RETN rs3745368 polymorphism and resistin level in Javanese ethnic Indonesian obese: a case-control study

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HIGHLIGHTS
Resistin level has a negative correlation with Body Mass Index.

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Abstract
Obesity has become a global public health problem. It occurs due to a positive energy balance leading to adipose tissue expansion. White adipose tissue was an endocrine organ which secreted resistin. Resistin also produced by immune cells due to low chronic level inflammation might cause higher resistin level in obese people. Polymorphism +62G>A RETN gene was reported has a relationship with low resistin level and A allele as a protective allele. This study aimed to determine genotype and allele frequency distribution concerning resistin level. Another objective aimed to know the correlation between resistin level with body mass index. The design of the research was a case-control study with 122 people (18-40 y.o.), divided equally in the case group (BMI ≥ 27 kg/m2) and control group (BMI 18.5-24.9 kg/m2) without diabetes mellitus. Blood was taken after fasting a minimal 8 hours. Plasma was used to measure the resistin level. DNA genotyping was analyzed using PCR-RFLP. Genotyping result showed three genotypes of RETN gene +62G>A polymorphism (GG, GA, AA). There was no significant difference in genotype and allele frequency distribution related to obesity status (p=0.680; p=1) and resistin level (p=0.537) between case and control group. There was no significant difference in resistin level between case and control group (p=0.770). Resistin level was correlated with BMI in obese group (p= 0.05; r= -0.25). The present study concludes that there is no significant difference in genotype and allele frequency distribution related to obesity status and resistin level. Resistin level has a negative correlation with BMI.

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1. **INTRODUCTION**

Obesity has become a global public health problem. The prevalence of obesity is roughly around 1.2 million and increased three-fold or more since 1980 in the Middle East, the Pacific Islands, Australasia, and China based on data from the World Health Organization (WHO). The prevalence of obesity and overweight in the Yogyakarta region is higher than the national average. The Javanese ethnic is the dominant ethnic in Yogyakarta.

Obesity occurs due to positive energy balance caused by weight gain. Adipose tissue expansion was significantly affected physiological response and can interfere in its function. Adipose tissue hypertrophy, ectopic fat deposition, hypoxia, and chronic stress occur in the state of obesity. White adipose tissue is known as an endocrine organ secreting signalling molecules called adipokines. Adipose tissue hypertrophy secreted pro-inflammatory adipokines. One of the pro-inflammatory adipokines elevated in an obese state is resistin. Changes in the chronic secretion of adipokines show adipose tissue dysfunction and developed into metabolic diseases, cardiovascular, inflammatory, and malignant disease. Hypertrophy of adipose tissue in obese individuals increases the infiltration of immune cells that secrete pro-inflammatory mediators, causing chronic low-level systemic inflammation.

Resistin is the link between obesity, insulin resistance to diabetes based on animal studies. On mice injected recombinant resistin, its expression was increased and interfere with glucose tolerance and insulin action. Some research shows that serum resistin levels in the obese subjects were higher than the normal subject, which positively correlated with changes in Body Mass Index (BMI) and visceral fat. However, other studies reported no association between serum resistin level with body fat percentage, visceral adipose tissue, and BMI. The role of resistin in obesity, insulin resistance and diabetes mellitus as a risk factor still controversial and the mechanisms underlying the expression, regulation, secretion, and resistin still unclear.

Genes encoding resistin is RETN gene. Several single nucleotide polymorphisms (SNPs) RETN gene related to levels of circulating resistin. Polymorphism +62G>A RETN gene (rs3745368) associated with low resistin levels. This study aimed to determine genotype and allele frequency distribution concerning resistin level in obese and control group. Another objective aimed to determine the difference of resistin level between obese and control group and to knowing the correlation between resistin level with body mass index. Research on polymorphism + 62G> A RETN in the Javanese ethnic had never been done before.

2. **MATERIALS AND METHOD**

The design of the research was a case-control study with 122 people (18-40 y.o.), divided equally in the case group (BMI ≥ 27 kg/m²) and the control group (BMI 18.5-24.9 kg/m²) according to obesity classification WHO the Asia Pacific. Subjects were Javanese ethnic and without diabetes mellitus. Blood was taken after minimal 8 hours fasting. Informed consents were obtained from all the subjects before their inclusion in the study. Studies were conducted under the guidelines set by The Medical and Health Research Ethics Committee (MHREC), Faculty of Medicine, Gadjah Mada University, Dr Sardjito General Hospital, Yogyakarta, Indonesia.

2.1 Anthropometric measurement

Obesity status was determined by body mass index (BMI). Height and body weight were measured without shoes, and the subjects wore light clothes. BMI of the obese subject was ≥ 27 kg/m², and the control subject was 18.50-24.99 kg/m².
2.2. Biochemical parameters
Fasting blood from subjects was collected in EDTA tube then is centrifuged. Blood plasma was used to measure fasting glucose using spectrophotometer with a commercially available kit (Dyasis), and resistin level was measured using ELISA method (RayBiotech).

2.3. Genotyping
DNA genotyping was analyzed using PCR-RFLP. Genotyping was carried out by PCR amplification of peripheral blood genomic DNA extracted using blood isolation kit (Promega) followed by restriction enzyme used in the previous study \(^{13}\). DNA was PCR amplified using the forward primer, \(5'\)-AGAGTCCACGCTCCTGTGTT-3' and the reverse primer, \(5'\)-TCATCATCAGCTCAGTT-3'. The amplified products were digested with a restriction enzyme, BseRI (New England BioLabs). PCR product size for AA was 238 bp and 21 bp (homozygous mutant allele), 259 bp for wild-type allele; 259, 238 and 21 bp for heterozygote allele.

2.4. Statistical analysis
Normality and variance of data were analyzed using Kolmogorov-Smirnov. Differences between variables were computed using Mann-Whitney and Kruskal-Wallis test. Genotype and allele frequency was determined using the chi-square test or Fisher exact test for an expected value less than 5. The Odds ratio was determined by logistic regression analysis. P-value was considered to be statistically significant \(p<0.05\). Correlation resistin level with BMI using Spearman Correlation.

3. RESULTS AND DISCUSSION
Subject’s baseline characteristics are presented in Table 1. The number of men and women was the same in both groups, with no significant difference in age. Anthropometric measurement, including BMI, was significantly higher in the obesity group than in control. Fasting plasma glucose and resistin level both groups showed were not significantly different.

<table>
<thead>
<tr>
<th>Table 1. Subjects baseline characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variables</td>
</tr>
<tr>
<td>Sex</td>
</tr>
<tr>
<td>Men</td>
</tr>
<tr>
<td>Women</td>
</tr>
<tr>
<td>Age (years)</td>
</tr>
<tr>
<td>18-20</td>
</tr>
<tr>
<td>21-30</td>
</tr>
<tr>
<td>31-40</td>
</tr>
<tr>
<td>Body Mass Index (kg/m(^2))</td>
</tr>
<tr>
<td>Fasting blood glucose (mg/dL)</td>
</tr>
<tr>
<td>HOMA-IR</td>
</tr>
<tr>
<td>Resistin (pg/mL)</td>
</tr>
</tbody>
</table>
Figure 1. Result of PCR-RFLP RETN gene polymorphism +62G>A. Lane M is a marker. PCR product was 259 bp (lane 1 and 8). Restricted fragment by BseRI enzymes was wild type or GG (lane 2, 4, 7, 9, 10, 12, 13, 14), heterozygote mutant or GA (lane 3, 5, and 6), and homozygote mutant or AA (lane 11). No 21 bp in the figure.

Allele and genotype frequency distribution was not statistically different (p=0.187 and p= 1) between groups as well as wild-type genotype and T allele carrier frequency (p= 0.680) (Table 2). Odds ratio test showed that SNP +62G>A has neither risk factor nor a protective role in the studied population.

Table 2. Genotype and Allele Distribution of RETN Gene Polymorphism +62G>A

<table>
<thead>
<tr>
<th>Variables</th>
<th>Obesity (n=61)</th>
<th>Control (n=61)</th>
<th>p</th>
<th>OR (CI 95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotype</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>59(96.72%)</td>
<td>57(93.44%)</td>
<td>0.680</td>
<td>2.070 (0.365-11.747)</td>
</tr>
<tr>
<td>GA+ AA</td>
<td>2(3.28%)</td>
<td>4(6.56%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>59(96.72%)</td>
<td>57(93.44%)</td>
<td>0.187</td>
<td>4.14 (0.449-38.172)</td>
</tr>
<tr>
<td>GA</td>
<td>1(1.64%)</td>
<td>4(6.56%)</td>
<td>0.00</td>
<td>(0.00-18.47)</td>
</tr>
<tr>
<td>AA</td>
<td>1(1.64%)</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Allele</th>
<th>GG (n=61)</th>
<th>GA (n=5)</th>
<th>AA (n=1)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>G</td>
<td>119(97.54%)</td>
<td>118(96.72%)</td>
<td>1</td>
<td>1.345 (0.295-6.138)</td>
</tr>
<tr>
<td>A</td>
<td>3(2.46%)</td>
<td>4(3.39%)</td>
<td></td>
<td>0.537</td>
</tr>
</tbody>
</table>

Resistin levels were not significantly different between obese group than the control group (Table 1). Table 3 shows that on the whole subject of the study, it has no significant differences in levels of resistin between genotype GG, GA, and AA (p = 0.537).

Table 3. Resistin Level among Genotypes

<table>
<thead>
<tr>
<th>Resistin level (pg/mL)</th>
<th>GG (n=116)</th>
<th>GA (n=5)</th>
<th>AA (n=1)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>366(3.55-1955)</td>
<td>142(15.42-1536)</td>
<td>776</td>
<td>0.537</td>
<td></td>
</tr>
</tbody>
</table>

Resistin level (Table 4) has no significant difference with the gene polymorphism RETN + 62G>A in this study. Resistin level in GG genotype had no significant difference between obese with control groups (p= 0.816) as well as individuals with GA genotype (p=1). Resistin level of genotype A allele carrier among the obese group and control group was also not significantly different (p=0.643).
Table 4. Resistin Level In Obese and Control Group

<table>
<thead>
<tr>
<th></th>
<th>Resistin Level (pg/mL)</th>
<th>P</th>
<th>GA+A</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GG</td>
<td>GA</td>
<td>AA</td>
<td></td>
</tr>
<tr>
<td>Obese (n=61)</td>
<td>374 (19.20-1645)</td>
<td>142</td>
<td>776</td>
<td>459</td>
</tr>
<tr>
<td>Control (n=61)</td>
<td>363 (3.55-1955)</td>
<td>0.364</td>
<td>0</td>
<td>378</td>
</tr>
<tr>
<td>p</td>
<td>0.816</td>
<td>-</td>
<td>0.830</td>
<td>0.643</td>
</tr>
</tbody>
</table>

Plasma resistin levels in the obese group were associated with body mass index. The relationship between plasma resistin levels with a body mass index in the obese group and the control group can be seen in the scatter diagram below.

Figure 2. Scatter diagram resistin level vs BMI in obese group

Figure 3. Scatter diagram resistin level vs BMI in obese group

Genotyping results showed three genotypes (GG, GA, AA) in the studied population (Figure 1). All three genotypes were also discovered in other Asian population, Caucasian, and the German population. Allele and genotype frequency distribution was not statistically different between groups as well as wild-type genotype and T allele carrier frequency (Table 2). The
present result showed no significant relationship between genotype and allele with obesity status. Results of other studies also showed no association between gene polymorphisms RETN +62G>A with the parameters of the metabolic syndrome and the An allele carriers had a body mass index higher than the G allele carrier. The results showed there was no significant relationship between the genotype and allele with obesity status. Results from other studies also showed no association between gene polymorphisms RETN +62G>A with the parameters of the metabolic syndrome. An allele carrier had a body mass index higher than G allele carrier. Obesity is caused by genetic factors, environmental, and other factors, such as decreased physical activity and increased food intake. Polygenic obesity results from the combined effects of multiple genes influenced by environmental factors. RETN gene variations differ in various ethnic groups and different samples. AA genotype is less common in Europe than in Asia. Environmental exposure differences (e.g., differences in dietary habits) and genetic background may also have a role in this polymorphism. In this study, genotype and allele frequency differences were not significant in the obesity group compared with normal BMI because gene polymorphism RETN + 62G>A might not directly be related to the status of obesity. Other research showed polymorphism +62G>A RETN gene associated with increased levels of adiponectin, resistin levels, markers of metabolic and body fat stores. This polymorphism was reported associated with hypertension and diabetes mellitus.

Polymorphism RETN gene +62G>A in this study was not related to resistin level. Asano (2010) compared plasma resistin level with other RETN gene polymorphism obtained that this polymorphism had p-value bigger among RETN gene polymorphism than other areas. So it can be said that polymorphism in the promoter region -537A>C (rs34124816) and -358G>A (rs3219175) has a more significant effect on resistin levels than in the 3' UTR (rs3745368). Promoter area polymorphisms affect resistin level. SNPs -358G>A and -638G>A has a strong linkage disequilibrium with -420C>G affected circulating resistin level among other SNPs at the same locus RETN. Polymorphism RETN gene -420C>G activity increased by inducing promoter activity bind to Sp1/3.

Transcription factor Sp1 and Sp3 bind specifically to DNA elements associated with -420G. Over-expression Sp1 and Sp3 increased the activity of gene promoter -420G RETN. Polymorphism RETN gene -638G>A (rs34861192) bind to SREB1c RETN as regulators of gene expression. This study was not finding significant differences may be due to gene SNP RETN +62G>A in this population has no linkage disequilibrium (LD) with functional genes that it directly influences resistin level. This study only screened +62G>A RETN gene, and therefore it cannot exclude the role of resistin other variant or variants of the nearby genes that linkage disequilibrium with SNP +62G>A RETN gene. LD structure differences may cause research of resistin level vary between populations.

Correlation resistin level with body mass index in this study showed resistin levels inversely related to BMI. A weak correlation between resistin levels with BMI was also found in the other study. Resistin levels in this study did not differ significantly between the obese with the control group. Others studies reported no association between serum resistin levels with the percentage of body fat, visceral fat tissue, and body mass index. Serum resistin reported unchanged after bariatric surgery despite significant weight loss. Increased serum resistin during weight loss in overweight subjects was also reported from other studies.

Resistin did not have a role in obesity and insulin resistance in human studies. Resistin mRNA in adipose tissue throughout the obese subjects increases, but lower resistin mRNA levels in isolated adipose tissue. No relationship of resistin gene expression in human adipose tissue with insulin resistance and BMI. Structure and physiological human resistin and mice were differences. Human resistin gene is located on chromosome 19p13.3 in an area that was not related to obesity and insulin resistance. Differences between rodents and humans resistin because there were significant
differences between the two species in the gene and protein structure, gene regulation, distribution in a specific tissue, and the induction of insulin resistance which the lack of evidence relating to the receptor resistin and mechanisms of action of downstream signaling.27

Resistin level results differences may because from various confounders such as age and sex, drug use, and measurement methods.28 Reference and sensitivity of the ELISA kit. In several studies, the cross-react with the ELISA kit RELM also affect the diversity of serum resistin concentrations. Other studies showed resistin, a hormone produced by adipose tissue that works not throughout the body. The expression of mRNA may cause this was not directly associated with the expression of proteins, which were modified post-transcriptional and post-translational affect resistin level. Serum resistin increased the average degradation of transcription through a negative feedback mechanism and the recruitment of translational inhibitors. Resistin secreted paracrine and act at the protein level. Effect of obesity on the expression of resistin in humans remains unclear.9

Resistin has a role in the pro-inflammatory process associated with obesity and insulin resistance. Resistin expression in peripheral blood mononuclear cells (PBMC) from several studies were affected by IL-1, TNF-α, and IL-6. The relationship between the expression, secretion of resistin, and other inflammatory markers include IL-6, leptin, and CRP were also reported in patients with severe inflammatory diseases.27 Increased production of pro-inflammatory cytokines TNF-α and IL-12 were obtained from macrophages incubation with recombinant human resistin.28 The study showed that this induction is mediated by transcription factor nuclear factor-kappa B (NF-κB). Low levels of tissue inflammation is a significant cause of insulin resistance induced by obesity and the molecular crosstalk in inflammation pathways and insulin signal delivery. Circulating resistin may be a central messenger between inflammation and insulin homeostasis.29 In this study, subjects were not in inflammation condition from clinical symptoms but not measured inflammatory markers so it cannot be measured the influence of inflammatory markers such as IL-1, TNF-α, and IL-6 which could also affect plasma resistin level.

4. CONCLUSION

There was no significant difference in genotype and allele frequency distribution related to obesity status and resistin level between the case and the control group. Resistin level has a negative correlation with BMI.

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REFERENCE


