RESEARCH ARTICLE

Correlation of Neopterin and TNF-α with Asymmetric Dimethylarginine in Metabolic Syndrome

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Abstract

BACKGROUND: A large number of obesity in the community increases the incidence of Metabolic Syndrome (MetS) that can increase the risks of heart disease, diabetes, and stroke. One of the possible causes of stroke is atherosclerosis. Atherosclerosis is initiated by the incidence of inflammation and endothelial dysfunction. Atherosclerosis is involved in an ongoing inflammatory response. At the beginning of atherosclerosis, when the endothel become inflamed, it expresses adhesion molecules that attract monocytes. The monocytes then migrate into the intima due to endothelial dysfunction. Activation of macrophage occurs in the process of inflammation as the earliest type of lesion of atherosclerosis. In this study, monocyte/macrophage activation is marked by Neopterin. In other process of atherosclerosis, vascular nitric oxide (NO) activity has a role as a potent endogenous vasodilator. In regulating the vascular tone, NO has a role to suppress vascular smooth muscle proliferation, inhibit platelet adhesion and aggregation, and interferes with the leukocyte-endothelial cell interaction (1). In MetS, hypercholesterolemia decreases NO activity. Asymmetric Dimethylarginine (ADMA) has been characterized as an endogenous, competitive inhibitor of NO synthase. In this study, the incidence of endothelial dysfunction is marked by ADMA. The aim of this study was to discover the role of Neopterin in MetS patients by evaluating the correlation between Neopterin and ADMA in MetS through tumor necrosis factor (TNF)-α or direct line.

METHODS: The study was cross sectional on 64 males with MetS aged 30-65 years. The measurements of Neopterin, ADMA, and TNF-α concentrations was done, respectively.

RESULTS: Neopterin concentration correlated with Log TNF-α concentration ($r = 0.311$, $p = 0.012$). There is no significant correlation between Neopterin and ADMA ($r = 0.012$, $p = 0.930$; ADMA and Log TNF-α ($r = 0.029$, $p = 0.821$).

CONCLUSIONS: There is no significant correlation between Neopterin and ADMA through TNF-α or direct line.

KEYWORDS: MetS, Neopterin, ADMA, NO, TNF-α

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Introduction

Increasing prevalence of obesity and insulin resistance due to sedentary lifestyle has increased the incidence of cardiovascular disease both in developed and developing countries. Abdominal obesity, insulin resistance and other conditions such as lack of activity, old age, and hormonal instability are some factors that contribute to the incidence of Metabolic Syndrome (MetS), which increased the risk of cardiovascular disease, stroke, and diabetes.

Atherosclerosis is an inflammatory disease. High plasma concentration of low-density lipoprotein (LDL) cholesterol is one of the principal risk factors for atherosclerosis (6). The pathogenesis of atherosclerosis consists of mechanism of endothelial dysfunction from the early beginning of lesion formation to the late complicated stages of unstable plaque. One biomarker of endothelial dysfunction is ADMA, an endogenous inhibitor of NO synthesis, has been implicated in the impairment of NO generation in a variety of cardiovascular diseases. NO activity has a role to regulate the vascular tone and suppress the vascular smooth muscle proliferation. So NO has a role to maintain vascular endothelium.

Inflammatory cytokine such as TNF-α is produced by the macrophage as an innate response to inflammation. Low production of TNF-α ($< 10^{-9}$ M) can cause endothelial cell to express cellular adhesion molecules, which is essential in early atherogenesis and function as binding sites for monocytes/macrophages. Once activated, the molecules adhere to the endothelial cells and start producing a number of pro-inflammatory substances such as cytokines and chemokines. The cytokines will further enhance attraction of other leukocytes to the endothelium, thus progressing lesion formation (7).

Several studies have stated that Neopterin as innate immune response in many diseases such as diabetes mellitus, foot ulcers (3), and ulcerative colitis (4). Neopterin is the exclusive product of monocytes/macrophages that has been stimulated by interferon-γ, a cytokine produced by activated T-lymphocytes and natural killer-cells. Therefore, neopterin provides an ideal diagnostic tool for monitoring the cell-mediated innate immune response in clinical diseases (7). In this study, we tried to confirm that Neopterin was produced by activated macrophages due to inflammatory process in atherosclerosis.

In this study, we only recruited MetS because we assumed that the initial process of atherosclerosis had already happened at the time of the study. We determined the concentrations of ADMA and Neopterin in order to evaluate the correlation between these biomarkers in MetS. We also determined TNF-α concentrations to assess the correlation between ADMA - TNF-α and Neopterin - TNF-α.

Methods

Subject Recruitment & Screening

This study used cross sectional method on a population of MetS, of which we calculated the minimal number of subjects of study was 62. Sixty four men with MetS (categories of the International Diabetes Federation (IDF), 2005) (12), aged 30–65, were recruited. Subjects with acute inflammation (hsCRP >10 mg/L), autoimmune disease, rheumatoid arthritis, multiple sclerosis, coeliasis disease, active pulmonary tuberculosis, chronic Hepatitis B, rheumatic fever, cancer and those taking glucocorticoid and immunomodulating cytokines drugs, were excluded.

All subjects agreed to sign informed consent prior to the study. The clinical protocols of this study were approved by the Health Research Ethics Committee of the Faculty of Medicine, Hasanuddin University.

Health Examination & Sample Collection

The anthropometric indicators were measured twice, the mean values of which were used in the analysis. Body weight (BW), height and waist circumferences (WC) were measured using a standardized method. Fasting blood specimens were collected in the morning between 07:00–10:00 am.

Sample Processing & Analysis

Sera were separated immediately by centrifugation and aliquots were frozen at < -20° C for batched analysis of fasting blood glucose, High Density Lipoprotein (HDL)-Cholesterol, Triglyceride, Erythrocyte Sediment Rate (ESR), leukocyte count, hsCRP, TNF-α, ADMA, and Neopterin. All biochemical analyses were performed at Prodia Clinical Laboratory. Serum and plasma for fasting blood glucose, HDL-cholesterol, triglyceride, ESR, leukocyte count, hsCRP concentration were measured using the Prodia Clinical Laboratory's routine chemistry procedures. Serum glucose was determined by hexokinase method, triglyceride was determined by GPO PAP (glycerol-3-phosphate oxidase-phenol aminophenazone) method, while HDL-cholesterol was assessed by homogenous method. Commercial kits and reagents for glucose and triglyceride measurements are from Roche and reagents of HDL-Cholesterol are from Daichi. Glucose, triglyceride, and HDL-cholesterol were measured in Modular P-800 system produced by Roche. Serum hsCRP was determined using sensitive immunoassay method. Commercially available kits and reagents were used on Immullite 2000 system produced by Siemens. Leukocyte count was measured using Sysmex XT 2000i system. Commercially available kits (Human TNF-α/TNFSF1A Immunoassay, R&D Systems, Inc., MN, USA) were used to measure TNF-α with quantitative sandwich enzyme immunoassay technique. To measure ADMA with ELISA reader, commercially available kit (DLD reagent Diagnostika GmbH, Hamburg, German)

was used. While for measuring Neopterin with ELISA reader, commercially available kit (Immuno-Biological Laboratories, USA) was used.

Data & Statistic Analysis

Data analysis was done using SPSS 11.0 statistical analysis software for Windows (SPSS Inc., Chicago, IL, USA). Univariate analysis was carried out to calculate mean, maximum, and minimum values and standard deviation. We used non parametric analysis of Kolmogorof-Smirnov to assess whether each variable was normally distributed or not. The associations among various measurements were analyzed with Pearson correlation test when the data were normally distributed and with Spearman's rho correlation when the data were not normally distributed. We considered $p < 0.05$ as significant correlation. Compared means were analyzed by one way Anova.

Results

Table 1. Baseline Characteristics of The Study Subjects

	Mean (±SD)	Min	Max	Sig. (2-tailed Kolm-Smir)
Systolic BP (mmHg)	126.25 ± 11.40	100.00	160.00	0.018
Diastolic BP (mmHg)	87.03 ± 8.80	70.00	110.00	0.003*
Glucose (mg/dL)	98.22 ± 22.95	48.00	211.00	0.081
HDL-Cholesterol (mg/dL)	37.89 ± 5.10	29.00	55.00	0.123
Triglyceride (mg/dL)	218.80 ± 120.30	74.00	864.00	0.094
ESR	9.34 ± 6.35	3.00	37.00	0.070
Leukocyte count	7.69 ± 1.65	5.20	12.40	0.034*
hsCRP (mg/L)	2.22 ± 2.14	0.20	9.67	0.002*
ADMA (mg/L)	0.721 ± 0.097	0.15	0.97	0.520
Neopterin (nmol/L)	8.90 ± 2.99	4.85	22.41	0.176
TNF-α (pg/mL)	4.49 ± 6.51#	1.41	47.67	0.000*

* Abnormal Distribution
# TNF-α concentrations between -2.02 – 11 pg/mL

Table 2. Correlation Among Variables

Variable	Neopterin r	ADMA r	Log TNF-α r
Systolic BP (mmHg) #	0.263*	0.11	0.05
Diastolic BP (mmHg) #	0.184	0.005	0.075
Fasting Glucose (mg/dL) ##	-0.072	0.207	-0.133
HDL-Cholesterol (mg/dL) ##	-0.042	0.025	-0.054
Triglyceride (mg/dL) ##	0.028	-0.097	0.423**
ESR ##	0.093	-0.215	0.051
Log Leukocyte count ##	-0.111	0.097	-0.037
Log hsCRP (mg/L) ##	0.108	-0.106	-0.054
Neopterin ##	1.000	0.012	0.311*
ADMA ##	0.012	1.000	0.029
Log TNF-α ##	0.311*	0.029	1.000

* $p < 0.005$, ** $p < 0.01$, # Spearman's Correlation, ## Pearson's Correlation

After being collected and analyzed, we categorized the data by inclusion criteria of MetS by IDF. Sixty four samples were measured. The subjects' characteristics are shown in Table 1. There was a significant correlation between Neopterin and Log TNF-α; but there was no significant correlation between TNF-α and ADMA, and between Neopterin and ADMA, as shown in Table 2.

Discussion

In this study, no significant correlation was noted in the relationship between Neopterin and ADMA. Although ADMA has an antagonist physiologic role with Neopterin, the result suggested that the expression of both proteins might be independent from each other. Neopterin stimulates inducible nitric oxide synthase (iNOS) gene expression at the mRNA level with a subsequent increase in NO production (2) and has antioxidant roles (5), whereas ADMA has an inhibiting role in NO production.

ADMA is derived from methylated arginine by protein arginine methyltransferase I enzyme (PRMT I). Expression of PRMT in endothelial cell increases as a consequent of oxidative stress. Beside that, ADMA is metabolized by dimethylarginine dimethylaminohidrolase enzyme (DDAH) to dimethylamin and arginine, and DDAH activity can be decreased by oxidative stress, so ADMA concentration increases and thus brings about NO production. Inflammation and obesity stimulate production of TNF-α hence enhancing atherosclerosis. This study did not examine the activity of enzyme that affects production of ADMA and Neopterin, thus the other factors were unknowledgeable. Recruitment of macrophages into cells would cause pro-inflammation condition. The macrophages would produce TNF-α that caused chronic inflammation.

In this study no significant correlation was found between neopterin and ADMA directly through TNF-α but there was a significant correlation between neopterin and log TNF-α ($r = 0.311$, $p = 0.012$) in the metabolic syndrome population. This could happen because production of ADMA as a marker of endothelial dysfunction in early atherosclerosis did not bring about production of Neopterin, the two processes of which are independent. The study did not measure NO concentration that can be directly allied to ADMA.

Conclusions

Our study has shown there was no correlation between Neopterin and ADMA either through TNF-α or direct line.

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