Original Article
Turk J Endocrinol Metab. 2021;25:174-183

# Plasma Levels of Soluble P-Selectin, Beta-Thromboglobulin and Platelet Indices in Patients with Prediabetes: Effects of Acute Hyperglycemic Stress

## Prediyabetik Hastalarda Oral Glukoz Yüklemesinin Trombosit İndeks Değerleri ile Plazma P-Selektin ve Beta-Tromboglobülin Düzeyleri Üzerine Akut Etkisinin Araştırılması

Nesibe Esra YAŞAR, Dildar KONUKOĞLU

Department of Medical Biochemistry, İstanbul University-Cerrahpaşa Faculty of Medicine İstanbul, TURKEY

*This study was presented and poster at the Association of Clinical Biochemistry Specialists, International Kongre&Lab Expo, 2-5 October 2019, Sapanca,* Turkey

## Abstract

**Objective:** To examine the effects of acute hyperglycemic stress on platelet activation by analyzing the plasma levels of P-selectin, beta-thromboglobulin, and platelet indices in an oral glucose tolerance test (OGTT). **Material and Methods:** The OGTT results were used to form four study groups: a control group (n=31) and a prediabetic group (n=103) comprising three subgroups according to the American Diabetes Association criteria for diabetes. Platelet count (PLT), mean platelet volume (MPV), platelet distribution width (PDW), and plateletcrit (PCT) were determined using the impedance method, and P-selectin and beta-thromboglobulin (β-TG) levels were determined by enzyme-linked immunosorbent assay. P<0.05 was considered significant. **Results:** No significant difference was observed in the baseline values of PLT, platelet indices, or plasma P-selectin level between the control and prediabetic groups. The baseline plasma β-TG level was significantly higher in the prediabetic group than in the control group (p<0.001). PLT, PCT and P-selectin levels significantly reduced during OGTT in both control (p<0.05 for each) and prediabetic groups (p<0.001 for each). The reduction in PCT (3.88%) and PLT (3.18%) after glucose loading was greater in the prediabetic group than in the control group (2.04% and 1.44%, respectively). The MPV decreased in the prediabetic group (p<0.05), while no significant decrease was observed in the control group. **Conclusion:** The platelet indices do not change in the prediabetic stage and the initiation of granule activation is independent of platelet morphology. Acute hyperglycemia appears to affect platelet morphology and function in both healthy and prediabetic subjects, and can be marked in prediabetic individuals.

**Keywords:** Platelet Activation; mean platelet volume; prediabetic state; hyperglycemia

## Özet

**Amaç:** Çalışmamızın amacı, prediyabetik hastalarda plazma P-selektin ve beta-tromboglobülin düzeyleri ile trombosit indekslerinin ilişkisinin incelenmesi ve oral glukoz yüklemesi ile oluşturulan akut hiperglisemik stresin trombosit aktivasyon belirteçleri üzerine etkisinin araştırılmasıdır. **Gereç ve Yöntemler:** Amerikan Diyabet Derneği kriterlerine göre; oral glukoz tolerans testi sonuçları baz alınarak kontrol (n=31) ve prediyabet (n=103) grupları oluşturuldu. Oral glukoz yüklemesinden önce ve yüklemenin 2. saatinde alınan kan örneklerinde trombosit sayısı [platelet count (PLT)], ortalama trombosit hacmi [mean platelet volume (MPV)], trombosit dağılım genişliği ve plateletkrit [plateletcrit (PCT)] impedans yöntemi ile P-selektin ve beta-tromboglobülin düzeyleri "Enzyme Linked İmmunabsorbent Assay" yöntemi ile belirlendi. İstatistiksel anlamlılık p<0,05 olarak değerlendirildi. **Bulgular:** Trombosit sayısı ve indeksleri ile plazma, P-selektin bazal değerleri açısından kontrol ve prediyabet grupları arasında anlamlı farklılık saptanmadı. Bazal plazma beta-tromboglobülin düzeyleri prediyabet grubunda, kontrol grubuna göre anlamlı yüksek bulundu (p<0,001). Glukoz yüklemesi sonrasında PLT, PCT ve P-selektin değerleri kontrol (her biri için p<0,05) ve prediyabet (her biri için p<0,001) gruplarında bazal değerlere göre anlamlı olarak azaldı. PCT ve PLT'deki azalma oranı prediyabetik grupta (%3,88 ve %3,18) kontrol grubuna (%2,04 ve %1,44) göre daha belirgindi. Prediyabet grubunda kontrol grubundan farklı olarak MPV'de de anlamlı azalma gözlendi (p<0,05). **Sonuç:** Çalışmamızın sonuçları, prediyabetik dönemde trombosit büyüklüğü henüz etkilenmeden granül aktivasyonunun başladığını ve trombosit indekslerinin prediyabetik dönemde değişmediğini düşündürmektedir. Akut hiperglisemi ise hem sağlıklı hem prediyabetik kişilerde, trombosit morfolojisi ve fonksiyonlarını etkilemektedir; bu etkilenme prediyabetik bireylerde daha belirgin olmaktadır.

**Anahtar kelimeler:** Trombosit aktivasyonu; ortalama trombosit hacmi; prediyabet; hiperglisemi

**Address for Correspondence:** Nesibe Esra YAŞAR, Republic of Turkey, Ministry of Health Banaz Public Hospital, Uşak, TURKEY
**Phone:** :+905534617049 **E-mail:** esrayasar2014@hotmail.com

Peer review under responsibility of Turkish Journal of Endocrinology and Metabolism.

**Received:** 13 Oct 2020 **Received in revised form:** 12 Feb 2021 **Accepted:** 03 Mar 2021 **Available online:** 23 Mar 2021



DOI:10.25179/tjem.2020-79546

## Introduction

Prediabetes is defined as a high fasting plasma blood glucose level but less than the limit defined as diabetes (1). In the prediabetic stage, patients have an impaired fasting glucose (IFG) and/or impaired glucose tolerance (IGT).

{Barbour Fernandes, 2018 #79}Type 2 diabetes mellitus (DM) develops in nearly 70% of long-term follow-up prediabetic patients. The risk of developing diabetes among prediabetic patients is approximately 5% to 10% (1). Hyperglycemia may have a role in the development of atherosclerosis through several mechanisms (2). The risk of conditions related to cardiovascular diseases such as obesity, dyslipidemia, and hypertension is frequently monitored in the prediabetic period (3-5).

Platelets play a crucial role in the development of atherogenesis and thrombus (6). Changes in platelet morphology and function have been shown to be associated with atherosclerosis and thrombus formation in conditions such as diabetes, acute coronary syndrome, sepsis, and stroke (7-11). Carbohydrate-rich nutrition and glucose loading can lead to platelet activation (12, 13), indicating that platelet functions may be affected in the prediabetic period.

Platelet indices, which reflect platelet morphology, can be determined in a routine complete blood count with no additional cost. The mean platelet volume (MPV) indicates the average platelet size, while platelet distribution width (PDW) reflects the heterogeneity of platelet size. Plateletcrit (PCT) is the percentage of blood coated by platelets (14). Larger platelets are more reactive, can express more surface molecules, and contain more secretory granules (15). Therefore, platelet indices, especially MPV, may be a marker of platelet activation (16).

P-selectin (P-sel), or CD62P, is a glycoprotein adhesion molecule stored in both the Weibel-Palade bodies of endothelial cells and the alpha-granules (α-granules) of platelets (17). After platelet activation, P-sel is rapidly translocated from the alpha-granule to the membrane and mediates leukocyte-platelet or leukocyte-endothelial cell adhesion. It is also released into circulation in a soluble form (sP-sel) (18). The plasma concentration of sP-sel increases in several inflammatory and thrombotic diseases and is also accepted as an indicator of increased cardiovascular risk (11, 19, 20).

One of the markers of platelet activation is beta-thromboglobulin (β-TG). β-TG is stored in alpha granules of platelets; it is an 81 amino acid tetrameric protein derived from the proteolytic cleavage of the platelet basic protein (21). Although β-TG is reported to have chemotactic activity for fibroblasts, the physiological effects are not yet fully understood. Recent studies have revealed that β-TG acts as a pro-coagulant by both increasing thrombin formation and regulating the factor X enzymatic activity (22).

Technological developments have enabled accurate measurement of platelet indices with hematology analyzers. The easy, rapid, and inexpensive detection of platelet indices has created a research area for studying diseases using these indices. Among these markers, the majority of studies have focused on MPV. Accurately measured platelet size is considered to be a good indicator of platelet function (23). On the other hand, sP-sel and β-TG, which are released from α-granules by platelet activation, affect the development and progression of the atherosclerotic process (17, 22, 24). The role of platelets in the development of vascular complications in diabetic patients has been discussed in the literature (25, 26). The present study investigates the effect of acute hyperglycemia induced with an oral glucose tolerance test (OGTT) on platelet function and activation in prediabetic patients.

## Material and Methods

### Subjects

In this cross-sectional study, the patient group comprised individuals who had undergone an OGTT between March 2017 and March 2018 at the Cerrahpaşa Medical Faculty Fikret Biyal Medical Biochemistry Laboratory. The study was conducted in accordance with the Helsinki Declaration of principles and approved by the Ethics Committee of İstanbul University-Cerrahpaşa, Cerrahpaşa Medical Faculty (date: 22.12.2016/ number: 68871907-604.01.01-462235). The study was explained verbally to all of the participants and written consent was obtained.

The patients were classified according to American Diabetes Association criteria. Patients who had a fasting plasma glucose (FPG) level of <100 mg/dL and an OGTT 2-hour plasma glucose (PG) level <140 mg/dL were classified as the control group (n=31). The prediabetic groups included the IFG group, an FPG level of 100-125 mg/dL and a 2-hour PG value <140 mg/dL (n=60); the IGT group, an FPG level <100 mg/dL and a 2-hour PG value of 140-199 mg/dL (n=14); and the IFG+IGT group, an FPG level of 100-125 mg/dL and a 2-hour PG level of 140-199 mg/dL (n=29).

The individuals included in the study did not have Type 1 or Type 2 DM, any malignancy, cardiovascular disease, chronic or acute inflammatory disease, kidney disease, severe anemia (hemoglobin <9 g/dL), or platelet count (PLT) disorder (PLT<100.000\mm$^3$ or >400.000\mm$^3$). The subjects did not use treatment agents (anticoagulant, antiaggregant, antihypertensive, oral contraceptive, or statin) and smoked no more than five cigarettes a day.

### Laboratory Analyses

A 75-g OGTT was performed between 8:00 and 10:00 am after 12 hours of fasting. Venous blood samples were collected into tubes containing anticoagulants ($K_3$ EDTA, 2 mL, 367836; Becton, Dickinson and Company, Franklin Lakes, NJ, USA) and serum separator tubes (SST II, 5 mL, 367953; Becton, Dickinson and Company, Franklin Lakes, NJ, USA) before and after 2-hour oral glucose testing.

PLT, MPV, PDW and PCT were determined by impedance method, within 2 hours in ethylenediaminetetraacetic acid (EDTA) whole blood (Unicel DxH 800; Beckman Coulter, Inc., Brea, CA, USA). A platelet histogram was obtained using a volume size of 2-30 fL. The mean of the curve was determined as MPV and the distribution width at the level of 20% was defined as PDW. PCT was calculated using the formula PLT * MPV / 100. The inter-assay coefficients of variation (CV) during the 12 months study period were in the range of 1.98-3.72%, 0.74-0.96%, 0.49-0.73%, and 2.05-4.2% for PLT, MPV, PDW, and PCT, respectively.

After the blood tubes containing EDTA were centrifuged at 5000 rpm for 5 minutes, the plasma samples were frozen and stored at <-20°C until the sP-sel and β-TG analysis were analyzed.

Plasma sP-sel (Human P-sel PicoKine ELISA Kit, EK0505; Boster Biological Technology, Pleasanton, CA, USA) and β-TG levels (Finetest Human β-TG ELISA Kit, EHO874; Wuhan Fine Biotech Co., Ltd., Wuhan, China) were measured using the sandwich enzyme-linked immunosorbent assay (ELISA) method. Intra-assay and inter-assay CVs were 5.8% and 4.2% for sP-sel and <8% and <10% for β-TG.

Plasma glucose (hexokinase method), serum urea (enzymatic-kinetic method), creatinine (kinetic Jaffe method), C-reactive protein (CRP; immunoturbidimetric method), total cholesterol, low-density lipoprotein cholesterol and high-density lipoprotein (LDL and HDL cholesterol; enzymatic-colorimetric method), and triglycerides (enzymatic-colorimetric method) levels were assayed using an autoanalyzer (Cobas c702; Roche Diagnostics, Basel, Switzerland). The serum insulin level was determined using the chemiluminescence method (Cobas c602; Roche Diagnostics, Basel, Switzerland). Glycated hemoglobin (HbA1c) was determined with the high-performance liquid chromatography method (Variant II Turbo; Bio-Rad Laboratories, Inc., Hercules, CA, USA). Body mass index (BMI) was calculated as the weight divided by the square of the height (kg/m$^2$). Homeostasis model assessment of insulin resistance (HOMA-IR) was calculated using the formula of [FPG (mg/dL) x fasting insulin (μU/mL)] / 405.

### Statistical Analysis

All statistical analyses were performed using IBM SPSS Statistics for Windows, Version 20.0 (IBM Corp., Armonk, NY, USA). Normal distribution was assessed using the Kolmogorov-Smirnov test. Analysis of variance was used for multiple-group comparison. Inter-group differences and intra-group differences were evaluated with independent and dependent samples t-tests, respectively. Pearson's correlation analysis was performed for parametric tests. The results are presented as mean±SD and a p value of <0.05 was considered statistically significant (95% confidence interval).

## Results

### Patient Characteristics and Routine Laboratory Tests

The general characteristics and routine laboratory test results of the control and prediabetic groups are mentioned in (Table 1). The prediabetic group had a significantly higher BMI, FPG, 2-hour PG, insulin, HOMA-IR, HbA1c, total cholesterol, LDL cholesterol and triglyceride level than the controls ($p<0.01$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.001$, $p<0.005$, $p<0.005$, and $p<0.05$, respectively).

### Baseline Values of PLT, Platelet Indices, Plasma sP-sel and β-TG

The baseline values of PLT, platelet indices, plasma sP-sel, and β-TG in the control and prediabetic groups are mentioned in (Table 2). The baseline plasma β-TG level was significantly higher in the overall prediabetic group ($p<0.001$) and the prediabetic subgroups (IFG: $p<0.001$, IGT: $p<0.05$, and IFG+IGT: $p<0.05$) than in the control group. There was no significant difference in the baseline PLT, platelet indices, or plasma sP-sel level between the prediabetic group or the prediabetic subgroups and the control subjects.

### PLT, Platelet Indices and Levels of Plasma sP-sel and β-TG 2 Hours After Glucose Loading

The PLT, platelet indices, plasma sP-sel, and β-TG values 2 hours after glucose loading in the control and prediabetic groups are presented in (Table 2). The prediabetic group had a significantly higher 2-hour plasma β-TG level than the control group ($p<0.05$). There was no significant difference in the 2-hour PLT, platelet indices, or plasma sP-sel levels between the prediabetic group or the prediabetic subgroups and the control subjects.

### Comparison of Groups Before and After Glucose Loading

The values of PLT, platelet indices, plasma sP-sel, and β-TG before and 2 hours after

Table 1. General characteristics and routine laboratory test results of the control and prediabetic groups (Mean±SD).

| | | | Prediabetic subgroups | | |
|---|---|---|---|---|---|
| | **Controls (n=31)** | **Prediabetic (n=103)** | **IFG (n=60)** | **IGT (n=14)** | **IFG+IGT (n=29)** |
| n (Female/male) | 31 (18/13) | 103 (68/35) | 60 (37/23) | 14 (8/6) | 29 (17/12) |
| Age (years) | 43.4±9.5 | 45.8±13.0 | 43.4±13.2 | 44.6±12.5 | 47.8±11.5 |
| BMI (kg/m$^2$) | 26.3±6.6 | 30.7±9.0 $^{a1}$ | 28.8±7.3 | 30.2±8.4 | 35.1±11.0$^{a4, b2}$ |
| Fasting plasma glucose (mg/dL) | 94.4±4.2 | 110.2±10.1 $^{a4}$ | 110.1±7.7$^{a4}$ | 95.3±3.5$^{b3}$ | 119.0±7.0$^{a4,b3,c3}$ |
| OGTT 2 h plasma glucose (mg/dL) | 97.0±18.6 | 129.1±39.0 $^{a4}$ | 101.4±18.8 | 154.3±22.3$^{a4, b3}$ | 176.4±19.8$^{a4,b3, c2}$ |
| Insulin (μU/mL) | 9.3±4.4 | 14.9±8.2 $^{a4}$ | 13.3±7.8$^{a3}$ | 16.8±8.3$^{a2}$ | 17.3±8.3$^{a4, b1}$ |
| HOMA-IR | 2.07±1.01 | 3.96±2.17 $^{a4}$ | 3.44±1.96$^{a4}$ | 4.01±1.97$^{a3}$ | 5.07±2.37$^{a4, b2}$ |
| Total cholesterol (mg/dL) | 171±54 | 201±44 $^{a3}$ | 192±35a1 | 234±39$^{a3, b3}$ | 207±40$^{a2, c1}$ |
| LDL cholesterol (mg/dL) | 108±45 | 132±36 $^{a3}$ | 122±32 | 156±39$^{a4, b2}$ | 139±36$^{a2, b1}$ |
| HDL cholesterol (mg/dL) | 51.6±16.1 | 52.6±20.8 | 53.9±24.6 | 56.6±13.0 | 49.3±9.2$^{c1}$ |
| Triglycerides (mg/dL) | 109±77 | 148±82$^{a1}$ | 131±73 | 179±85$^{a2, b1}$ | 167±91$^{a1}$ |
| Urea (mg/dL) | 24.9±8.5 | 28.0±7.8 | 27.3±7.7 | 27.4±10.7 | 26.5±6.0 |
| Creatinine (mg/dL) | 0.68±0.15 | 0.72±0.14 | 0.73±0.14 | 0.74±0.18 | 0.68±0.11 |
| CRP (mg/dL) | 2.9±3.8 | 2.9±3.0 | 2.8±3.2 | 2.5±1.7 | 3.5±3.0 |
| HbA1c (%) | 5.40±0.43 | 5.80±0.47$^{a3}$ | 5.68±0.43$^{a1}$ | 5.68±0.48 | 6.07±0.42$^{a4, b2, c1}$ |

a: Comparison with the control group; $a^1$: $p<0.05$ $a^2$: $p<0.01$ $a^3$: $p<0.005$ $a^4$: $p<0.001$
b: Comparison with the IFG group; $b^1$: $p<0.05$ $b^2$: $p<0.005$ $b^3$: $p<0.001$
c: Comparison with the IGT group; $c^1$: $p<0.05$ $c^2$: $p<0.005$ $c^3$: $p<0.001$
SD: Standard deviation; BMI: Body mass index; HOMA-IR: Homeostasis model assessment of insulin resistance; IFG: Impaired fasting glucose; IGT: Impaired glucose tolerance; OGTT: Oral glucose tolerance test; CRP: C-reactive protein

Table 2. PLT, platelet indices, plasma sP-sel, and β-TG values at baseline and 2 hours after glucose loading in the control and prediabetic groups (Mean±SD).

| | Controls (n=31) | | Prediabetic (n=103) | | Prediabetic subgroups | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | IFG (n=60) | | IGT (n=14) | | IFG+IGT (n=29) | |
| | Baseline | 2-h | Baseline | 2-h | Baseline | 2-h | Baseline | 2-h | Baseline | 2-h |
| PLT ($10^3$/mm3) | 250±71 | 246±72$^{c1}$ | 253±63 | 244±58c3 | 247±66 | 241±63$^{c1}$ | 266±58 | 251±50$^{c2}$ | 258±58 | 246±52$^{c2}$ |
| MPV (fL) | 9.03±0.84 | 8.99±0.85 | 8.96±0.84 | 8.90±0.90$^{c1}$ | 8.88±0.82 | 8.78±0.87$^{c1}$ | 9.26±0.89 | 9.24±0.91 | 8.98±0.87 | 8.95±0.93 |
| PDW (%) | 16.9±0.5 | 16.8±0.5 | 16.7±0.5 | 16.7±0.5 | 16.7±0.5 | 16.7±0.5 | 16.8±0.5 | 16.8±0.5 | 16.7±0.4 | 16.8±0.4 |
| PCT (%) | 0.222±0.053 | 0.217±0.053$^{c1}$ | 0.224±0.050 | 0.215±0.046$^{c3}$ | 0.217±0.052 | 0.210±0.051$^{c2}$ | 0.245±0.051 | 0.230±0.040$^{c1}$ | 0.229±0.040 | 0.218±0.038$^{c3}$ |
| sP-sel (ng/mL) | 23.4±7.7 | 19.9±6.5$^{c1}$ | 22.8±9.4 | 20.4±8.2$^{c1}$ | 23.1±9.9 | 20.9±9.0$^{c1}$ | 23.8±10.6 | 22.5±8.0 | 21.5±7.4 | 18.1±5.8$^{c2}$ |
| β-TG (ng/mL) | 8.6±2.1 | 8.7±3.0 | 11.1±4.5$^{a2}$ | 10.3±3.2$^{b}$ | 11.3±5.0$^{a2}$ | 10.4±3.2$^{b}$ | 11.0±3.4$^{a1}$ | 11.0±3.5$^{b}$ | 10.3±3.5$^{a1}$ | 9.4±3.1 |

a: Comparison with the baseline values of the control group; $a^1$: p<0.05 $a^2$: p<0.001
b: Comparison with 2 hours after glucose loading values of the control group; $^b$: p<0.05
c: Within-group comparisons of the before and after glucose loading values; $c^1$: p<0.05 $c^2$: p<0.005 c3: p<0.001
SD: Standard deviation; IFG: Impaired fasting glucose; IGT: Impaired glucose tolerance; MPV: Mean platelet volume; PCT: Plateletcrit; PDW: Platelet distribution width; PLT: Platelet count; sP-sel: Soluble P-selectin; β-TG: Beta(β)-thromboglobulin.

glucose loading in the control and prediabetic groups are mentioned in (Table 2). The PLT, PCT, and sP-sel levels were significantly lower after glucose loading in both the control (p<0.05 for each) and prediabetic group (p<0.001 for each). In the prediabetic group, the MPV decreased significantly after glucose loading (p<0.05), while it was not the case in the control group. The percentage decrease in the PLT level was significantly greater in the prediabetic group (3.18%) than in the control group (1.44%) (p<0.05). Although, the percentage decrease in the PCT level was greater in the prediabetes patients (3.88%) than in the control subjects (2.04%), the difference was not reach the level of significance. In the IFG, IGT, and IFG+IGT groups, the decrease in PLT was 2.2%, 5%, and 4.2%, respectively, and the rate of decrease of PCT was 3.2%, 5.2%, and 4.6%, respectively. The decrease in PLT was significantly greater in the IGT and IFG+IGT groups than in the control group (p<0.01 and p<0.01, respectively). Moreover, the decrease in PCT was greater in the IFG+IGT group than in the control group (p<0.05).

## Correlation Analysis

Correlations between the baseline values of platelet indices, plasma sP-sel, and β-TG in the control and prediabetic groups are shown in (Table 3). The PLT value correlated negatively with the MPV and PDW, and a positively with the PCT in both the control (r=-0.613, p<0.01; r=-0.574, p<0.01; r=0.952, p<0.01, respectively) and prediabetic groups (r=-0.433, p<0.01; r=-0.526, p<0.01; r=0.917, p<0.01, respectively). A negative correlation was observed between the PDW and the PCT (control: r=-0.490, p<0.01; prediabetic: r=-0.388, p<0.01), and a positive correlation between the PDW and the MPV (control: r=0.589, p<0.01; prediabetic: r=0.487, p<0.01). The MPV and the PCT correlated negatively only in the control group (r=-0.367, p<0.01). In addition, the HbA1c value correlated positively with the PLT and the PCT (r=0.523, p<0.01; r=0.395, p<0.05) and negatively with the MPV and the PDW (r=-0.539, p<0.01; r=-0.538, p<0.01) in the control group. There was no correlation between platelet indices, sP-sel, and β-TG in either the control or prediabetic groups.

## Discussion

The present study examined the plasma level of P-sel and β-TG and platelet indices in subjects with prediabetes. The effect of acute hyperglycemic stress on platelet indices and functions was also investigated using an OGTT. No significant difference

Table 3. Correlation coefficients between the baseline values of platelet indices, plasma sP-sel and β-TG in the control and prediabetic groups.

| | PLT ($10^3$/$mm^3$) | | MPV (fL) | | PDW (%) | | PCT (%) | |
|---|---|---|---|---|---|---|---|---|
| | Controls | Prediabetics | Controls | Prediabetics | Controls | Prediabetics | Controls | Prediabetics |
| PLT ($10^3$/$mm^3$) | - | - | -0.613 [a2] | -0.433 [a2] | -0.574 [a2] | -0.526 [a2] | 0.952 [a2] | 0.917 [a2] |
| MPV (fL) | -0.613 [a2] | -0.433 [a2] | - | - | 0.589 [a2] | 0.487 [a2] | 0.367 [a2] | -0.056 |
| PDW (%) | -0.574 [a2] | -0.526 [a2] | 0.589 [a2] | 0.487 [a2] | - | - | -0.490 [a2] | -0.388 [a2] |
| PCT (%) | 0.952 [a2] | 0.917 [a2] | 0.367 [a2] | -0.056 | -0.490 [a2] | -0.388 [a2] | - | - |
| sP-sel (ng/mL) | 0.143 | 0.027 | -0.043 | 0.114 | -0.053 | 0.044 | 0.129 | 0.082 |
| β-TG (ng/mL) | 0.146 | 0.019 | 0.115 | 0.058 | 0.094 | 0.111 | 0.243 | 0.198 |
| HbA1c (%) | 0.523 [a2] | 0.040 | -0.539 [a2] | -0.050 | -0.538 [a2] | -0.004 | 0.395 [a1] | 0.030 |

a[1]: Correlation is significant at the 0.05 level
a[2]: Correlation is significant at the 0.01 level
MPV: Mean platelet volume; PCT: Plateletcrit; PDW: Platelet distribution width; PLT: Platelet count; sP-sel: Soluble P-selectin; β-TG: Beta(β)-thromboglobulin.

was found between the controls and prediabetic subjects in the baseline values of PLT, indices, or plasma sP-sel level. However, the basal β-TG level showing platelet activation was higher in the prediabetic group when compared with the control group. After glucose loading, the PLT, PCT, and sP-sel levels were lower in both the control and prediabetic groups. A reduction in the PCT and the PLT was prominent in the prediabetic group. The MPV also decreased in patient with prediabetes.

Although several studies have examined platelet activation in diabetic patients (25, 26), the results of platelet activation and platelet indices have been contradictory and inconclusive in prediabetic patients. In a meta-analysis, Zaccardi et al., (27) reported that although both the MPV and the PDW increased, the PLT did not change in Type 2 DM. They also observed that both the PLT and the MPV levels increased in patients with IFG, but only the PLT increased in patients with metabolic syndrome. However, in another retrospective analysis of 13,021 patients, a high MPV was observed in diabetic patients. A positive correlation between the MPV and glucose and the MPV and HbA1c levels was observed in diabetics, but not in non-diabetics (15). Beyan et al. (28) suggested that platelet aggregation and platelet indices are not correlated and that those platelet indices alone are not sufficient to determine a pathological condition. De Luca et al. (29) divided MPV values into three levels (<10.6, 10.6-11.3, and >11.4 fL) and compared with platelet aggregation results, and found no significant relationship; suggesting that the MPV is not associated with platelet reactivity and is not an indicator of cardiovascular risk in diabetic patients. Tavil et al. (30) and Halbmayer et al. (31) also reported that the MPV is not a risk indicator for cardiovascular events. In our study, subjects with IFG or IGT or both were evaluated as a prediabetic group based on OGTT results. We observed that baseline platelet counts and indices in prediabetics were not significantly different from those of the controls. These findings suggest that hyperglycemic status alone with no diabetes is not sufficient to affect PLT and morphology in a prediabetic state.

Our results also revealed that the β-TG level showing baseline platelet activation was elevated in the prediabetic group when compared with the control group. However, the plasma sP-sel level was not significantly different between the two groups. In most studies, platelet function was investigated by simply measuring the MPV, and other platelet activation markers were not assessed. The results of our study suggest that although the MPV level did not change significantly in prediabetic patients, the plasma β-TG level increased. Our findings suggest that thrombocyte activation occurs in the prediabetic period and that the increase in platelet activation is independent of platelet size.

Postprandial hyperglycemia is an independent risk factor for the development of car-

diovascular events in diabetic patients. It has been shown that postprandial hyperglycemia is related to increased oxidative stress, endothelial damage, and platelet activation (32). Spectre et al. (32) found activated platelets in Type 2 diabetic patients after carbohydrate-rich nutrition and no such activation in the control group. Yngen et al. (33) observed that adenosine diphosphate-induced P-sel expression in Type 2 diabetic patients was greater after meals compared with baseline values and that this was associated with an increase in platelet reactivity. They also suggested that the increase in platelet activation occurred long before the onset of diabetes and may be associated with early diabetic vascular lesions. We examined the impact of acute hyperglycemia induced by oral glucose administration on platelet indices and functions. In control and prediabetic groups, the PLT, PCT, and sP-sel levels decreased following oral glucose loading. Interestingly, the reduction in the PCT and the PLT was higher in the prediabetic group. The MPV decreased after glucose loading in the prediabetic group; and not in the control group. We found that 2-hour PLT and PCT values were lower than the baseline values in each of the prediabetic subgroups. This decrease was marked in the IGT group.

Our findings showed that platelet morphology changed with an increased PG level. We observed that acute hyperglycemia led to a decrease in the PLT in all of the prediabetic and the control groups. Since the PCT is derived from the PLT and MPV levels, it provides more precise information about platelet quality and quantity (34). A reduction in the PCT level after glucose loading supports our conclusion that PLT decreases due to acute hyperglycemic stress. The effect of hyperglycemia on platelets is based on highly complex mechanisms. Few studies have focused on the effects of acute hyperglycemia on platelets. In previous studies, granule content and the surface antigens of platelets were investigated as activation markers, rather than the count and indices of platelets. The increase in the PLT reported in studies suggesting that hyperglycemia increases the MPV values by causing osmotic swelling has been explained by the defective relationship between MPV and PLT in diabetic patients. The physiological response to an increment in PLT is a decrement in MPV (27). Our findings of a negative association between the PLT and MPV levels, and the positive association between the PLT and PCT levels, are important because these findings demonstrate the consistency of the indices. The reduction in the PLT and PCT levels (and in MPV levels in prediabetics) may be due to platelet swelling caused by the osmotic effect of acute hyperglycemia followed by lysis of platelets. The fact that PLT and PCT levels decreased in most of the subjects with IGT suggests that the platelets of prediabetic patients with IGT are more sensitive to the osmotic effect of hyperglycemia than that of other prediabetic subjects. In healthy subjects, insulin prevents platelet activation, and this effect is inhibited by the decrease in the number of insulin receptors on the platelet surface in patients with Type 2 DM (35). Davison et al. (36) demonstrated that the number of activated leukocytes (such as monocytes and neutrophils) and platelets increase in diabetic patients, resulting in the increased platelet-leukocyte aggregate formation and cardiovascular complications. Our study indicated that oral glucose loading induced platelet activation and caused a decrease in platelet volume due to the release of granule contents. In addition, a decrease in PLT and sP-sel plasma levels may have occurred due to the binding of circulating sP-sel to its receptors and the formation of active platelet-leukocyte aggregates. The decrease in PLT level with an OGTT was greater in the prediabetic group than in the control group, may be because of impaired glucose uptake and use by platelets in prediabetic patients. We also did not observe any relationship between platelet indices and other platelet activation markers. Therefore, we suggest that platelet indices and activation markers could be change independently.

Most studies in the literature include common limitations, such as ethnic characteristics, the neglect of medical treatments that influence the platelet function, type of anticoagulant used, inappropriate transport and preservation of samples, long waiting periods before the analysis, or a single measurement of MPV. Over a period of 2 hours, platelets swell in EDTA blood and their size

varies (16). Furthermore, in observational and cross-sectional studies, there is supportive evidence for a link between abnormal gluco-metabolic status, poor glycemic control, and platelet activity, rather than a causal relationship between MPV levels and diabetes. Often, the MPV level is reported within the reference intervals (6.9-10.8 fL). Analytical CVs of platelet indices were not provided in some retrospective studies lasting 2 to 4 years; therefore, it becomes difficult to evaluate values within reference ranges as an increase in the prediabetic period. In our study, there was no change in any device or method and the quality control results for platelet indices were appropriate (CV <5%). We also optimized preanalytical conditions that could affect platelet indices. EDTA blood samples were transported at room temperature and analyzed within 2 hours. A pneumatic tube system was not used, as it could affect on platelet indices.

### Study limitations

Limitations of this study include a small patient population, non-homogeneous distribution of prediabetic subgroups, and a single measurement of parameters. Additionally, most of the patients evaluated as prediabetic had one or more metabolic syndrome components, which may be related to our findings.

## Conclusion

Our results suggest that the platelet indices do not change in the prediabetic stage and granule activation had begun without platelet morphology just affected. Acute hyperglycemia appears to affect platelet morphology and function in both healthy and prediabetic subjects. This effect can be marked in prediabetic individuals.

### Acknowledgments

This study was supported by Scientific Research Projects Unit of Istanbul University (project number: 24475). *The authors wish to thank Özlem YALÇIN and Jermin BENLİOĞLU for their technical assistance.*

## Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

## Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

## Authorship Contributions

Idea/Concept: Nesibe Esra Yaşar, Dildar Konukoğlu; Design: Nesibe Esra Yaşar, Dildar Konukoğlu; Control/Supervision: Nesibe Esra Yaşar, Dildar Konukoğlu; Data Collection and/or Processing: Nesibe Esra Yaşar; Analysis and/or Interpretation: Nesibe Esra Yaşar; Literature Review: Nesibe Esra Yaşar; Writing the Article: Nesibe Esra Yaşar, Dildar Konukoğlu; Critical Review: Dildar Konukoğlu; References and Fundings: Scientific Research Project Unit of İstanbul University; Materials:Nesibe Esra Yaşar.

## References

1. Tabák AG, Herder C, Rathmann W, Brunner EJ, Kivimäki M. Prediabetes: a high-risk state for diabetes development. Lancet. 2012;379:2279-2290. [Crossref] [PubMed] [PMC]
2. Katakami N. Mechanism of Development of Atherosclerosis and Cardiovascular Disease in Diabetes Mellitus. J Atheroscler Thromb. 2018;25:27-39. [Crossref] [PubMed] [PMC]
3. Beulens J, Rutters F, Rydén L, Schnell O, Mellbin L, Hart HE, Vos RC. Risk and management of pre-diabetes. Eur J Prev Cardiol. 2019;26:47-54. [Crossref] [PubMed]
4. Iraj B, Salami R, Feizi A, Amini M. The profile of hypertension and dyslipidemia in prediabetic subjects; results of the Isfahan Diabetes Prevention program: A large population-based study. Adv Biomed Res. 2015;4:27. [Crossref] [PubMed] [PMC]
5. Liu HH, Cao YX, Li S, Guo YL, Zhu CG, Wu NQ, Gao Y, Dong QT, Zhao X, Zhang Y, Sun D, Li JJ. Impacts of Prediabetes Mellitus Alone or Plus Hypertension on the Coronary Severity and Cardiovascular Outcomes. Hypertension. 2018;71:1039-1046. [Crossref] [PubMed]
6. Rumbaut RE, Thiagarajan P. Platelet-Vessel Wall Interactions in Hemostasis and Thrombosis. San Rafael (CA): Morgan & Claypool Life Sciences; 2010. [PubMed]

7. Biradar SB, Kashinakunti SV, Manjula R. Platelet volume indices in acute coronary syndrome-a case control study. Int J Adv Med. 2017;3:349-352. [Crossref]
8. Chen X, Fang L, Lin H, Shen P, Zhang T, Li H, Li X, Yu M, Xu C, Zhang J, Lu F, DU X, Hu R, Zhong J. The Relationship between Type 2 Diabetes and Platelet Indicators. Iran J Public Health. 2017;46:1211-1216. [PubMed] [PMC]
9. Wan JL, Ma ZW. The Value of Mean Platelet Volume for Prognosis of Patients with Acute Cerebral Infarction. Clin Lab. 2017;63:1801-1807. [Crossref] [PubMed]
10. Hanaganahalli SB, Sreeram S, Bompada M, Kuppannagari SK, Suresh PK, Philipose CS. Is MPV a Predictive Marker for Neonatal Sepsis? A Pilot Study. J Pediatr Hematol Oncol. 2018;40:548-552. [Crossref] [PubMed]
11. Ridker PM, Buring JE, Rifai N. Soluble P-selectin and the risk of future cardiovascular events. Circulation. 2001;103:491-495. [Crossref] [PubMed]
12. Sudic D, Razmara M, Forslund M, Ji Q, Hjemdahl P, Li N. High glucose levels enhance platelet activation: involvement of multiple mechanisms. Br J Haematol. 2006;133:315-322. [Crossref] [PubMed]
13. Rao AK, Chouhan V, Chen X, Sun L, Boden G. Activation of the tissue factor pathway of blood coagulation during prolonged hyperglycemia in young healthy men. Diabetes. 1999;48:1156-1161. [Crossref] [PubMed]
14. Budak YU, Polat M, Huysal K. The use of platelet indices, plateletcrit, mean platelet volume and platelet distribution width in emergency non-traumatic abdominal surgery: a systematic review. Biochem Med (Zagreb). 2016;26:178-193. [Crossref] [PubMed] [PMC]
15. Shah B, Sha D, Xie D, Mohler ER 3rd, Berger JS. The relationship between diabetes, metabolic syndrome, and platelet activity as measured by mean platelet volume: the National Health And Nutrition Examination Survey, 1999-2004. Diabetes Care. 2012;35:1074-1078. [Crossref] [PubMed] [PMC]
16. Leader A, Pereg D, Lishner M. Are platelet volume indices of clinical use? A multidisciplinary review. Ann Med. 2012;44:805-816. [Crossref] [PubMed]
17. Alfonso F, Angiolillo DJ. Targeting p-selectin during coronary interventions: the elusive link between inflammation and platelets to prevent myocardial damage. J Am Coll Cardiol. 2013;61:2056-2059. [Crossref] [PubMed]
18. Henry M, Davidson L, Cohen Z, McDonagh PF, Nolan PE, Ritter LS. Whole blood aggregation, coagulation, and markers of platelet activation in diet-induced diabetic C57BL/6J mice. Diabetes Res Clin Pract. 2009;84:11-18. [Crossref] [PubMed]
19. Gokulakrishnan K, Deepa R, Mohan V, Gross MD. Soluble P-selectin and CD40L levels in subjects with prediabetes, diabetes mellitus, and metabolic syndrome-the Chennai Urban Rural Epidemiology Study. Metabolism. 2006;55:237-242. [Crossref] [PubMed]
20. Perkins LA, Anderson CJ, Novelli EM. Targeting P-selectin adhesion molecule in molecular imaging: p-selectin expression as a valuable imaging biomarker of inflammation in cardiovascular disease. J Nucl Med. 2019;60:1691-1697. [Crossref] [PubMed] [PMC]
21. Gurney D, Lip GY, Blann AD. A reliable plasma marker of platelet activation: does it exist? Am J Hematol. 2002;70:139-144. [Crossref] [PubMed]
22. Egan K, van Geffen JP, Ma H, Kevane B, Lennon A, Allen S, Neary E, Parsons M, Maguire P, Wynne K, O' Kennedy R, Heemskerk JWM, Áinle FN. Effect of platelet-derived β-thromboglobulins on coagulation. Thromb Res. 2017;154:7-15. [Crossref] [PubMed]
23. Vizioli L, Muscari S, Muscari A. The relationship of mean platelet volume with the risk and prognosis of cardiovascular diseases. Int J Clin Pract. 2009;63:1509-1515. [Crossref] [PubMed]
24. Bielinski SJ, Berardi C, Decker PA, Kirsch PS, Larson NB, Pankow JS, Sale M, de Andrade M, Sicotte H, Tang W, Hanson NQ, Wassel CL, Polak JF, Tsai MY. P-selectin and subclinical and clinical atherosclerosis: the Multi-Ethnic Study of Atherosclerosis (MESA). Atherosclerosis. 2015;240:3-9. [Crossref] [PubMed] [PMC]
25. Carr ME. Diabetes mellitus: a hypercoagulable state. J Diabetes Complications. 2001;15:44-54. [Crossref] [PubMed]
26. Vinik AI, Erbas T, Park TS, Nolan R, Pittenger GL. Platelet dysfunction in type 2 diabetes. Diabetes Care. 2001;24:1476-1485. [Crossref] [PubMed]
27. Zaccardi F, Rocca B, Pitocco D, Tanese L, Rizzi A, Ghirlanda G. Platelet mean volume, distribution width, and count in type 2 diabetes, impaired fasting glucose, and metabolic syndrome: a meta-analysis. Diabetes Metab Res Rev. 2015;31:402-410. [Crossref] [PubMed]
28. Beyan C, Kaptan K, Ifran A. Platelet count, mean platelet volume, platelet distribution width, and plateletcrit do not correlate with optical platelet aggregation responses in healthy volunteers. J Thromb Thrombolysis. 2006;22:161-164. [Crossref] [PubMed]
29. De Luca G, Verdoia M, Cassetti E, Schaffer A, Di Giovine G, Bertoni A, Di Vito C, Sampietro S, Aimaretti G, Bellomo G, Marino P, Sinigaglia F; Novara Atherosclerosis Study (NAS) group. Mean platelet volume is not associated with platelet reactivity and the extent of coronary artery disease in diabetic patients. Blood Coagul Fibrinolysis. 2013;24:619-624. [Crossref] [PubMed]
30. Tavil Y, Sen N, Yazici HU, Hizal F, Abaci A, Cengel A. Mean platelet volume in patients with metabolic syndrome and its relationship with coronary artery disease. Thromb Res. 2007;120:245-250. [Crossref] [PubMed]
31. Halbmayer WM, Haushofer A, Radek J, Schön R, Deutsch M, Fischer M. Platelet size, fibrinogen and lipoprotein(a) in coronary heart disease. Coron Artery Dis. 1995;6:397-402. [Crossref] [PubMed]
32. Spectre G, Östenson CG, Li N, Hjemdahl P. Postprandial platelet activation is related to postprandial plasma insulin rather than glucose in patients with type 2 diabetes. Diabetes. 2012;61:2380-2384. [Crossref] [PubMed] [PMC]

33. Yngen M, Ostenson CG, Hjemdahl P, Wallén NH. Meal-induced platelet activation in Type 2 diabetes mellitus: effects of treatment with repaglinide and glibenclamide. Diabet Med. 2006;23:134-140. [Crossref] [PubMed]
34. Akpinar I, Sayin MR, Gursoy YC, Karabag T, Kucuk E, Buyukuysal MC, Aydin M, Haznedaroglu IC. Plateletcrit. A platelet marker associated with saphenous vein graft disease. Herz. 2014;39:142-148. [Crossref] [PubMed]
35. Ferreira IA, Mocking AI, Feijge MA, Gorter G, van Haeften TW, Heemskerk JW, Akkerman JW. Platelet inhibition by insulin is absent in type 2 diabetes mellitus. Arterioscler Thromb Vasc Biol. 2006;26:417-422. [Crossref] [PubMed]
36. Davison GM, Nkambule BB, Mkandla Z, Hon GM, Kengne AP, Erasmus RT, Matsha TE. Platelet, monocyte and neutrophil activation and glucose tolerance in South African Mixed Ancestry individuals. Sci Rep. 2017;7:40329. [Crossref] [PubMed] [PMC]