Level of adipokines and insulin resistance in obese Javanese population

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ABSTRACT
Obesity is a condition involving low-level chronic inflammation as indicated by increased levels of C-reactive protein (CRP), tumor necrosis factor alpha (TNF-α), interleukin (IL) and other inflammatory markers in the blood. Some endocrine mediators, such as paracrine and autocrine play an important role in regulating the function of adipocytes, especially related to insulin sensitivity. The purpose of this study is to determine the level CRP, TNF-α, IL-6, resistin and insulin resistance in the obese Javanese population. This was a preliminary study involving 120 people, consisting of 60 obese subjects and 60 non obese subjects as controls. Lipid profiles, CRP, TNF-α, IL-6, resistin levels were determined with ELISA methods, whereas insulin resistance was calculated by HOMA IR index. The study found that the glucose, insulin, CRP levels and HOMA-IR of obese subjects were significantly higher than those non obese subjects (p<0.05). However, the TNF-α, IL-6 and resistin levels were not significantly different between obese and non obese subjects (p>0.05).

In conclusion, the CRP levels ad insulin resistance in obese Javanese population were higher compared with those non obese.

Keywords: obese - insulin resistance – CRP - TNF-α, IL-6 - resistin
INTRODUCTION

In 2013, Ministry of Health, Republic of Indonesia reported 18.8% of population in 12 major cities in Indonesia were overweight and 3.7% of them were obese. Obesity involves a state of low-level chronic inflammation as indicated by increased levels of C-reactive protein (CRP), tumor necrosis factor-alpha (TNF-α), interleukin, resistin and other inflammatory markers in the blood. Some endocrine mediators such as, paracrine and autocrine derived from adipose tissue play an important role in regulating the function of adipocytes, especially those related to insulin. Significant complications of obesity, especially insulin resistance include many of the risk factors of type 2 diabetes mellitus (type 2 DM).2,3

Adipokines (adipocitokines) include TNF-α, IL-6, resistin and CRP are secreted primarily in adipose cells in mice and mononuclear cells in humans as a result of inflammation process. Adipokines play an important role in regulating energy, glucose and fat homeostasis and maintain fasting blood glucose levels by modulating hepatic insulin.4 Low levels of adipokines are already in circulation, but there are increased levels in people with insulin resistance, type 2 DM and cardiovascular disease. Hypoadiponectinemia increases the risk of coronary artery disease, and shows that adipokine plays a role in metabolic syndromes.5 It was reported that the increase of resistin levels not only stimulates the expression but also degrades of low density lipoprotein-cholesterol (LDL-cholesterol) receptors in liver cells.6

Resistin is an antagonist of insulin and mediates insulin-signaling pathways playing a role in the pathogenesis of insulin resistance. Excessive resistin expression in adipose tissues of obese people might act to mediate the modulation of insulin sensitivity.7 Experiments with exogenous administration of TNF-α in animals induced insulin resistance while decreasing TNF-α improve insulin sensitivity. There is increasing levels of TNF-α in patients with diabetes correlated with Body Mass Index (BMI) and Homeostatic Model Assessment of Insulin Resistance (HOMA-IR). This relationship was stronger in women than men.8 Over production of cytokine is correlated with the initial onset of inflammation and is a prognostic indicator of inflammation due to obesity and metabolic syndromes. IL-6 as a mediator of inflammatory, acting as an autocrine regulator of adipocytes may cause the negative effects on metabolic processes.9 This was a preliminary study in order to evaluate the levels of adipokines and insulin resistance in obese Javanese people in Yogyakarta Special Region, Indonesia.

MATERIALS AND METHODS

Subjects

This was an observational study with a cross sectional design in order to compare the adipokines levels and insulin resistance in obese with non obese Javanese people in Indonesia. One hundred and twenty subjects consisting 60 obese subjects and 60 non obese subjects were involved in this study. Subjects with body mass index (BMI) > 25 for obese and 18.5 to 23 for non obese with the ages from 18 to years were included in this study. Subjects who taking corticosteroid and antibiotics were excluded from this study. The protocol of this study has been approved by the Medical Health Research Ethics Committee, Faculty of Medicine, Universitas Gadjah Mada, Yogyakarta.

Protocol

Each subject received written and verbal explanations concerning the background, objectives and benefit of the study before
signing an informed consent form. After obtaining written informed consent, all subjects were screened through a detailed questionnaires, medical history and physical examination. Age, sex, height, weight, BMI, waist to hip ratio, blood pressure, family history and present medications were recorded for each subject.

Each subject who fulfilled the inclusion and exclusion criteria, blood sample was collected for analysis. Blood glucose level was estimated by glucose oxidase-peroxidase method, cholesterol was estimated by cholesterol oxidase-peroxidase method, high density lipoprotein-cholesterol (HDL-cholesterol) was estimated by the glycerol-3-phosphate oxidase-peroxidase-N-methylanilin propan-sulphonate sodic method and triglyceride was estimated by GPO-POD-ESPT method using auto analyzer. Serum CRP, TNF-α, IL-6, resistin and insulin levels were measured using ELISA method. The insulin resistance was estimated by the HOMA-IR index calculation as follow : (insulin (µU/mL) x [glucose(mmol/L)/22.5]). Subjects were considered having insulin resistance if HOMA IR value >2.0.10

Statistical analysis

Data of obese and non obese groups were presented as mean ± standard deviation (SD) and analyzed by student t test. The statistical analysis was considered significant if p value <0.05.

RESULTS

One hundred and twenty subjects consisting 60 obese and 60 non obese were involved in this study. The characteristics of subjects are presented in TABLE 1. Significantly higher in the body weight, BMI, waist/hip ratio and diastolic blood pressure between obese and non obese groups were observed in this study (p<0.05). The higher waist circumference value in the obese group compared to non obese group indicated that they have abdominal obesity.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Obese</th>
<th>Non obese</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Male</td>
<td>36</td>
<td>29</td>
<td></td>
</tr>
<tr>
<td>• Female</td>
<td>24</td>
<td>31</td>
<td></td>
</tr>
<tr>
<td>Age (year)</td>
<td>21.1 ± 2.8</td>
<td>20.9 ± 3.7</td>
<td>0.170</td>
</tr>
<tr>
<td>Body weight (kg)</td>
<td>83.9 ± 14.6</td>
<td>53.4 ± 8.1</td>
<td>0.000</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.627 ± 0.079</td>
<td>1.607 ± 0.068</td>
<td>0.147</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>31.5 ± 3.5</td>
<td>20.6 ± 2.3</td>
<td>0.000</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>96.7 ± 11.3</td>
<td>72.3 ± 7.8</td>
<td>0.000</td>
</tr>
<tr>
<td>Hip circumference (cm)</td>
<td>110.7 ± 8.3</td>
<td>89.3 ± 6.9</td>
<td>0.000</td>
</tr>
<tr>
<td>Waist/hip ratio</td>
<td>0.872 ± 0.069</td>
<td>0.812 ± 0.082</td>
<td>0.000</td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>113.8 ± 10.7</td>
<td>111.9 ± 10.7</td>
<td>0.333</td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>76.3 ± 7.5</td>
<td>73.3 ± 8.7</td>
<td>0.050</td>
</tr>
</tbody>
</table>
TABLE 2 showed lipid profile and HOMA IR value of obese and non-obese groups. Significantly higher in the blood glucose and insulin levels as well as HOMA IR value in the obese group compared to non-obese group were observed in this study (p<0.05). Moreover, significantly higher in triglyceride level and significantly lower in HDL-cholesterol level in obese group compared to non-obese group were also reported (p<0.05).

TABLE 2. Level blood glucose, insulin, HOMA IR and lipid profile (mean ± SD) in obese and non-obese groups

<table>
<thead>
<tr>
<th>Variable</th>
<th>Obese (mg/dL)</th>
<th>Non obese (mg/dL)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood glucose (mg/dL)</td>
<td>85.4 ± 29.1</td>
<td>71.6 ± 19.6</td>
<td>0.003</td>
</tr>
<tr>
<td>Insulin (µL U/mL)</td>
<td>33.19 ± 22.00</td>
<td>16.27 ± 14.61</td>
<td>0.000</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>3.63 ± 3.11</td>
<td>1.53 ± 1.49</td>
<td>0.000</td>
</tr>
<tr>
<td>Cholesterol (mg/dL)</td>
<td>156.6 ± 32.2</td>
<td>170.6 ± 19.6</td>
<td>0.121</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>143.4 ± 26.1</td>
<td>120.0 ± 22.9</td>
<td>0.000</td>
</tr>
<tr>
<td>HDL-cholesterol (mg/dL)</td>
<td>35.39 ± 11.35</td>
<td>54.62 ± 15.51</td>
<td>0.000</td>
</tr>
<tr>
<td>LDL-cholesterol (mg/dL)</td>
<td>92.52 ± 29.29</td>
<td>91.61 ± 65.57</td>
<td>0.921</td>
</tr>
</tbody>
</table>

TABLE 3 showed adipokines level in obese and non-obese groups. Significantly higher in CRP and resistin levels in obese group compared to non-obese group were observed (p<0.05). In contrast, no significantly different in TNF-α and IL-6 in both groups were reported.

TABLE 3. Level of CRP, TNF-α, IL-6 and resistin (mean ± SD) in obese and non-obese subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>Obese (mg/L)</th>
<th>Non obese (mg/L)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>CRP (mg/L)</td>
<td>1.543 ± 0.976</td>
<td>1.044 ± 0.256</td>
<td>0.000</td>
</tr>
<tr>
<td>TNF-α (pg/mL)</td>
<td>58.32 ± 97.52</td>
<td>59.43 ± 53.52</td>
<td>0.939</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>6.19 ± 8.59</td>
<td>8.75 ± 7.77</td>
<td>0.089</td>
</tr>
<tr>
<td>Resistin (pg/mL)</td>
<td>1,552.6 ± 306.3</td>
<td>1,250.0 ± 288.9</td>
<td>0.000</td>
</tr>
</tbody>
</table>

DISCUSSION

Obesity is associated with a low-grade inflammatory process in the white adipose tissue (WAT) that leads to increase of circulation of inflammatory markers such as IL-6, CRP, and TNF-α. In this study (TABLE 2), significantly higher blood glucose, insulin, triglyceride levels and HOMA-IR value in the obese subjects compared with those in non-obese subjects were found. In contrast, significantly lower HDL-cholesterol in the obese subjects compared with those in non-obese subjects were found. In addition, significantly higher in CRP and resistin levels in the obese subjects compared with those in non-obese subjects were also found (TABLE 3).

Increases of these inflammation markers are caused by two phases. In the first phase, the fat tissue of obese patients becomes resistant to insulin because of the effects of several adipokines. In the second stage, this resistance occurs in other tissues and leads to increased levels of glucose and insulin. This increasing, together with increased levels of adipokines as clinical conditions due to obesity increase
oxidative stress, endothelial dysfunction, blood pressure and disorders of lipoprotein metabolism, all of which are harmful to health. Inflammation of the WAT is due to obesity because macrophage infiltration is caused by the development of tissue mass.

Other pathways of increasing levels of adipokines occur in response to hypoxia, because the mass of WAT expands in obesity, and as a result, the adipocytes become further from the blood vessels causing a relative lack of oxygen. Hypoxia then causes the stimulation of production and release of inflammatory cytokines, chemokines and angiogenic factors to stimulate blood flow and improve vascularization. Adipokine secretion is activated by extracellular and intracellular stress. Among the extracellular factors, free fatty acid (FFA) is a primary inductor of these pathways and in the obese, chronically FFA increases. Innate immunity receptors, such as toll-like receptor-4 and -2 (TLR-4 and -2), expressed in WAT (mainly by adipocytes, preadipocytes, macrophages and endothelial cells) are involved in the inflammatory process associated with obesity. FFA and other molecules produced by hypoxic conditions during obesity activate these receptors, particularly TLR4. FFA also activate macrophages, especially CD11c+, through TLR-4, exacerbating pro-inflammatory activity.

The CRP levels in obese group in this study was significantly higher than that in non obese groups. This result is consistent with other previous research findings. CRP levels were associated with decreased levels of HDL-cholesterol and insulin resistance, and increased BMI, waist circumference, blood pressure, triglyceride, blood glucose, insulin, HOMA-β. In addition, HOMA-IR showed a significant correlation between CRP levels and metabolic syndrome, including adiposity, hyperinsulinemia and insulin resistance. Recent research shows that there is a strong correlation between fasting insulin with CRP levels. In women, the increase of CRP levels are correlated with the increase of fat mass.

In this study, significantly higher resistin levels in the obese group compared with that non obese group were found. Miyamoto et al. reported that subjects with premature atherosclerosis have higher plasma resistin levels compared with subjects with established atherosclerosis. In addition, increased resistin expressions were found in type 2 DM, insulin resistance and obesity. However, Norata et al. failed to detect any change in resistin levels in atherosclerotic conditions. Resistin may increase the susceptibility of metabolic syndrome by regulating adiponectin secretion from adipocytes and enhancing hepatic gluconeogenesis by inhibiting the enzymes involved in gluconeogenesis through AMP-activated protein kinase activation.

In this study, no significantly different in the IL-6 and TGF-α levels in obese group compared with those in non obese group were found. This result is similar with other previous study. Although IL-6 appears to be increasing in obesity and decreased in response to weight loss, our results showed no significantly different at baseline or in response to diet. Subcutaneous adipose tissue is estimated to release about 30% more IL-6 in systemic and visceral adipose tissue. However, small changes of IL-6 level in obese subjects show the levels of IL-6 in obesity only about 10% of total IL-6 that is produced by fat cells.

Some reports indicated that in metabolic syndrome patients, the IL-6 levels increased with BMI, however its mechanism is still unclear. In this study, the TNF-α levels were not significantly associated with body weight, waist circumference, or percentage of body.
fat at baseline. These results are in contrast to previous study showing the TNF-α level were associated with obesity.\textsuperscript{32} In addition, TNF-α levels did not show a significantly decline in response to weight loss or diet. They were not significantly different after the weight loss of 7.5% at 10 weeks. It is indicated that adipose tissue has only a small effect on the regulation of TNF-α levels. Furthermore, it is reported that TNF-α appears to act locally in human fat tissue\textsuperscript{33} and it is not secreted from adipose tissue into the blood. Previous study indicated changes in IL-6 or TNF-α after weight loss (5-9 kg) with dietary intervention and exercise.\textsuperscript{34} Changes in IL-6 and TNF-α were significantly correlated with a weight loss diet for nine months in the group with low calorie diet.\textsuperscript{30} This result may explain the relationship between IL-6 and TNF-α in which IL-6 provides proinflammatory activity and increases the TNF-α levels. This possibility shows that although the weight loss did not improve the IL-6 and TNF-α in the circulation, there appears to be a significant clinical response demonstrating weight loss gives a reduction in inflammation. High levels of TNF-α are also seen in metabolic syndrome patients.\textsuperscript{35} The relationship between high levels of TNF-α and metabolic syndrome is associated with TNF-inducing NH2-terminal kinase c-jun to mediate phosphorylation of IRS-1 serine. This relationship determines the normal inhibition of tyrosine phosphorylation of IRS-1 and insulin signaling.\textsuperscript{36}

**CONCLUSION**

In conclusion, the CRP and resistin levels as well as insulin resistance are higher in obese Javanese population compared to those non obese. Further genotype study to identify hereditary factors that may contribute to insulin sensitivity and resistance are recommended.

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