Analysis of the Relationship Between Soluble Cd40 Ligand and Homeostasis Model Assessment of Insulin Resistance in Obese Non-Diabetic Subjects

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KEYWORDS
Obesity, HOMA-IR, sCD40L,

ABSTRACT:
Introduction: Obesity is a complex multifactorial disease characterized by the accumulation of body fat, leading to adverse effects on one’s well-being. Obesity has a strong association with insulin resistance. Insulin resistance can lead to a range of health issues including type II diabetes mellitus, cardiovascular disease, and other metabolic syndrome diseases. A method that can be employed to evaluate insulin resistance is the measurement of HOMA-IR. sCD40L is the soluble form of the CD40L molecule that has been released on the cell surface through proteolysis.

Objectives: The objective of the present study is to determine the correlation between sCD40L and HOMA-IR in non-DM subjects.

Methods: The method utilized was observational analytic with a cross-sectional design involving 70 non-DM subjects, 36 obese subjects, and 34 non-obese subjects, comprising 30 males and 40 females.

Results: The findings exhibited a significant difference in the value of HOMA-IR between obese and non-obese research subjects (p=0.001). No significant difference, however, was observed in sCD40L levels between the two groups (0.117). In addition, no correlation was observed between HOMA-IR and sCD40L levels in non-diabetes mellitus subjects (r= 0.081 p=0.507).

Conclusions: Between HOMA-IR values and sCD40L levels in non-diabetes mellitus subjects, no significant correlation was found.

1. Introduction

Indonesia is currently grappling with diverse health challenges, one of which is the issue of obesity. Basic Health Research (Riskesdas) data in 2018 revealed that the prevalence of obesity in toddlers was 3.8%, while individuals 18 years and older experienced a prevalence of 21.8%. The target obesity rate for 2024 continues to be unchanged at 21.8%[1]. Obesity is a complex multifactorial disease characterized by the accumulation of body fat that causes negative impacts on health. [2] Obesity is strongly linked to insulin resistance, dyslipidemia, hypertension, and type II diabetes mellitus[3]. It is always linked to the accumulation of fat or adipose tissue. The accumulation of fat tissue in the central part of the body triggers increased production of excessive free fatty acids, contributing to their heightened transfer to the liver through portal vein drainage. The excessive amount of free fatty acids in the liver triggers visceral fat to release inflammatory cytokinesis through the portal vein. Consequently, this initiates insulin resistance within the liver and an uncontrollable surge in glucose production. Insulin resistance can lead to various health issues including type II diabetes mellitus, cardiovascular disease, and other metabolic syndrome diseases. [4]

In insulin resistance, low-grade systemic inflammation sets off dysfunction in the hemostatic system through
various mechanisms, such as increased activation of platelets. Both chronic hyperglycemia and hyperinsulinemia can contribute to an elevated expression of CD40 ligand (CD40L) in circulating platelets (5). In cases of insulin resistance, low-grade systemic inflammation also triggers the activation of various other cells such as B cells, T cells, as well as monocytes and endothelial cells which will then trigger the release of sCD40L into the circulation; this process may exacerbate the resistance of insulin and contribute to the onset of type II diabetes and other health problems(6).

Soluble CD40 Ligand refers to a form of the CD40L molecule released on the cell surface through a process called proteolysis. This process involves enzymatic cutting of the CD40L molecule bound to the surface of the cell, enabling it to circulate in body fluids in a soluble form (7). The presence of insulin resistance is one of the contributing factors to the increased level of plasma sCD40L in obese patients. (8)

Several methods can be employed to evaluate the level of insulin resistance, one of which is by assessing the Homeostasis Model of Insulin Resistance (HOMA-IR). HOMA-IR can describe insulin resistance. In individuals diagnosed with pre-diabetes, heightened secretion of insulin serves as compensation for the rise in HOMA-IR by pancreatic beta cells. (9)

Patients with insulin resistance exhibited a notably higher level of soluble CD40 Ligand (sCD40L) when compared to those without insulin resistance(8). Another study stated that compared to the non-IGT-group, the average levels of sCD40L were observed to be significantly higher in the Impaired glucose tolerance (IGT) group(10). The findings of this study differ from those conducted by Sameh & Wassim (2016), which revealed that no correlation was found between sCD40L and body mass index. Furthermore, the current study exhibits a negative correlation with HOMA-IR in female subjects with polycystic ovarian syndrome (PCOS), regardless of their obesity status. (11). Meanwhile, a study by Byun et al. also found lower sCD40L values in obese children with insulin resistance compared to the control group. (12)

High HOMA-IR values arise from numerous factors linked to metabolic syndrome, such as obesity, hypertension, and dyslipidemia. The insulin resistance commonly observed in obese individuals is connected to heightened HOMA-IR levels that are likely to exhibit a positive correlation with sCD40L values (9). Considering this context, the authors are interested in investigating and analyzing the relationship between sCD40L and HOMA-IR in obese non-diabetes mellitus subjects.

2. Objectives
Considering this context, the authors are interested in investigating and analyzing the relationship between sCD40L and HOMA-IR in obese non-diabetes mellitus subjects.

3. Methods
Design and Population of the Research
The current study employed a cross-sectional study design. All adults who willingly volunteered for the research were determined as the research population. A total of 70 non-DM individuals were included, comprising 30 males and 40 females. The inclusion criteria encompassed non-DM males and females aged 18-40 years who consented to take part in the study. The exclusion criteria were pregnant women, having a history of DM, having an infection, suffering from malignancy, taking anti-platelet aggregation drugs, taking corticosteroid drugs, icteric, lipemic, or hemolyzed serum samples. The study took place at the Clinical Pathology Laboratory of Hasanuddin University Hospital, Makassar, and Hasanuddin University Medical Research Center (HUM-RC) Laboratory. It was carried out after receiving ethical approval from the Health Research Ethics Commission (KEPK) of Hasanuddin University Hospital (RSPTN UH) with ethical number 858/UN4.6.4.5.31/PP36/2023.

Laboratory Procedure
This study involved documenting the participants’ identities, and their height (m) and weight (Kg) were assessed. Body Mass Index (BMI) was measured using the weight/(height) formula², with obesity defined as BMI ≥ 25 kg/m2. Blood samples were collected in the morning after an 8-12 hours fast using a red cap tube without anticoagulant. The samples were left for 15-30 minutes in a vacuum tube to induce clotting. Following this, centrifugation at 3000 rpm for 10-15 minutes was carried out to separate the serum from the blood cell components. The serum obtained was stored at -200C. Fasting glucose was tested using ABX Pentra 400 with enzymatic method, while insulin levels were measured using Cobas® e411 with Electrochemiluminescence Immunoassay (ECLIA) method. The analysis of sCD40L was conducted through the Enzyme-linked Immunosorbent Assay (ELISA) method, employing the
MyBioSource insert kit. A microplate reader (Type 357, Thermo Fisher Scientific, Shanghai, China) was utilized to assess the absorbance of the ELISA test result.

**Data Analysis**

The Statistical Package for the Social Sciences (SPSS) version 22 was employed to analyze the data. Meanwhile, the Kolmogorov-Smirnov normality test was utilized to assess the data distribution of sCD40L and HOMA-IR. For non-diabetic subjects, the relationship between sCD40L and HOMA-IR was examined using the Pearson correlation test for normally distributed data and Spearman correlation test for non-normally distributed data. Statistical test results are significant if the p value <0.05.

4. Results

A total of 70 research subjects were involved, consisting of 30 males and 40 females. From the results of the study, there were 36 individuals with obesity non-DM and 34 individuals with non-obesity non-DM shown in Table 1.

**Table 1.** Frequency Distribution of Gender and Group

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Category</th>
<th>Non diabetes mellitus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>Male</td>
<td>30</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>40</td>
</tr>
<tr>
<td>Group</td>
<td>Obese</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>Non-obese</td>
<td>34</td>
</tr>
</tbody>
</table>

As can be seen in Table 2, the study participants’ ages ranged from 20 years to 40 years, with a mean age of 31.34 years, mean body weight of 64.33 kg, mean height of 159.04 cm, BMI of 25.31 kg/m², mean waist circumference of 88.24 cm, mean HOMA-IR score of 2.58 and mean sCD40L level of 1.83 ng/mL.

**Table 2.** Descriptive Analysis of Age Variable, sCD40L Levels, and HOMA-IR Values

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>n</th>
<th>Mean±SD</th>
<th>Median</th>
<th>Min-Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (Year)</td>
<td>70</td>
<td>31.34±3.80</td>
<td>31</td>
<td>20-40</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>70</td>
<td>64.33±4.45</td>
<td>63.3</td>
<td>41.2-130.1</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>70</td>
<td>159.04±7.76</td>
<td>157</td>
<td>145-178</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>70</td>
<td>25.31±4.51</td>
<td>25</td>
<td>15.3-45</td>
</tr>
<tr>
<td>Waist Circumference (cm)</td>
<td>70</td>
<td>88.24±11.18</td>
<td>89</td>
<td>61-133</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>70</td>
<td>2.58±1.26</td>
<td>2.39</td>
<td>0.53-5.87</td>
</tr>
<tr>
<td>sCD40L (ng/mL)</td>
<td>70</td>
<td>1.83±0.40</td>
<td>1.82</td>
<td>0.38-2.74</td>
</tr>
</tbody>
</table>

**Normality Test**

Based on the Kolmogorov-Smirnov test in Table 3 conducted on all subjects, the HOMA-IR value in non-DM subjects exhibited a p-value of 0.002 <α (0.05), indicating that the data is not normally distributed. Meanwhile, the p-value of sCD40L levels in all non-DM subjects was 0.200>α (0.05), which suggests that the data is normally distributed. For obese non-diabetes mellitus (DM) subjects, the obtained p-value for HOMA-IR levels was 0.124>α (0.05), and the p-value of sCD40L levels was 0.103>α (0.05), indicating normal data distribution. Conversely, in non-obese subjects without DM, the p-value of HOMA-IR value was 0.020<α (0.05), suggesting non-normal data distribution. In contrast, the p-value of sCD40L level was 0.544>α (0.05), indicating a normal data distribution.

**Table 3.** Normality Test of sCD40L Level and HOMA-IR Value of Research Subjects

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Normality Test</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Statistic</td>
<td>N</td>
</tr>
<tr>
<td>HOMA-IR (Overall)</td>
<td>0.132</td>
<td>70</td>
</tr>
<tr>
<td>sCD40L (Overall)</td>
<td>0.070</td>
<td>70</td>
</tr>
<tr>
<td>HOMA-IR (OB)</td>
<td>0.952</td>
<td>36</td>
</tr>
<tr>
<td>sCD40L (OB)</td>
<td>0.950</td>
<td>36</td>
</tr>
<tr>
<td>HOMA-IR (Non OB)</td>
<td>0.923</td>
<td>34</td>
</tr>
<tr>
<td>sCD40L (Non OB)</td>
<td>0.973</td>
<td>34</td>
</tr>
</tbody>
</table>

Notes: *p = Kolmogorov-Smirnov test, **p = Shapirrio Wilk test, OB = Obese, Non-OB = Non-obese.

**Comparison Test**

Based on the normality test conducted, the HOMA-IR value for non-obese subjects is the only dataset that does not exhibit a normal distribution. Therefore, to assess differences in HOMA-IR value between obese and non-obese subjects, the Mann-Whitney test was employed (Table 4). Then, a p value of 0.001 was obtained from the test conducted. Given that p<α (0.05), conclusion can be drawn that a very significant difference was found in the HOMA-IR values between obese and non-obese subjects within the non DM group.

Notes: **BMT = Body Mass Index, HOMA-IR = Homeostasis Model Assessment Insulin Resistance, sCD40L = Soluble CD40 Ligand**
The scatterplot image above revealed that the data distribution does not form a linear relationship pattern between HOMA-IR values and sCD40L levels. This indicates that there is no correlation or relationship between the variables of HOMA-IR values and sCD40L levels in non-DM subjects.

5. Discussion
This study took place between August and November 2023. The results revealed a highly significant difference in the value of HOMA-IR between obese and non-obese subjects in the non-DM group. HOMA-IR is an index used to measure insulin resistance. A high HOMA-IR value signifies the presence of insulin resistance. This aligns with the research conducted by Obiageli (2021), indicating a significant increase in HOMA-IR among the obese group when compared to the normal control group (13).

The increase in HOMA-IR values among obese subjects in comparison to non-obese subjects is attributed to dysfunction in the adipose organ associated with obesity. Adipocytes can produce several hormones and chemicals called adipocytokines that can affect insulin sensitivity. Increased fat levels in fat cells can cause changes in adipocytokine secretion and exacerbate insulin resistance, increasing HOMA-IR values (14). In addition, decreased mitochondrial function and increased reactive oxygen species (ROS) are also factors that play a part in causing insulin resistance (15).

The independent T-test obtained a p-value of 0.117 (p>0.05), suggesting no significant difference between the value of sCD40L in both obese and non-obese subjects in the non-DM group. This is because most respondents in the obese group are still within a mild obesity condition (average BMI 25.31 kg/m²), which may not have led to a significant systemic inflammation for high sCD40L in circulation. According to the findings from the study conducted by Guldiken et al (2016), patients with severe obesity (IMT≥35 kg/m²) exhibited significantly higher sCD40L values compared to obese patients (IMT 30-34.9 kg/m²) and non-obese subjects (IMT<25 kg/m²) (16). Another study conducted by Unek (2010) also found that obese subjects (BMI≥30 kg/m²) had higher sCD40L values than overweight subjects (BMI 25 kg/m² to 29.9 kg/m²) (17).

Based on the Spearman correlation test, a p-value of 0.507 was obtained. Since 0.507 > α (0.05), it can be concluded that there is no significant correlation between HOMA-IR values and sCD40L levels in non-DM subjects.

### Table 4. Comparison Test between HOMA-IR Value and sCD40L Levels in Subjects with Obesity and Non-Obesity

<table>
<thead>
<tr>
<th>Group</th>
<th>N</th>
<th>Mean±SD</th>
<th>Median</th>
<th>Min-Max</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOMA-IR (OB)</td>
<td>36</td>
<td>3.09±1.33</td>
<td>3.11</td>
<td>1.24-5.87</td>
<td>0.001*</td>
</tr>
<tr>
<td>HOMA-IR (Non OB)</td>
<td>34</td>
<td>2.04±0.93</td>
<td>1.74</td>
<td>0.53-3.84</td>
<td>0.117**</td>
</tr>
<tr>
<td>sCD40L (ng/mL) (OB)</td>
<td>36</td>
<td>1.90±0.47</td>
<td>1.92</td>
<td>0.38-2.74</td>
<td></td>
</tr>
<tr>
<td>sCD40L (ng/mL) (Non OB)</td>
<td>34</td>
<td>1.75±0.31</td>
<td>1.72</td>
<td>0.92-2.43</td>
<td></td>
</tr>
</tbody>
</table>

Notes: *p = Mann-Whitney Test, **p = T Independen Test
As can be seen in Table 4, the statistical tests comparing the sCD40L level between obese and non-obese subjects in the non-DM group using the independent T-test revealed a p-value of 0.117. As the p-value is greater than α (0.05), it can be inferred that there is no significant difference observed in the value of sCD40L between individuals with obesity and non-obesity in the non-DM group.

### Correlation Test

Table 5 depicts the Spearman correlation test carried out between HOMA-IR values and sCD40L levels in non-DM subjects obtained a p value of 0.507. Since 0.507 > α (0.05), it was concluded that there is no significant correlation between HOMA-IR values and sCD40L levels was found in non-DM subjects.

### Table 5. Correlation Test of HOMA-IR Values and sCD40L Levels in Overall Non-Diabetes Mellitus Subjects

<table>
<thead>
<tr>
<th>Variable</th>
<th>sCD40L (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOMA-IR Value</td>
<td>r = 0.81</td>
</tr>
<tr>
<td>p = 0.507</td>
<td></td>
</tr>
<tr>
<td>n = 70</td>
<td></td>
</tr>
</tbody>
</table>

Data exploration was carried out using scatterplot graphs to determine the linear relationship pattern between HOMA-IR value and sCD40L levels in non-DM subjects.
results of the current study may be influenced by various variables, one of which is smoking. The release of nicotine from smoking activities can contribute to hemodynamic effects and various cardiovascular occurrences (18). Cardiovascular diseases result from the rupture of atherosclerotic plaques and the formation of thrombus. Dyslipidemia, endothelial dysfunction, as well as platelet hyperreactivity, can lead to atherosclerosis and an increased risk of thrombotic vascular events. Platelet hyperactivation is associated with the release of sCD40L, influencing its level in circulation (19).

Extended periods of obesity will worsen insulin resistance. Persistent low-grade systemic inflammation impedes insulin action within the insulin signaling pathway, disrupts glucose homeostasis, and leads to the occurrence of systemic dysregulation. Generally, sustained obesity and excessive nutrition over an extended period result in insulin resistance and chronic low-grade systemic inflammation through lipotoxicity (20). Hypertension is also related to the levels of sCD40L. Soluble CD40 ligand (sCD40L) contributes to the pathogenesis of vascular damage linked to risk factors and is closely associated with inflammation, thrombosis, and angiogenesis. The newly available data point to the vasoactive peptide angiotensin II as a promoter and enhancer of inflammation activation induced by CD40/CD40L ligation in human blood vessel cells (21). Angiotensin II, a vasoactive peptide associated with blood vessel constriction, induces an increase in blood pressure as its level rises. Consequently, sCD40L levels will increase in hypertensive patients (22).

The consumption of antihypertensive medications affects blood vessels, not only reducing blood pressure (exhibiting a pleiotropic effect) but also influencing sCD40L levels (22). This research is consistent with the findings reported by Han et al., (2010), stating that either combination therapy or the use of losartan alone significantly decreases the level of plasma sCD40L (23). The consumption of antihypertensive is what will then affect or even reduce the levels of sCD40L circulating in the body. The involvement of physical activity, such as exercise, can also have an impact on this study. With or without weight loss, physical activity can reduce the risk of cardiometabolic disorders, partially improve insulin sensitivity, and lower blood pressure (24). This corresponds with the study carried out by Hilberg et al.’s research (2021), which asserts that moderate-intensity exercise inhibits platelets, whereas vigorous exercise promotes the aggregation and activation of platelets (25). Other studies suggest that aerobic exercise in adults dealing with overweight or obesity and cardiometabolic disorders is successful in decreasing postprandial glucose and insulin levels (26).

The effect of platelet count can also potentially influence the level of sCD40L. Abnormal platelet counts can disrupt the balance of sCD40L in the body. An increase in platelet count may be associated with an increased production or release of sCD40L (27). Another study by Calabro (2009) suggests that the adipose hormone resistin produced by adipose tissue in obesity can enhance the expression of sCD40L and tissue factors in human coronary endothelial cells (28). CD40L and platelets have a complex relationship in the immune and inflammatory systems. An increase in platelet count (thrombocytosis) can potentially elevate the concentration of sCD40L in the blood (29).

6. Conclusion

According to the findings in this study, it can be inferred that there is no significant correlation between HOMA-IR and sCD40L levels in non-diabetic subjects.

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Conflict of Interest

All authors affirm that there are no conflict of interest in this study.

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