RESEARCH ARTICLE

Correlation between Circulating Levels of Pro-Inflammatory Cytokines TNF-α and Vascular Calcification Inhibitor Matrix Gla Protein in Obese Men

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Abstract

BACKGROUND: Adult obesity is rapidly increasing in the world including Indonesia. Tumor necrosis factor α (TNF-α) was chronically elevated in obese adipose tissue. TNF-α, a pleiotropic cytokine and also a regulator of bone formation, may might represent an important link between obesity and vascular calcification. Elegant genetic studies in mice and human have highlighted the important roles for Matrix Gla Protein (MGP) as an inhibitor of vascular calcification. The aim of this study was to examine the correlation between circulating levels of pro-inflammatory cytokines TNF-α and vascular calcification inhibitor MGP in obese men.

METHODS: This was an observational cross sectional study including 40 central obese men (waist circumference ≥ 90 cm) aged 31-60 years old. Serum MGP and serum TNF-α concentrations were quantified by ELISA principle. Fasting plasma glucose was assessed using hexokinase methods, triglyceride by GPO-PAP methods, and creatinine by Jaffe methods. All assays were performed according to the manufacture instruction.

Statistical analysis was performed with SPSS for windows ver 16. Univariate analysis were performed to analyze mean, maximum, minimum value and SD. Pearson correlation statistic were performed to determine the correlation between variables. Significance value were define as alpha level = 0.05 based on two-tailed tests.

RESULTS: The cross-sectional study (n = 40) showed that the advancing age was correlated with plasma TNF-α concentration (r = 0.348; p = 0.028). The mean concentration of TNF-α and MGP were 8.323 and 8.368, respectively. We found a significant negative correlation between TNF-α with MGP (r = -0.425; p = 0.006) and a significant correlation between TNF-α and triglyceride (r = 0.375; p = 0.017).

CONCLUSIONS: Circulating level of TNF-α was inversely correlated with MGP concentration in obese men. This finding suggested that high level TNF-α leads to low MGP concentration obese men, hence, limits inhibitory capacity in vascular calcification.

KEYWORDS: hypertension, obesity, vascular calcification, MGP, TNF-α.

Introduction

Obesity is a major contributor to the global burden of chronic disease and disability. In developing countries like Indonesia, data from national basic health research 2007 showed that over nutrition was found among all age groups, on a double digit scale, with similar magnitude in urban and rural areas.(1)

Obesity is a major risk factor for cardiovascular disease, and adipocyte-derived factors might contribute

to or ameliorate obesity-associated pathologies such as vascular dysfunction and a chronic inflammatory state.(2)

Increased plasma levels of the primary inflammatory cytokine TNF-α, have been demonstrated in patients with obesity. There is evidence to support the synthesis of TNF-α in adipose tissue. This may contribute to both the maintenance of a chronic low-grade inflammatory state in obese patients. Thus inflammation may be involved in the triggering of vascular calcification. TNF-α has a particular interest in vascular calcification because it has been found to promote osteogenic differentiation and calcification of vascular cells. It may potentially lead to a positive feed-back loop, further stimulating macrophage activation and calcification.(3). Although, TNF-α has been detected in both human and mouse atherosclerotic lesions, its contribution to vascular calcification has not been assessed.

Vascular calcification was once considered only a passive process of dead and dying cells, worked from laboratories worldwide has now highlighted that arterial biomineralization is an actively regulated form of calcified tissue metabolism.(4) Genetic and biochemical studies have established MGP as the first protein known to act as a calcification inhibitor *in vivo*. The role of MGP in calcification is complex. MGP functions as a noggin-like protein by inhibiting BMP/BMPR2 interactions as well as by binding BMP2 directly. Once the BMPR2 and BMPR1 receptors interact through ligand binding its downstream effects are achieved through the upregulation of key osteogenic transcription factors including Msx2, Cbfa1, and osterix. Expression of transcription factors in VSMCs serves as an early initial step in vascular calcification. (5) Association between TNF-α and MGP is unknown. Present study aim to examine correlation between TNF-α and MGP.

Methods

This study protocol has been approved by the institutional review boards: the ethical clearance from the Health Research Ethics Committee Faculty of Medicine Padjajaran University Dr Hasan Sadikin General Hospital Bandung, (No: 25/FKUP-RSHS/KEPK/Kep/EC/2008). All participants provided written informed consent.

This study included 40 Indonesian obese men (waist circumferences ≥ 90 cm) aged 31-60 years. Subject with symptoms of infection, or any underlying acute inflammation were excluded. Heights, body weights, blood pressure and waist circumferences were measured by standard methods and the body mass index (BMI) was calculated according to the standard formula.

ASSAY OF BIOCHEMICAL MARKERS

Serum handling

Blood was collected by venipuncture in serum tubes (10 ml; BD Vacutainer Systems) and in sodium citrate (10 ml; BD Vacutainer Systems) and stored for 20 min at room temperature before centrifugation. Serum and plasma were sub sampled in aliquots and frozen at -70°C until test were performed.

Serum MGP concentrations were quantified with Human MGP Matrix GLA Protein kit (Biomedica Vienna, Austria), based on the competitive ELISA principle, where antibodies against non-phosphorylated MGP coated on the microtiter plate as has been described before. Serum TNF-α concentrations were quantified with the kit from IMMULITE 1000 TNF-α based on ELISA principle.

Measurements of fasting plasma glucose was carried out by hexokinase methods (Roche diagnostics), triglyceride with GPO-PAP methods (Roche diagnostics), creatinine by Jaffe methods. All assays were performed according to manufacturers instruction.

STATISTICAL ANALYSIS

Statistical analysis was performed using SPSS for windows ver 16. Univariate analysis were performed to analyze mean, maximum and minimum value and SD. Pearson correlation statistic were performed to determined the correlation between variables. Significance value were define as alpha level 0.05 based on two-tailed tests.

Results

Subjects characteristics including age, body mass index, waist circumference, blood pressure, creatinine serum, cholesterol HDL, Triglyceride, MGP and TNFα were shown in Table 1. According to the data shown in Table 1, there were no outliers data, showed from the standard deviation values which were lower than the mean values.

We found a positive correlation in this study between age and TNF-α ($r = 0.348$; $p = 0.028$), also a significant negative correlation between TNF-α and MGP ($r = -0.425$; $p = 0.006$). This study also showed a significant correlation between TNF-α and triglyceride ($r = 0.375$; $p = 0.017$) (Table 2). We found no significant correlation between MGP and all variables except TNF-α.

Table 1. Characteristics of 40 patients with obese investigated in the present study.

Variable	Mean	±	SD
Age (years)	46.325	±	7.166
Body Mass Index	27.989	±	3.403
Waist Circumference (cm)	99.638	±	6.129
Systolic Blood Pressure (mmHg)	133.750	±	15.922
Diastolic Blood Pressure (mmHg)	95.500	±	11.972
Creatinine (mg/dL)	0.898	±	0.142
Glomerular Filtration Rate (72 ml/min/1.72m^2)	115.023	±	21.065
Fasting Plasma Glucose (mg/dL)	101.200	±	43.787
Cholesterol-HDL (mg/dL)	41.375	±	8.356
Triglyceride (mg/dL)	199.025	±	145.345
MGP (nmol/L)	8.368	±	1.005
TNF-α (pg/mL)	8.323	±	4.351

Table 2. Correlation between MGP, TNF-α and other parameters in present study

Variable	MGP (nmol/L) r	TNF-α (pg/mL) r
Age (years)	-0.220	0.348*
Body Mass Index	0.171	-0.249
Waist Circumference (cm)	0.117	-0.170
Systolic Blood Pressure (mmHg)	0.278	0.028
Diastolic Blood Pressure (mmHg)	0.087	0.055
Creatinine (mg/dL)	0.010	0.089
Glomerular Filtration Rate (72 ml/min/1.72m^2)	0.118	-0.347
Fasting Plasma Glucose (mg/dL)	-0.082	0.134
Cholesterol-HDL (mg/dL)	0.087	-0.296
Triglyceride (mg/dL)	-0.187	0.375*
MGP (nmol/L)		-0.425**

*p-value < 0.05
**p-value < 0.01

Discussions

Correlation between Age and TNF-α

Our study demonstrated that plasma TNF-α concentration was positively correlated with advancing age (r = 0.348; p = 0.028). These results were similar to studies performed by Chorinchath *et al*. (6), Han *et al*. (7), and Morin *et al*.(8) which were showed that plasma TNF-α level was increased with advancing age in mice. Cross-sectional study by Paolisso *et al*. showed that advancing age was correlated with plasma TNF-α concentration (r = 0.64, P < 0.001).(9)

Why plasma TNF-α concentration increases with advancing age remains to be determined. Our volunteers were obese men subjects. Most likely, plasma TNF-α concentration parallels the age-related increasing in body fatness. In our study, the relationship between plasma TNF-α and age was assessed only by a cross-sectional design, so a cause-effect relationship cannot be drawn.

Correlation between TNF-α and serum triglyceride

In our study found the significant positive correlation between TNF-α and triglyceride (r = 0.375; p = 0.017) (Table 2). Other study in rats by Feingold *et al*. showed that TNF-α administration can promote serum triglycerides level. TNF-α induced hyperlipidemia was suggested to be the result of increasing hepatic lipogenesis and lipolysis rather than decreased peripheral clearance (10). In our study, a significant positive correlation between TNF-α and triglyceride (r = 0.375; p = 0.017) strengthen the role of cytokines in inducing hyperlipidemia.

Correlation between TNF-α and MGP

TNF-α a pleiotropic cytokine, has been shown to play a role in both vascular and bone pathophysiology. TNF-α is mainly secreted by macrophages in response to factors such as oxidized LDL (11), acetylated LDL (12), physically damaged extracellular matrix (13), or bacterial infection (14). *In vivo*, TNF-α induces arteriosclerosis-like lesions in coronary arteries (15). TNF-α also regulates bone turnover, inhibiting osteoblastic function (16,17,18) and stimulating bone resorption (19).

The previous study by Tintut *et al*. showed that TNF-α enhanced *in vitro* calcification of vascular cells, providing further evidence that vascular and bone calcification shares regulatory factors. (20) Other study by Jono *et al*. reported an association between serum MGP levels and coronary artery calcification, detected by EBCT, in 115 subjects with suspected coronary artery disease and normal renal function. Patients with coronary artery calcification had lower serum MGP levels compared to those with no calcium in the coronary tree, suggesting the potential role of MGP on prevention of vascular calcification.(21)

Our data demonstrated a significant negative correlation between MGP and TNFα (r = -0.425; p = 0.006). This result suggested that elevated plasma TNF-α and decreased MGP might provide a further contribution to the calcification of vascular in obese male. However, the relationship between inflammation and MGP insufficiency was less clear. In a series studies, Yao *et al*. identified that MGP was a Gla-dependent inhibitor of BMP2 and BMP4, an osteogenic morphogens that up-regulate AKP2 expression.(22,23,24) Yao *et al*. went on to show that interleukin (IL) 6, an inflammatory cytokine that was important in diabetic vascular diseases, increased the expression and secretion of heat shock protein (HSP) 70, an endogenous MGP binding protein and antagonist of MGP function that was highly expressed in calcifying atherosclerotic plaques. Thus, by inducing HSP70, inflammatory signals could potentiate vascular BMP2/4 actions by nullifying MGP.(24)

Conclusions

Circulating level of TNF-α was increase and MGP concentration was decrease in obese men. This finding suggested that high level of TNF-α leads to low MGP concentration in obese men, hence, limiting the inhibitory capacity in vascular calcification.

Further studies were needed to elucidate the mechanisms of TNF-α in suppressing MGP as a novel link in vascular calcification.

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