ABSTRACT

Aim: in this study we report the relationship between fasting plasma insulin, C-reactive protein, adiponectin levels and the components of metabolic syndrome.

Methods: for the diagnosis of metabolic syndrome we used the modified NCEP ATP criteria for Asian people. Complete routine physical examination were performed to all subjects including blood pressure, waist circumference. After 12-hours fasting, blood sample was taken for fasting plasma glucose, fasting insulin, and lipid profiles. Fasting insulin was determined by RIA, adiponectin by ELISA, and hs-CRP by sensitive immunometric assay. According to NCEP ATP III, the metabolic syndrome consist of five components. Subjects fulfill the studied criteria were divided into five groups according to the component/components they had.

Results: during the study, 118 adult individuals can be covered, including 88 patients (68.8%) with metabolic syndrome. They consist of 19 subjects who had only 1 component, 21 subjects had 2 components, 31 subjects with 3 components, 34 subjects with 4 components, and 23 subjects had 5 components. All subjects were overweight/obese (BMI > 23 kg/m²), but patients with metabolic syndrome were significantly more obese compared to the non-metabolic syndrome (p < 0.05). There was no significant difference in age among all individuals. Fasting insulin, hs-CRP levels is increasing with the increasing number of components of metabolic syndrome, being higher among those with 3-5 components, while adiponectin decreasing with the number of the components. Fasting insulin 3.5+1.1uU/ml, 3.6+1.4uU/ml, 5.9+1.8uU/ml, 7.8+2.1uU/ml, 7.9+2.3uU/ml respectively, hs-CRP 2.8+1.2mg/L, 5.6+3.4mg/L, 7.4+4.4mg/L, 9.0+4.7mg/L, 9.5+3.9mg/L, and adiponectin 9.1+3.5ng/ml, 8.6+1.6ng/ml, 3.4+1.2ng/ml, 3.2+1.3ng/ml, 2.8+0.9ng/ml in 3, 4, 5 components respectively.

Conclusion: there is a relationship between the number of components of metabolic syndrome and the increasing levels of fasting insulin, and hs-CRP, and low levels of adiponectin. These may explain in part the risk of cardiovascular events among individuals with metabolic syndrome.

Key words: fasting insulin, hs-CRP, adiponectin, metabolic syndrome.

INTRODUCTION

Metabolic syndrome represents a constellation of several risk factors, lipid and nonlipid risk factors of metabolic origin. This syndrome is closely linked to insulin resistance in which the normal action of insulin is impaired. This syndrome has been identified as multidimensional risk factors for the development of cardiovascular events. There is no doubt that subjects with metabolic syndrome are obese or at least they are overweight. In obese subjects not only because they may have more traditional cardiovascular risk factors such as dyslipidaemia, dysglycemia, and hypertension, but they also have other non-traditional risk factors. Adipose tissue is no longer regarded as a fat storage deposit. Recently it has been recognized as an endocrine organs which secretes several bioactive protein known as adipocytokines including tumor necrosis factor-α (TNF-α), adiponectin, plasminogen-activator inhibitor-1 (PAI-1), that may play a direct role in the pathogenesis of vascular diseases. The high fasting insulin level/hyperinsulinemia in obese subjects has been proved to be an independent cardiovascular risk factor. For these reasons, subjects with metabolic syndrome are at increased risk to have cardiovascular morbidity and also mortality.

Low levels of adiponectin, an adipocyte-specific plasma Erotein, has been found to be a predictor for the development of type 2 diabetes and metabolic syndrome. High levels of adiponectin has been proved to have antiatherogenic effect by modulating insulin action and insulin resistance, effect on lipid metabolism
particularly increasing HDL-cholesterol, and has anti-inflammatory properties. High sensitive C-reactive protein (hsCRP) is a well established systemic marker for inflammation. Several epidemiological studies have been conducted to evaluate the relation between hsCRP and the metabolic syndrome. These studies proved that high levels of hsCRP (> 3 mg/L) correlate with the incidence of metabolic syndrome as well as the morbidity and mortality of cardiovascular disease. These two markers, low adiponectin and high levels of hsCRP, have a reciprocal effect on the pathogenesis of atherosclerosis.

The aims of this study were to evaluate the relationship of fasting plasma insulin, adiponectin, and hsCRP levels with the components of metabolic syndrome.

METHODS

East Indonesia Diabetes Epidemiology Group (EIDEG) is a group of physicians who participate for the screening of type 2 diabetes in their private offices or hospital clinics. For this study, data was collected from the EIDEG data in the period of January 2005 to June 2005. The study had been approved by The Ethics Committee of Faculty of Medicine of Hasanuddin University, and all subjects gave written informed consent. Patients with known diabetes were excluded from the screening. A total of one hundred and twenty eight subjects were enrolled in this study. Complete physical examinations were done to all subjects including measurement of waist circumference. Blood samples were taken in the morning after 12 hours fasting. For biochemical examinations, blood samples were sent to the central laboratory Prodia, while fasting insulin levels were measured in the Biomedic Laboratory using RIA method.

Fasting plasma glucose was measured by enzymatic colorimetric hexokinase method, triglycerides by enzymatic calorimetric GOP PAP method, HDL-cholesterol by enzymatic method using Cholestest N HDL (Daichii Pure Chemicals Co.Ltd). Adiponectin levels were determined by enzymatic colorimetric ELISA, and hs-CRP levels using chemiluminescent method.

In this study, NCEP A TP III 2001 criteria for metabolic syndrome was used, with the modification of waist circumference according to the WHO criteria for Asian people. This syndrome consist of abnormal waist circumference for male > 90 cm, and female > 80 cm, blood pressure > 130/ > 85 mmHg, triglyceride > 150 mg/dl, HDL in male < 40 mg/dl, female < 50 mg/dl, and fasting plasma glucose > 110 mg/dl. Metabolic syndrome was diagnosed if the subject has > 3 of the components. Subjects were divided into five groups according to the component/components they had. Group 1 those who had only one component, group 2 had two components, group 3 had three components, group 4 had four components, and group 5 those with five components.

Data are presented as means ± SD or as a proportion. To compare the distribution ofdermographic and biochemical characteristics between groups, one-way Anova (SPSS software package version 11.5) was used. To calculate the correlation between fasting insulin, hs-CRP, and adiponectin levels and the component of the metabolic syndrome, Pearson Correlation test was applied. Considered to be significant if the p value < 0.05.

RESULTS

As defined by the modified NCEP-ATP III criteria used in this study, of the 128 subjects, 19 subjects had only 1 component, 21 subjects had 2 components, 31 subjects with 3 components, 34 subjects with 4 components, and 23 subjects had 5 component. Totally there were 88 subjects (68.8%) fulfilling the criteria of metabolic syndrome. There was no significant difference in age among those five groups. All subjects were overweight/obese (BMI > 23 kg/m²), but those with metabolic syndrome were significantly more obese compared to the non-metabolic syndrome subjects (p < 0.05), being the most in the group five. The waist circumference was significantly larger in group 4 and 5 compared to the other three groups. The systolic blood pressure were significantly higher in group 3 to group 5 (those with metabolic syndrome) compared to group 2 and 1 (p < 0.05) but not in diastolic blood pressure (p > 0.05). (Table 1)

Table 2 showed there was a strong linear increase in the fasting insulin and hs-CRP levels as the number of components of metabolic syndrome increased. The mean of fasting insulin levels among those with component 1,2,3,4, and 5, were 3.16±2.53 uU/ml, 4.57±2.41 uU/ml, 7.45±3.09 uU/ml, 12.62±6.78 uU/ml, and 15.68±7.85 uU/ml respectively (p < 0.05). There was no significant difference of adiponectin levels between the group 1 and group 2 (p > 0.05), as these two groups were not those with the metabolic syndrome. Interestingly, within the groups of those with metabolic syndrome (group 3, 4, and 5) there was a significant decrease of adiponectin levels between the group 1 and group 2 (p > 0.05), as these two groups were not those with the metabolic syndrome. Comparing the groups of those without metabolic syndrome (group 1 and 2) and those with metabolic syndrome (group 3, 4, 5), the adiponectin levels in the groups of those with metabolic syndrome were significantly lower (p < 0.05).
Figure 1 displays the distribution of fasting insulin levels among 128 subjects after they were classified according to their total number of components of the metabolic syndrome. As shown, there is a strong linear increase in fasting insulin levels with an increase of the number of metabolic syndrome, although the difference is only between those with metabolic syndrome compared to those without metabolic syndrome, being the highest in group 5.

Figure 2 showed a strong linear decrease in adiponectin levels as the number of components of the metabolic syndrome increasing, this decreasing was sharply consistent among the subjects with 3 to 5 components of metabolic syndrome; median adiponectin levels in group 3, 4, and 5 components of metabolic syndrome were 11.74+6.44, 8.21+4.57, and 5.72+2.93 ng/ml.

Table 1. Demographic and Biochemical Characteristics in 128 Subjects

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1 (n=19)</th>
<th>2 (n=21)</th>
<th>3 (n=31)</th>
<th>4 (n=34)</th>
<th>5 (n=23)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>49.74±9.09 a</td>
<td>53.38±8.36 a</td>
<td>51.42±9.58 a</td>
<td>48.00±9.18 a</td>
<td>50.09±9.42 a</td>
</tr>
<tr>
<td>BMI (kg/m2)</td>
<td>23.22±2.69 a</td>
<td>24.98±3.58 a</td>
<td>27.60±2.34 ab</td>
<td>29.24±3.55 bc</td>
<td>30.10±5.30 bc</td>
</tr>
<tr>
<td>Waist circ (cm)</td>
<td>79.62±5.85 a</td>
<td>84.05±4.99 a</td>
<td>84.09±10.97 a</td>
<td>90.30±10.02 a</td>
<td>98.41±5.32 a</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>122.55±12.88 a</td>
<td>130.21±11.88 a</td>
<td>141.51±11.85 ab</td>
<td>159.41±10.82 bc</td>
<td>158.10±10.55 bc</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>77.89±8.55 a</td>
<td>84.2±12.17 a</td>
<td>83.5±11.8 a</td>
<td>84.5±10.32 a</td>
<td>84.5±10.32 a</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>92.84±11.45 a</td>
<td>94.98±15.51 a</td>
<td>136.95±45.41 a</td>
<td>204.93±50.80 b</td>
<td>219.22±62.23 b</td>
</tr>
<tr>
<td>HDL (mg/dl)</td>
<td>58.69±10.14 a</td>
<td>54.78±9.81 a</td>
<td>48.52±7.00 b</td>
<td>37.86±9.34 b</td>
<td>33.12±6.38 b</td>
</tr>
<tr>
<td>TG (mg/dl)</td>
<td>109.92±35.46 a</td>
<td>169.70±47.67 ab</td>
<td>219.94±87.86 bc</td>
<td>336.68±135.27 cd</td>
<td>311.79±86.92 d</td>
</tr>
</tbody>
</table>

Superscript with the identical unsure on the same row means no significant difference (p > 0.05) and superscript with different unsure significantly difference (p < 0.05).

Table 2. Fasting Insulin, hs-CRP and Adiponectin Levels According to Number of Components of Metabolic Syndrome

<table>
<thead>
<tr>
<th>Variables</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting insulin (uU/ml)</td>
<td>3.16±2.53 a</td>
<td>4.57±2.41 a</td>
<td>7.45±3.09 ab</td>
<td>12.62±7.78 b</td>
<td>15.68±7.85 b</td>
</tr>
<tr>
<td>Adiponectin (ng/ml)</td>
<td>17.19±5.38 a</td>
<td>17.37±6.44 ab</td>
<td>11.74±6.44 bc</td>
<td>8.21±4.57 c</td>
<td>5.72±2.93 c</td>
</tr>
<tr>
<td>Hs-CRP (mg/L)</td>
<td>1.95±1.53 a</td>
<td>2.85±1.46 ab</td>
<td>4.13±2.61 ab</td>
<td>7.59±2.85 bc</td>
<td>7.74±3.05 c</td>
</tr>
</tbody>
</table>

Superscript with the identical unsure on the same row means no significant difference (p > 0.05) and superscript with different unsure significantly difference (p < 0.05).
Figure 3 displays the distribution of hs-CRP levels among 128 subjects according to their total number of the components of metabolic syndrome they have. There was a strong linear increase in hs-CRP levels as the number of the component increased, the highest levels were in those with 4 and 5 components with median of 7.59+2.85, and 7.74+3.05 mg/L.

We also compare the percentage of high levels hs-CRP (> 3 mg/L) among subjects with and without metabolic syndrome. Among those with metabolic syndrome (group 3,4,5 as one group) the percentage of high hs-CRP was significant higher compared to the non-metabolic syndrome (group 1,2 as one group) (p < 0.5%). Among the metabolic syndrome groups (group 3, 4, 5) 88 subjects, 73 subjects have high risk levels or 83.0% of them, while in the non-metabolic syndrome group (group 1, 2) 40 subjects, only 14 subjects have high risk levels or 35.0% (Figure 5).

**DISCUSSION**

Most subjects with metabolic syndrome are obese. It has been reported that in obese individual there is a dysfunction of adipocyte, often lead to the upregulation of certain cytokines such as IL-6 and TNF-α. These adipocytokines have been shown to have the propensity to cause the disturbance of phosphorilation of insulin receptor, therefore even with the increment of insulin, it can not activate the glucose transporter. This condition is known as insulin resistance. Insulin resistance in the liver will cause the activation of gluconeogenesis which eventually will result in the increase level of fasting plasma glucose level.

In addition, it has been reported that there was a close relationship between insulin resistance and obesity with low level of HDL cholesterol and high triglyceride. 12-14 In this study, there were increment of fasting plasma level and triglyceride as the number of metabolic syndrome risk factors increase. On the contrary, inverse pattern was observed with HDL cholesterol. Insulin
resistance condition in metabolic syndrome might cause over production of insulin (hyperinsulinemia) in order to keep the plasma glucose homeostasis. We have found that as the number of metabolic syndrome component increase, there was concomitant increase of fasting insulin level, and the difference is significant (p<0.05).

Several studies have proved that low levels of serum adiponectin may predict the development of type 2 diabetes5,15 and cardiovascular disease.16 In addition, adiponectin has been shown to predict the occurrence of metabolic syndrome as reported by Choi et al6 in Korea, who conducted study in the elderly subjects.

Several studies have reported that low level of adiponectin is a predictor of the risk of type 2 diabetes mellitus as well as cardiovascular events. Engeli et al17 studied obese woman, Matsubara et al18 studied subjects with degree of body mass index, reported the same conclusion that there was a correlation between low level of adiponectin with insulin resistance. In this study, we found the similar result, where most of the metabolic syndrome subjects participated in the study were obese and showed relatively lower level of adiponectin as compared to the non metabolic syndrome subjects. As the number of metabolic syndrome component increase, the level of adiponectin is decreasing.

One of the important markers of prolonged vascular inflammation is the measurement of hs-CRP. Based on the American Heart Association, the measurement of hs-CRP can be divided to < 1 mg/dl indicating lower risk, 1-3 mg/dl moderate risk, and > 3 mg/dl high risk of getting cardiovascular events.18 Freeman et al19 have reported that hs-CRP can also be used to predict the occurrence of type-2 diabetes mellitus. Pradhan et al20 reported that the increase of hs-CRP level is a marker of developed newly diagnosed diabetes mellitus patients. The study by Sattar et al21 and also Laaksonen et al all have showed that hs-CRP also predict the occurrence of metabolic syndrome. The relationship between diabetes mellitus and metabolic syndrome are closely related to the cardiovascular events.

Ridker et al22 in a follow-up studied of 14,719 healthy women found that there is a strong linear increase in hs-CRP levels as the number of the total components of the metabolic syndrome increased. Their final conclusions was, besides the total number of components of metabolic syndrome, baseline hs-CRP levels add clinically relevant prognostic information concerning future development of cardiovascular disease. The same results was reported by Sattar et al22 from The West of Scotland Coronary Prevention Study. They reanalyzed subjects with metabolic syndrome and the levels of hs-CRP .Coronary heart disease event and also newly diabetes mellitus was higher among metabolic syndrome subjects with hs-CRP > 3 mg/L. Similar to the result reported by Ridker et al22, the result of the current study showed that level of hs-CRP increase as the number of risk factors increased. This study also showed that those with metabolic syndrome have higher percentage of high hs-CRP levels compared to those without metabolic syndrome.

Ouichi et al23 proved that there is a reciprocal association between of hs-CRP as an inflamamtory marker and adiponectin as an anti-atherogenic substance. In this study this reciprocal association can also be seen in those with metabolic syndrome (group 3,4,5). This support the function of adipocyte in obese subjects secrete substances which my play important role in the pathogenesis of atherosclerosis. This increased release of IL-6 in obese subjects will stimulate the production of hs-CRP in the liver.

In the present study, there were only 128 patients involved from our EIDEG study, yet at least it has brought about the overall picture of relationship between fasting insulin level, adiponectin and hsCRP with the total number of components of metabolic syndrome.

CONCLUSION
In conclusion, from this study we demonstrate that the number of metabolic syndrome components determine the level of fasting insulin, adiponectin and hs-CRP levels. These data also provide facts that in subjects with metabolic syndrome (3 to 5 components), low levels ofadiponectin as well as high levels of high risk hs-CRP are always present. Further large study is needed to elucidate this reciprocal changes between adiponectin and high risk hs-CRP in subjects with metabolic syndrome.

ACKNOWLEDGEMENT
The authors would like to thanks Clinical Laboratory Prodia Jakarta for the support of adiponectin examination, and to Dr. Ilhamdjaja Pattelongi PhD and Dr. Gatot Lawrence PhD for their assistance in statistical analysis.

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