Scientific Foundation SPIROSKI, Skopje, Republic of Macedonia
Open Access Macedonian Journal of Medical Sciences. 2022 Feb 15; 10(B):351-356.
https://doi.org/10.3889/oamjms.2022.8202
eISSN: 1857-9655
Category: *B - Clinical Sciences*
Section: *Endocrinology*

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

Eva Decroli[1]*, Rudy Afriant[2], Alexander Kam[1], Upi Puspita[3]

[1]Department of Internal Medicine, Endocrinology and Metabolic Subdivision, Medical Faculty, Andalas University, Dr. M. Djamil General Hospital, Padang, Indonesia; [2]Department of Internal Medicine, Hematology-Oncology Subdivision, Medical Faculty, Andalas University, Dr. M. Djamil General Hospital, Padang, Indonesia; [3]Depatment of Internal Medicine, Medical Faculty, Andalas University, Dr. M. Djamil General Hospital, Padang, Indonesia

Edited by: Ksenija Bogoeva-Kostovska
Citation: Decroli E, Afriant R, Kam A, Puspita U. Correlation between Insulin Resistance with Soluble CD40 Ligand and Plasminogen Activator Inhibitor-1 Plasma in Pre-diabetic Patients. Open-Access Maced J Med Sci. 2022 Feb 15; 10(B):351-356. https://doi.org/10.3889/oamjms.2022.8202
Keywords: Insulin resistance; HOMA IR; sCD40 ligand; PAI-1; Pre-diabetes
***Correspondence:** Eva Decroli, Department of Internal Medicine, Dr. M. Djamil General Hospital/ Medical Faculty, Andalas University, Padang, Indonesia. E-mail: evadecroli@med.unand.ac.id
Received: 07-Dec-2021
Revised: 27-Jan-2022
Accepted: 05-Feb-2022

Funding: This study was supported by Andalas University through LPPM (Contract No. 16/UN.16.02/Fd/PT.01.03/2021)
Competing Interest: The authors have declared that no competing interest exists

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 levels 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 three variables was HOMA-IR had a significant effect on PAI-1 levels through sCD40L ($t = 2.010$, $p < 0.05$, 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 blood glucose [FBG] [mg/dl] × 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 basic data, including patient characteristics and laboratory examinations. Numerical data are displayed in the form of mean and standard deviation. Categorical variables are expressed in terms of frequency and percentage. The numerical data were tested for normality using the Shapiro–Wilk test, and Spearman correlation was performed. The data were processed computerized. The statistical test used for correlation between HOMA-IR and PAI-1 levels through sCD40L was path analysis. In analyzing the path diagram structure, SmartPLS version 3.0 software was used.

Results

There were 30 samples with a mean age of 31.47 (5.03) years old, consisting of 19 (63%) men and 11 (37%) women. The mean body mass index (BMI) was 33.49 (4.90) kg/m^2. In this study, 100% of subjects had central obesity, with the mean waist circumference was 112.47 (14.95). The mean of HbA1C was 5.90 (0.20), FBG was 90.67 (9.91) mg/dl, blood glucose level 2 h after oral glucose tolerance test (postprandial blood glucose) was 109.77 (15.92) mg/dl, fasting serum insulin was 16.50 (4.758) IU/ml, HDL mean level was 45.57 (8.374) mg/dl, and triglycerides were 134.13 (61.65) mg/dl. The subjects in this study were pre-diabetic patients with central obesity, we got 30% of the subjects had dyslipidemia, and 40% had hypertension. This is described in Table 1.

Table 1: Characteristic of pre-diabetic patient

Characteristic	n (%)	Mean (SD)
Sex		
Man	19 (63)	
Woman	11 (37)	
Age (years)		31.47 (5.03)
21–30	11 (37)	
31–40	19 (63)	
Body mass index (kg/m^2)		33.49 (4.90)
Weight		96.00 (17.07)
Height		169.10 (8.31)
Waist circumference>90 cm for males and>80 cm for females	100	112.47 (14.95)
Systolic blood pressure (mmHg)		124.83 (10.38)
<130 mmHg	18 (60)	
>130 mmHg	12 (40)	
Diastolic blood pressure (mmHg)		80.33 (6.15)
<85 mmHg	24 (80)	
>85 mmHg	6 (20)	
HbA1C		5.903 (0.20)
Fasting blood glucose (mg/dL)		90.67 (9.91)
Blood glucose 2 h OGTT (mg/dL)		109.77 (15.92)
Fasting serum insulin (mIU/L)		16.50 (4.76)
HDL cholesterol (mg/dL)		45.57 (8.37)
≥40 mg/dL in males and ≥50 mg/dL in female	21 (70)	
≤40 mg/dL in males and ≤50 mg/dL in female	9 (30)	
Triglycerides (mg/dL)		134.13 (61.65)
<150 mg/dL	21 (70)	
≥150 mg/dL	9 (30)	

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.

PAI-1 levels of more than 8,4 ng/mL (7 IU/ml) were found in 70% of subjects, where the average PAI level was 10.25 (3.72). This PAI-1 cutoff describes PAI-1 activity and the reference value was 1–7 IU/ml. The PAI-1 activity was defined as the levels of active free PAI-1. About 30% pre-diabetes patients had low PAI-1 levels from the cutoff (<8,4 ng/mL). This is described in Table 2.

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

Variable	n=30	
	n (%)	Mean (SD)
HOMA-IR		3.69 (1.12)
sCD40 ligand		4495.7 (1136.3)
≤4000 pg/ml	11 (37)	
≥4000 pg/ml	19 (63)	
PAI-1		10.25 (3.72)
≤8,4 ng/ml	9 (30)	
≥8,4 ng/ml	21 (70)	

*Cutoff point: PAI-1 was <7 IU/ml (8.4 ng/ml) (Benyamin *et al*., 2016) and sCD40: 62.5–4000 pg/ml (R and D Systems).[11,15]

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 ($r = 0.636$, $r^2 = 0.404$).

Figure 1b shows that there was a strong and significant correlation between levels of HOMA-IR and PAI-1 in pre-diabetic patients ($r = 0.742$, $r^2 = 0.550$, $p < 0.05$). Based on Figure 1c, there was a moderate and significant correlation between levels of sCD40L and PAI-1 in pre-diabetic patients ($r = 0.592$, $r^2 = 0.350$, $p < 0.05$).

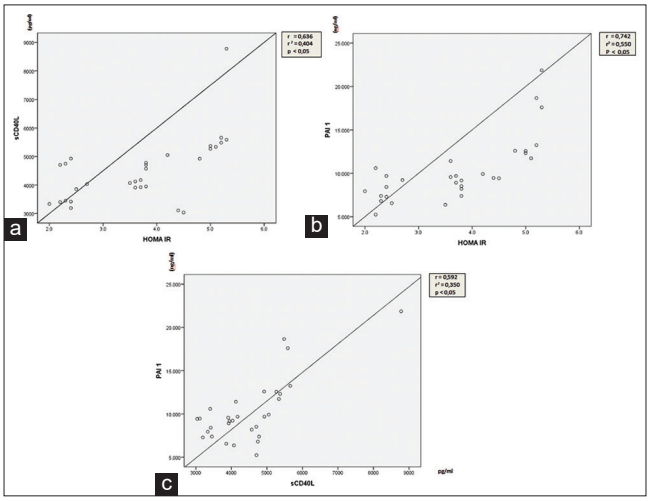

Figure 1: Graph of correlation (a) between HOMA-IR with CD40 ligand (sCD40L) levels, (b) between HOMA-IR with plasminogen activator inhibitor-1 (PAI-1) levels, (c) between sCD40L with PAI-1 levels

The path diagram model of the correlation between three variables is shown in Figure 2, while the detailed data collection for variables and indicators is shown in Table 3.

Table 3: The correlation between HOMA-IR levels with sCD40L and PAI-1 levels in pre-diabetic patients

Hypothesis	T-statistics	p-value	SLF
HOMA-IR→sCD40L	5.189	<0.001*	
HOMA-IR→PAI-1	2.849	0.020*	
sCD40L→PAI-1	2.325	<0.001*	
HOMA-IR→sCD40L®PAI-1	2.010	0.045*	0.286

*T-statistic>1.96 significant; P<0.05 significant, SLF: Structure loading factor, sCD40L: sCD40 Ligand, PAI-1: Plasminogen activator inhibitor-1.

From the structural model of three variables, 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). The determinant coefficient structure loading factor (SLF) of these three correlations is 0.286.

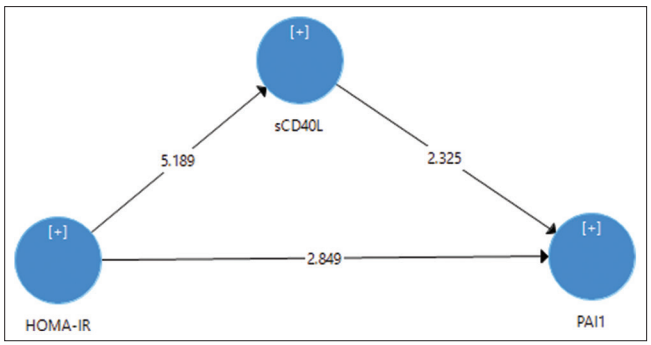

Figure 2: Path analysis diagram between HOMA-IR with sCD40 ligand and plasminogen activator inhibitor-1 in pre-diabetic patients

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-diabetes 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]. Linna *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

1. IDF Diabetes Atlas. International Diabetes Federation (IDF). 9th ed. Brussels, Belgium: IDF; 2019.
2. Perkeni, Konsensus Pengelolaan dan Pencegahan Diabetes Melitus Tipe 2 di Indonesia; 2019.
3. Decroli E, Kam A, Dillasamola D. The percentage of depressive symptoms in patients with Type 2 Diabetes Mellitus in M Djamil General Hospital Padang, Indonesia. J Res Pharm. 2019;23(2):292-7.
4. Decroli E, Efendi YP, Kam A, Manaf A, Syahbuddin S. Description of Insulin Resistance and Beta Cell Pancreas Dysfunction in Prediabetic Patients, ICoMHER; 2018. https://doi.org/10.4108/eai.13-11-2018.2283632
5. Ghani M, Ralph A. Pathophysiology of prediabetes. Curr Diab Rep. 2009;9(3):193-9.
6. Decroli E, Manaf A, Syahbuddin S, Waspadji S, Dillasamola D. The role of survivin and raf-1 kinase against enhancement of pancreatic beta-cell apoptosis in patients with Type 2 diabetes mellitus. Asian J Pharm Clin Res. 2018;11(11):344-7. https://doi.org/10.22159/ajpcr.2018.v11i11.27671
7. Decroli E, Manaf A, Syahbuddin S, Syafrita Y, Dillasamola D. The correlation between malondialdehyde and nerve growth factor serum level with diabetic peripheral neuropathy score. Open Access Maced J Med Sci. 2019;7(1):103-6. https://doi.org/10.3889/oamjms.2019.029
   PMid:30740170
8. Kleinherenbrinka W, Oseib E, den Hertogc HM, Zandbergen AA. Prediabetes and macrovascular disease: Review of the association, influence on outcome and effect of treatment. Eur J Intern Med. 2018;55:6-11. https://doi.org/10.1016/j.ejim.2018.07.00
   PMid:30007840
9. Beulens JW, Rutters F, Ryden L, Schnell O, Mellbin L, Hart HE, *et al.* Risk and management of pre-diabetes. Eur J Prev Cardiol. 2019;26(2S):47-54. https://doi.org/10.1177/2047487319880041
   PMid:31766914
10. Ciccone MM, Scicchitano P, Cameli M, Cecere A, Cortese F, Dentamaro I, *et al.* Endothelial function in pre-diabetes, diabetes and diabetic cardiomyopathy: A review. J Diabetes Metab. 2014;5(4):1-10.
11. Kacker S, Saboo N, Sorout J. Prediabetes: Pathogenesis and adverse outcomes. Int J Med Res Prof. 2018;4(2):1-6.
12. Brannick B, Wynn A, Dagogo-Jack S. Prediabetes as a toxic environment for the initiation of microvascular and macrovascular complications. Exp Biol Med. 2016;241:1323-31.
13. Michel NA, Zirlik A, Wolf D. CD40L and its receptors in atherothrombosis-an update, front. Cardiovasc Med. 2017;4:1-18.
14. Santilli F, Simeone P, Liani R. The Role of Platelets in Diabetes Mellitus. Department of Medicine and Aging and Haemostasis and Thrombosis Unit. Amsterdam, Netherlands: Elsevier; 2019. p. 469-503.
15. Pusuroglu H, Akgul O, Erturk M, Uyarel H, Bulut U, Akkaya E, *et al.* Predictive value of elevated soluble CD40 ligand in patients undergoing primary angioplasty for ST-segment elevation myocardial infarction. Coron Artery Dis. 2014;25(7):558-64.
16. Li J, Wang Y, Lin J, Wang D, Wang A, Zhao X, *et al.* Soluble CD40L is a useful marker to predict future strokes in patients with minor stroke and transient ischemic attack. Stroke. 2015;46(7):1990-2.
17. Tony AA, Tony EA, Mohammed WS, Kholef EF. Evaluation of plasma levels of neopterin and sCD40L in patients with acute ischemic stroke in upper Egypt: Can they surrogate the severity and functional outcome? Neuropsychiatr Dis Treatment. 2019;15:575-86. https://doi.org/10.2147/NDT.S177726
   PMid:30863079
18. Benyamin AF, Minhajat R, Bayu D, Saleh S, Harjianti T. Association between PAI-1 activity levels and t-PA antigen with glycemic status in prediabetic population. Acta Med Indones. 2016;48(3):200-6.
   PMid:27840354
19. Kearney K, Tomlinson D, Smith K, Ajjan R. Hypofibrinolysis in diabetes: A therapeutic target for the reduction of cardiovascular risk. Cardiovasc Diabetol. 2017;16(1):34. https://doi.org/10.1186/s12933-017-0515-9
   PMid:28279217
20. Rajput R, Rajput M, Mishra S, Ahlawat P. Prevalence of metabolic syndrome in prediabetes. Metab Syndr Relat Disord. 2019;17(8):406-10. https://doi.org/10.1089/met.2019.0010
   PMid:31305207
21. Salazar M, Horacio A, Walter G, Marcelo A, Carlos E, Betty C, *et al.* Insulin resistance: The linchpin between prediabetes and cardiovascular disease. Diab Vasc Dis Res. 2016;13(2):157-63. https://doi.org/10.1177/1479164115610057
   PMid:26802220
22. Andes LJ, Cheng YJ, Rolka DB, Gregg EW, Imperatore G. Prevalence of prediabetes among adolescents and young adults in the United States, 2005-2016. JAMA Pediatr. 2020;174(2):e194498. https://doi.org/10.1001/jamapediatrics.2019.4498
   PMid:31790544
23. Mandob DE, Sabine P. Metabolic syndrome prevalence among prediabetic and normoglucotolerant women. J Diabetes Metab. 2016;7:1-5.
24. Barbu E, Popescu MR, Popescu AC, Balanescu SM. Phenotyping the prediabetic population-a closer look at intermediate glucose status and cardiovascular disease. Int J Mol Sci. 2021;22(13):6864. https://doi.org/10.3390/ijms22136864
   PMid:34202289
25. Gupta AK, Menon A, Brashear M, Johnson WD. Prediabetes: Prevalence, pathogenesis, and recognition of enhanced risk. In: Nutritional and Therapeutic Interventions for Diabetes and Metabolic Syndrome. Vol. 2. Amsterdam, Netherlands: Elsevier; 2018. p. 15-32.
26. Ahsan S, Ahmed SD, Jamali SN, Imran M, Haque MS, Qasim R. Frequency and risk of metabolic syndrome in prediabetics versus normal glucose tolerant subjects: A comparative study. J Pak Med Assoc. 2015;65(5):496-500.
   PMid:26028383
27. Gateva A, Assyov Y, Tsakova A, Kamenov Z. Soluble CD40L is associated with insulin resistance, but not with glucose tolerance in obese nondiabetic patients. Arch Physiol Biochem. 2016;122(3):161-5. https://doi.org/10.3109/13813455.2016.1160934
   PMid:26934129
28. Linna H, Suija K, Rajala U, Herzig KH, Karhu T, Jokelainen J. The association between impaired glucose tolerance and soluble CD40 ligand: A 15-year prospective cohort study. Aging Clin Exp Res. 2016;28(6):1243-9. https://doi.org/10.1007/

s40520-015-0524-z

PMid:26749117

29. Michel NA, Zirlik A, Wolf D. CD40L and its receptors in atherothrombosis-an update. Front Cardiovasc Med. 2017;4:40. https://doi.org/10.3389/fcvm.2017.00040

PMid:28676852

30. Yuan M, Fu H, Ren L, Wang H, Guo W. Soluble CD40 ligand promotes macrophage foam cell formation in the etiology of atherosclerosis. Cardiology. 2015;131(1):1-12. https://doi.org/10.1159/000374105

PMid:25825037

31. Grant PJ. Diabetes mellitus as a prothrombotic condition. J Intern Med. 2007;262(2):157-72. https://doi.org/10.1111/j.1365-2796.2007.01824.x

PMid:17645584

32. Laryushina Y, Parakhina V, Turgunova L, Sheryazdanova D, Dosmagambetova S, Turmukhambetova A, *et al.* Association of plasminogen activator inhibitor-1 and cardiovascular events development in patients with prediabetes. Open Access Maced J Med Sci. 2021;9(B):726-33.