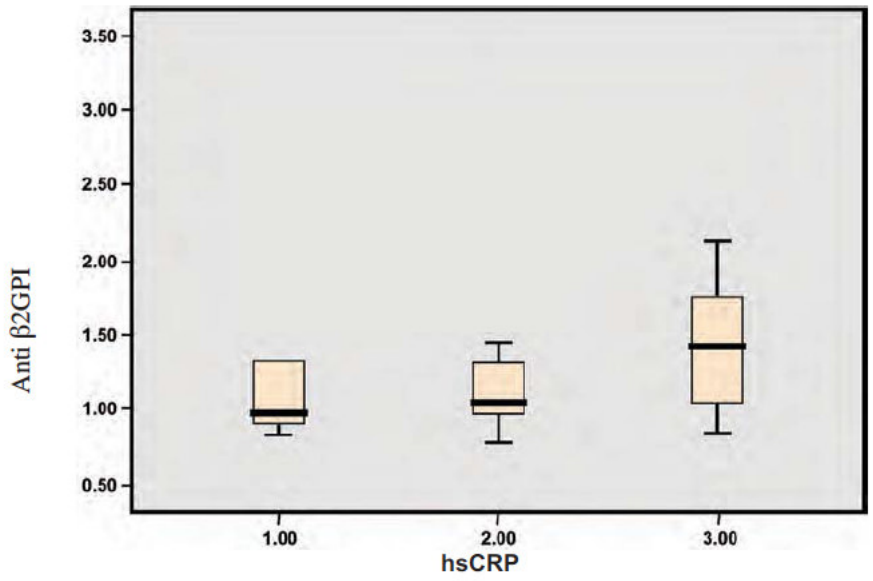

Figure 1. Differences of Anti-β2GPI antibody based on hsCRP category.

Discussion

Recent data indicate that autoimmunity and inflammation in autoimmune subjects can influence the progression of atherosclerosis. Our present study was conducted to see whether there was relationship between inflammation and autoimmune factors in MetS. This study was done to compare the group of subjects with MetS with that of non-MetS subjects, from which no significant difference was found in the values of variables (Table 1). We assume this finding was due to the fact that the subjects in this study were screened among obese individuals according to IDF 2005's criteria with waist circumference ≥ 80 cm for female and ≥ 90 cm for male.

In the group of MetS we found a significant positive correlation ($p < 0.05$; $r = 0.406$) between hsCRP and anti-β2GPI antibody. This suggests that inflammation in MetS subjects could initiate atherosclerosis, not only by causing injured vascular endothelium or by increasing atherosclerosis lesions including mobilization and formation of foam cells, but also by activating other cell presenting antigen that could activate T and B lymphocytes. The above process would produce antibodies such as anti-β2GPI antibody that could enhance ox-LDL uptake by macrophages.

As we know MetS has an association with low grade inflammation as shown by hsCRP. There are three categories in hsCRP levels, each having different risk factors for the incidence of athreosclerosis; the first category with level < 1 mg/L has low risk, the second category with level between 1-3 mg/L has mild risk, and the third category with level between 3-10 mg/L has high risk factors on incidence of atherosclerosis (16,17). This study showed that the levels of anti-β2GPI antibodies were elevated in hsCRP with the third category, and this suggests that antiβ2GPI antibody may have an important role as risk factors for incidence of atherosclerosis in MetS.

CRP is one of the early diagnostic markers for inflammation, it is also a prognostic marker of atherosclerosis, and plays a role in pathogenesis. CRP directly injures vascular endothelium, increasing the lesions of atherosclerosis including mobilization and formation of foam cell (14).

MetS is associated with obesity, insulin resistance, atherosclerosis and type-2 diabetes as well as with low grade inflammation. A report has shown that individuals who have a risk of atherosclerosis also has more inflammatory markers such as Interleukin 6 (IL-6), Tumor Necrosis Factor-α (TNF-α) and CRP (4, 15).

Inflammatory process that initiate the occurence of atherosclerosis starts from activation of macrophages and other cells presenting antigen will activate T and B lymphocytes, and this process produces antibodies and T cells of auto-antigen such as β2GPI (9).

Conclusions

This study showed that concentration of anti-β2GPI antibody were elevated in hsCRP with high risk factor for the incidence of atherosclerosis and there was significant positive correlation between hsCRP and anti-β2GPI antibody in subject with MetS.

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