Correlation between Insulin Resistance with Soluble CD40 Ligand and Plasminogen Activator Inhibitor-1 Plasma in Pre-diabetic Patients

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Abstract

BACKGROUND: The main condition of pre-diabetes is insulin resistance that can lead to a prothrombotic state.

AIM: This study aims to correlate insulin resistance with soluble CD40 ligand (sCD40L) and plasminogen activator inhibitor-1 (PAI-1) plasma in pre-diabetic patients.

METHODS: This study is an analytic observational study with a cross-sectional approach. HOMA-IR assessed insulin resistance, and prothrombotic factors were assessed by PAI-1 and soluble CD40L. PAI-1 and sCD40L were examined by ELISA. These indicators were assessed on 30 pre-diabetic patients.

RESULTS: Thirty subjects included in this study with a mean age of 31.47 (5.03) years old, consist of 19 (63%) men and 11 (37%) women. The mean HOMA-IR was 3.69 (1.12), PAI-1 was 10.25 (3.72) ng/mL, and the PAI-1 levels were increased (>8.4 ng/mL) in 70% of the subjects. The mean of sCD40L levels was 4495.7 (1136.3) pg/ml, and sCD40L levels were increased (>4000 pg/ml) in 63% of subject. There was a significant correlation between HOMA-IR and sCD40L (r = 0.636, p < 0.05) and between of HOMA-IR and PAI-1 (r = 0.742, p < 0.05). Moderate correlation was found between sCD40L levels and plasma PAI-1 (r = 0.592, p = 0.05) in pre-diabetic patient. The correlation between these variables was HOMA-IR had a significant effect on PAI-1 levels through sCD40L (t = 2.01, p < 0.05, structure loading factor = 0.286).

CONCLUSION: Insulin resistance has a strong and significant correlation with sCD40L and PAI-1 levels in pre-diabetic patients.

Introduction

Pre-diabetes is the forerunner of diabetes. Pre-diabetes is defined as a condition when serum blood glucose is higher than the normal value but not higher enough for diabetes. The prevalence of pre-diabetes has increased in the last decade worldwide. The International Diabetes Federation in 2019 reported that the prevalence of pre-diabetes was 373.9 million or 7.5% of the adult population aged 20–79 years worldwide. In Indonesia, there was 29.1 million population with pre-diabetes in 2019 [1], [2], [3], [4].

The main condition causing pre-diabetes is insulin resistance and increased dysfunction of the beta-cell pancreas that can lead to a prothrombotic state. HOMA-IR can describe insulin resistance. Decroli et al. stated that increased insulin secretion compensates for increased HOMA-IR by beta-cell pancreas in pre-diabetic patients [4], [5], [6], [7]. Various studies have proven that in pre-diabetic patients, a prothrombotic state has taken place, which will cause micro- and macro-vascular complications [8], [9], [10], [11], [12].

Low-grade systemic inflammation in insulin resistance triggers hemostatic system disorders in many mechanisms, including increased platelet activation, increased coagulation, impaired fibrinolysis, and endothelial dysfunction. Chronic hyperglycemia and hyperinsulinemia increase CD40 ligand (CD40L) expression in circulating platelets. Soluble CD40L is causing platelet activation and aggregation, thereby promoting the growth and stability of thrombus formation [13], [14]. Clinically, sCD40L levels reflect increased platelet activation and correlate with thrombotic events, and various studies have reported it [15], [16], [17]. In the state of insulin resistance, hypofibrinolysis occurs due to an increase in plasminogen activator inhibitor-1 (PAI-1). PAI-1 is produced by endothelial cells and adipose tissue, and it has been considered a significant inhibitor of fibrinolysis in insulin resistance conditions [18], [19].
This study aims to correlate insulin resistance with soluble sCD40L and plasma PAI-1 in pre-diabetic patients.

Methods

This study was an analytic observational study with a cross-sectional approach. The research was conducted in Medical Faculty, Andalas University, Padang, Indonesia, for 6 months. The sample in this research is appropriate with inclusion and exclusion criteria. The sampling method used was random sampling. Inclusion criteria are pre-diabetic patients with insulin resistance and a family history of having type 2 diabetes mellitus. Exclusion criteria are patients with diabetes mellitus, pre-diabetes with medication, cerebrovascular disease, chronic kidney disease, autoimmune disease, sepsis, malignancy, hepatic cirrhosis, post-trauma, on antiplatelet medication, and antifibrinolytic drugs.

The insulin resistance levels can be assessed using several methods, one of which was assessing the value of HOMA-IR. Prothrombotic factors were assessed by PAI-1 and soluble CD40L.

Assessment of HOMA-IR value got from formula (fasting serum insulin [mIU/L]/22.5). Subjects who meet the criteria for pre-diabetes and HOMA IR >2 continue to assess PAI-1 and sCD40L.

Descriptive analysis was performed on the primary data, which included patient characteristics and laboratory examinations. The mean of HOMA-IR on pre-diabetic patients was 3.69 (1.12). The mean of sCD40L levels in pre-diabetic subjects was 4495.7 (1136.3), and triglyceride levels from the cutoff (<8,4 ng/mL). This is described in Table 1.

Descriptive analysis was performed on the primary data, which included patient characteristics and laboratory examinations. The mean of HOMA-IR on pre-diabetic patients was 3.69 (1.12). The mean of sCD40L levels in pre-diabetic subjects was 4495.7 (1136.3), and 63% of them had sCD40L levels greater than 4000 pg/ml, 37% obtained normal results. It means that not all pre-diabetes patients have high sCD40L levels, even though the average of sCD40L levels is above normal. In this study, 2/3 of pre-diabetes patients had sCD40L above normal.

Table 1: Characteristic of pre-diabetic patient

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n (%)</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Man</td>
<td>19 (63)</td>
<td></td>
</tr>
<tr>
<td>Woman</td>
<td>11 (37)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>21–30</td>
<td>11 (37)</td>
<td></td>
</tr>
<tr>
<td>31–40</td>
<td>19 (63)</td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>33.49 (4.90)</td>
<td></td>
</tr>
<tr>
<td>Weight</td>
<td>96.00 (17.57)</td>
<td></td>
</tr>
<tr>
<td>Height</td>
<td>169.10 (8.31)</td>
<td></td>
</tr>
<tr>
<td>Waist circumference&gt;90 cm for males and&gt;80 cm for females</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mmHg)</td>
<td>124.83 (10.38)</td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure (mmHg)</td>
<td>80.33 (6.15)</td>
<td></td>
</tr>
<tr>
<td>&lt;85 mmHg</td>
<td>24 (80)</td>
<td></td>
</tr>
<tr>
<td>&gt;85 mmHg</td>
<td>6 (20)</td>
<td></td>
</tr>
<tr>
<td>HbA1C</td>
<td>5.903 (0.20)</td>
<td></td>
</tr>
<tr>
<td>Fasting blood glucose (mg/dL)</td>
<td>90.67 (9.91)</td>
<td></td>
</tr>
<tr>
<td>Blood glucose 2 h OGTT (mg/dL)</td>
<td>109.77 (15.92)</td>
<td></td>
</tr>
<tr>
<td>Fasting serum insulin (mIU/L)</td>
<td>16.50 (4.76)</td>
<td></td>
</tr>
<tr>
<td>HDL cholesterol (mg/dL)</td>
<td>45.57 (8.37)</td>
<td></td>
</tr>
<tr>
<td>&gt;40 mg/dL in males and ≤50 mg/dL in female</td>
<td>21 (70)</td>
<td></td>
</tr>
<tr>
<td>&lt;40 mg/dL in males and ≤50 mg/dL in female</td>
<td>9 (30)</td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mg/dL)</td>
<td>134.13 (61.65)</td>
<td></td>
</tr>
<tr>
<td>≤150 mg/dL</td>
<td>21 (70)</td>
<td></td>
</tr>
<tr>
<td>&gt;150 mg/dL</td>
<td>9 (20)</td>
<td></td>
</tr>
</tbody>
</table>

Table 2: Level of PAI-1 and sCD40 ligand in pre-diabetic patient

<table>
<thead>
<tr>
<th>Variable</th>
<th>n=30</th>
<th>Mean (SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HOMA-IR</td>
<td>3.69 (1.12)</td>
<td></td>
</tr>
<tr>
<td>sCD40 ligand</td>
<td>4495.7 (1136.3)</td>
<td></td>
</tr>
<tr>
<td>≤4000 pg/ml</td>
<td>11 (37)</td>
<td></td>
</tr>
<tr>
<td>&gt;4000 pg/ml</td>
<td>19 (63)</td>
<td></td>
</tr>
<tr>
<td>PAI-1</td>
<td>10.25 (3.72)</td>
<td></td>
</tr>
<tr>
<td>≤8.4 mg/mL</td>
<td>9 (30)</td>
<td></td>
</tr>
<tr>
<td>&gt;8.4 mg/mL</td>
<td>21 (70)</td>
<td></td>
</tr>
</tbody>
</table>

From Figure 1a, the analysis showed that there was a significant correlation between HOMA-IR and sCD40L levels in pre-diabetic patients (p < 0.05) with a positive and strong correlation (r = 0.636, r² = 0.404).
Discussion

In this study, the mean age of the patients was 31.47 (5.03) years. This result was younger than the studies obtained. Rajput et al. reported that the mean age of people with pre-diabetes was 42.14 (11.77) [20]. Research by Salazar et al. reports that the mean age is 55 (14) [21]. The study of Andes et al. in line with our study, Andes reported that one in four young adults (19–34 years) in the United States suffers from pre-diabetes where the number of men is twice that of women. Andes also reported obesity as a strong predictor of an increased risk of pre-diabetes in young adults [22]. In this study, 100% of the study samples suffered from central obesity (>90 cm for men and >80 cm for women). The study of Rajput et al. reported >80% of pre-diabetes with central obesity. Rajput et al. also concluded that an increase in waist circumference had a strong predictive value for the occurrence of metabolic syndrome among pre-diabetic subjects. Waist circumference is more correlated with regional body fat distribution than BMI [20]. Overall, 40% of the samples had hypertension in pre-diabetic patients. Mandob and Sabine reported that 59% of pre-diabetic patients suffer from hypertension [23].

The average HbA1C in this study was 5.90 (0.2), with a mean of FBG 90.67 (9.91) mg/dL and a postprandial blood glucose of 109.77 (15.92). HbA1c measurement had 49% sensitivity and 79% specificity for pre-diabetes, while FBG had 25% sensitivity and 94% specificity. HbA1C alone identified 14% of individuals diagnosed with impaired glucose tolerance (IGT), 9% with impaired fasting glycemia, and 33% with both disorders [24]. In this study, there is a discrepancy between the results of the HbA1C examination with the results of FBG and postprandial blood glucose. This can be caused by many mechanisms, including hemoglobin variants, differences in erythrocyte age, iron levels, and distribution of glucose across the erythrocyte membrane. In pre-diabetes, the measurement of FBG and postprandial blood glucose alone is not describe the actual situation of plasma blood glucose, because in pre-diabetes patients, there is often a sudden increase and decrease in blood sugar due to an unhealthy diet, so the HbA1C examination can represent a longer blood glucose state.

In this study, 30% of pre-diabetic patients suffered from dyslipidemia. The results of this study are in line with the research of Benjamin et al. (2016) where the mean triglycerides in pre-diabetes are 143.43 (35.49) and HDL is 48.39 (15.66) [18]. Research by Gupta et al. (2018) found that the mean triglycerides were 164.2 (3.4) and the mean HDL for males was 45.7 (0.5) and females was 53.9 (0.9) [25]. Rajput et al. reported 36% of pre-diabetic patients with low HDL levels and 68% with triglycerides >150 mg/dL [20].
Overall, we found that many subjects in this study suffer from metabolic syndrome. The incidence of metabolic syndrome in the study of Rajput et al. was much higher where it was found in 63% of pre-diabetic subjects [20]. Similar study by Ahsan et al. was conducted in Karachi, Pakistan, where metabolic syndrome was found in 57, 5% of the subjects had pre-diabetes [26]. The most common abnormality among pre-diabetic subjects was an increase in waist circumference/central obesity, accompanied by elevated triglycerides and low HDL values. Therefore, metabolic syndrome has emerged when pre-diabetes occurs. Metabolic syndrome is associated with atherogenic dyslipidemia, pro-inflammatory states, and risk factors for cardiovascular disease.

Insulin resistance started a dysglycemic state in pre-diabetes. In this study, we found that mean HOMA-IR level was 3.69 (1.12) in the pre-diabetic patient. This is in line with the research of Salazar and Gupta. Salazar et al. reported that the mean HOMA-IR in pre-diabetes was 3.4 (1.7) [21]. Gupta et al. reported that the mean HOMA IR level was 3.7 (0.1) and significantly increased in all pre-diabetic subjects with p < 0.001 [25]. In this study, a high mean of HOMA-IR was found due to many factors of the metabolic syndrome. This factor affects HOMA-IR levels, such as obesity, hypertension, and dyslipidemia.

The mean of sCD40L level was 4495.7 (1136.3) pg/ml in pre-diabetic patients, and 63% of them had sCD40L levels increased more than 4000 pg/ml. Gateva et al. in 2016 reported significantly higher levels of sCD40L in patients with insulin resistance compared to those without (6.4 ± 3.7 vs. 4.1 ± 2.4 ng/ml, p = 0.025) and only a tendency toward higher levels in pre-diabetes compared to normoglycemic patients (5.9 ± 3.6 vs. 5.3 ± 3.4 ng/ml) [27]. Linnà et al. also reported that the mean level of sCD40L was significantly higher in the IGT group than the non-IGT group at the baseline (0.42 vs. 0.27 ng/mL) [28]. The results of the study were a significant correlation between HOMA-IR levels and sCD40L levels in pre-diabetic patients (p < 0.05) with a positive correlation direction and strong correlation (r = 0.636). This increase indicates that at the pre-diabetes stage, there has been an increase in prothrombotic factors, in this case, sCD40L. Soluble CD40L is expressed on activated platelets. In a state of dysglycemia, platelets become hyperactive which is characterized by an increased in platelet adhesion, activation, and aggregation. Hyperactive platelets are a major determinant of the prothrombotic state. CD40L plays a key role in the pathophysiology of multicellular vascular events such as thrombosis, inflammation, and atherosclerosis [29], [30]. The value of r square in the study was 0.404, which means that HOMA-IR levels affected sCD40L by 40%, while other factors influenced the rest. sCD40L levels are influenced by factors that affect platelets, such as infection, ischemia, autoimmune, malignancy, tissue injury, or trauma [31].

From this study, the mean level of PAI-1 in pre-diabetic patients was 10.25 (3.72). Based on the previous research, Benyamin et al. explained that the cutoff limit for higher levels of PAI-1 activity was >7 IU/ml (8.4 ng/ml). In our study, PAI-1 levels of more than 8.4 ng/mL were found in 70% of subjects. Benyamin also reports from his research that PAI-1 activity was significantly higher in subjects with IGT, IFBG, and IFBG than NGT (p = 0.024) [18]. A significant and strong correlation between HOMA-IR and PAI-1 levels in pre-diabetic patients was found (r = 0.742, p < 0.05). Increased HOMA-IR in pre-diabetic patients causes an increase in PAI-1 levels. PAI-1 was one of the prothrombotic factors. Another study that discussed the correlation between insulin resistance and PAI-1 was by Laryushina et al. in 2021. Laryushina reported a correlation between HOMA-IR and PAI-1 plasma (r = 0.59, p < 0.001) [32]. In insulin resistance conditions, low-grade systemic inflammation causes impaired fibrinolysis and increased prothrombotic factors, especially PAI-1 levels. Insulin resistance stimulates gene transcription upstream of the PAI-1 transcription start site. The most common cause of high plasma PAI-1 concentrations in the general population is insulin resistance syndrome [31]. The value of r square in this study was 0.550, which means that the level of HOMA-IR affects PAI-1 by 55%, and other factors influenced 45%. PAI-1 was an acute-phase reactant, which was influenced by many factors, including pro-inflammatory cytokines (IL-1, IL-6, and TNF-α), growth factors (tissue growth factor-β [TGF-β]), and hormones (insulin, glucocorticoids, and adrenaline) [31].

The analysis showed that there was a significant correlation between sCD40L levels and PAI-1 (p < 0.05) with a moderate correlation (r = 0.592). So far, we have not found a correlation between sCD40L levels and PAI-1 in pre-diabetic patients. PAI-1 can be affected by pro-inflammatory cytokines (IL-1, IL-6, and TNF-α). Besides being directly affected by pro-inflammatory cytokines, PAI-1 is affected by cytokines and chemokines produced by the binding of CD40L to its target cells [29], [30], [31], [32].

There was a significant correlation between the three variables where HOMA-IR gave a significant positive effect on PAI-1 levels through sCD40L (t = 2.010, p < 0.05). These three relationships’ determinant coefficient SLF is 0.286. In other words, HOMA-IR, which affects the results of PAI-1 through sCD40L, is 28%. In contrast, the remaining 72% is influenced by other factors. These two prothrombotic biomarkers are influenced by many aspects but are still activated simultaneously due to an increase in the inflammatory state caused by insulin resistance [27], [28], [32].

After detecting an increase in plasma sCD40L and PAI-1 levels in pre-diabetic patients, clinicians should increase their awareness of the thrombotic risk in pre-diabetes. Once a diagnosis of pre-diabetes is made, prothrombotic factor testing is recommended to prevent the worsening of prothrombotic levels.
Conclusion

Insulin resistance has a strong and significant correlation with sCD40L and PAI-1 levels in pre-diabetic patients.

References

PMid:30740170.
PMid:30007840.
PMid:31766914.
PMid:30863079.
PMid:27840354.
PMid:28279217.
PMid:31305207.
PMid:26802220.
PMid:31790544.
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