The Relationship Between Platelet Morphology and Neutrophil β2 (CD18) Integrins in Patients with Type 2 Diabetes

C Top, H Terekeci, O Öncül, F Demirel, S Özkan

Citation

Abstract

Aims: To evaluate the relationship between platelet morphology and neutrophil β2 (CD18) integrins in patients with type 2 diabetes.

Methods: Twenty-five type 2 diabetic patients (aged 60.2 ±12.4 years, BMI: 29.5±5.1 kg/m²) and 14 non-diabetic healthy controls (aged 51.4 ±16.8 years, BMI: 25.7±5.4 kg/m²) were enrolled in the study. After a 12-h overnight fast, all subjects underwent a diagnostic protocol including the serum fasting insulin, C-peptid, hsCRP, HbA1c. The neutrophil β2 (CD18) integrin expression assay were performed by flow-cytometry as a unit of relative fluorescence intensity (RFI). The insulin sensitivity were assessed using the homeostasis model assessment (HOMA) indexes and QUICKI. The correlation analysis were performed to investigate the relationship between platelet morphology and other parameters.

Results: The study results showed that there were statistically significant difference between study patients (type 2 diabetic patients) and healthy control subjects in accordance to serum concentration of insulin (12.2 ± 7.4 μU/mL vs 5.9 ± 2.7 μU/mL, p<0.01), HOMA scores (4.5 ± 3.0 vs 1.3 ± 0.6, p<0.001), QUICKI scores (0.55 ± 0.3 vs 0.69 ±0.12, p<0.01). There were no statistically significant difference between study patients (type 2 diabetic patients) and healthy control subjects in accordance to CD18 expression (RFI) (2.60 ± 0.93 mg/dl vs 3.41 ± 1.33, p>0.05), platelet count (245.3±71.0x10⁶/ml vs. 238.9±62.8x10⁶/ml, p>0.05), mean platelet volume (8.5±1.3 fL vs 8.6±0.9 fL, p>0.05), platelet distribution width (16.2±0.6 vs 16.1±0.7, p>0.05) (Table 1). The correlation analyses (Pearson) have shown that in diabetic subjects, there was a statistically significant correlation between CD18 expression and MPV (r=-0.44, p<0.05). There was no statistically significant correlation between CD18 expression and platelet count (r=0.38, p>0.05), PDW (r=-0.14, p>0.05), fasting serum insulin (r=0.21, p>0.05), HOMA scores (r=0.14, p>0.05), QUICKI scores (r=-0.13, p>0.05), hs-CRP levels (r=-0.14, p>0.05).

Conclusions: The present study demonstrated that there were significant correlation between neutrophil β2 (CD18) integrin expression and MPV. These results have shown that there may be a relationship between platelet morphology changes and neutrophil activation. This relationship may be important for developing cardiovascular complications in diabetic patients.

INTRODUCTION

CD18 (integrin β2 subunit) forms a heterodimer with members of the CD11 group and is expressed on all leukocytes. The intensity of CD18 expression on fetal granulocytes was less than adults, although it increased with fetal age. The intensity of expression of CD18 was higher in the elderly population (1,3). Members of the β2 integrin family are the dominating integrins expressed on leukocytes, and they play a major role in leukocyte cell-cell and cell-matrix adhesions during inflammation and other immune responses. Beta 2 integrins are signaling receptors, but they are also targets of and are functionally affected by intracellular signals (1,2,3).

Neutrophils and platelets interact both physically and metabolically during inflammation and thrombosis, but the mechanisms responsible for their adhesion remain incompletely understood. Neutrophil-platelet adhesion can be initiated by specific activation of either the neutrophil or the platelet. Neutrophil-platelet adhesion uses both platelet P-selectin and the neutrophil β2 integrin CD11b/CD18 when
the cells are primarily or secondarily activated \((s_{1677940,2211,112})\).

Diabetic hyperglycaemia causes a variety of pathological changes in macro and microvascular system. Platelet-neutrophil interactions are an important target of hyperglycaemic effects, but the mechanisms underlying these interactions are not fully understood. One of the possible mechanisms that is involved in the pathogenesis of diabetic vascular complication is the altered platelet morphology and function due to hyperglycaemia that is reported in patients with diabetes mellitus. They are likely to be associated with the pathological neutrophil-platelet interactions, microthrombus formation and prothrombotic state \((s_{1313,132,127,171,191,192})\). Previous clinical studies have shown that the increased platelet hypersensitivity and accelerated rate of platelet production in subjects with type 2 diabetes may lead to change of platelet morphology (a greater number of very large and hypersensitive younger platelets and a more abundant fraction of small exhausted platelets). Platelets have an increased potency to adhere and aggregate in diabetic subjects. Platelet index changes and leukocyte CD18 expression can be used to monitor platelet and leukocyte activation, respectively \((s_{1017,186})\). In this study, we evaluated whether there was a relationship between platelet morphology and neutrophil \(\beta_2\) (CD18) integrins in patients with type 2 diabetes or not.

**SUBJECTS AND METHODS**

**PATIENTS**

We studied twenty-five type 2 diabetic patients (aged 60.2 ±12.4 years, BMI: 29.5±5.1 kg/m\(^2\)) and 14 non-diabetic healthy controls (aged 51.4 ±16.8 years, BMI: 25.7±5.4 kg/m\(^2\)). Patients with the concomitant any other chronic inflammatory disease that can effects the neutrophil and platelet activation were excluded from the study. None of the subjects were on any medication that causes altered inflammatory responses.

**METHODS**

Sample Preparation: Venous blood samples were collected from all study and control subjects into tubes (preservative-free) with sodium heparin. Within 0.5 h after sampling, the neutrophils were seperated from whole blood by methods of Lyse-Wash. All reagents used were equilibrated at room temperature for 0.5 h before use. 50 ?l whole blood was collected in a 12x75 mm steril polypropylene centrifuge tubes (BD Biosciences, Cat.no.:352052) and followed by adding 10 ?l FITC-labeled (fluorescein isothiocyanate) mouse monoclonal antibodies to human CD18 antigen or matching isotype controls. The tubes were mixed by vortex mixer, and allowed to stand at room temperature for 20 minutes. 2ml lysing solution (Cal-lyse, Cat.no:GAS010) was added to these tubes, and than incubated at room temperature for 12 minutes. After that, it was centrifuged at 1,400g for 5 minutes. The upper layer was removed from the tubes. 2 ml FACS flow solution was added, and than centrifuged at 1,400g for 5 minutes. The upper layer was removed again from the tubes. Samples was immediately analyzed by flow cytometry.

Assay of CD 18 expression by flow cytometry: This procedure was performed at flow cytometry laboratory of GATA Haydarpasa Training Hospital. Samples were analyzed on a FACSCalibur flow cytometer (Becton Dickinson). The data was analyzed using CellQuest software (BD Biosciences). During this analysis, FITC-labeled mouse monoclonal antibodies to human CD18 antigen (Caltag Lab.,Burlingame, CA, Product Code:MHCD1801) and matching isotype controls (Mouse IgG1 FITC) (Caltag Lab.,Burlingame, CA, Product Code:MG101) were used. It was analyzed antibody-stained cells on flow cytometerThe right angle side scatter (SSC) versus forward angle light scatter (FSC) was collected to reveal the neutrophil cell cluster. A gate was drawn for the neutrophil cluster on the FSC/SSC histograms. The neutrophil \(\beta_2\) (CD18) integrin expression is than measured as a unit of relative fluorescence intensity.

Plasma glucose concentration was measured by the glucose oxidation method. Serum insulin and C-peptide was determined by immuno-enzymatic method (Beckman, ImmunoTech, IRMA GH ).

The estimate of insulin resistance by HOMA ( Homeostasis Model Assessment ) score was calculated with the formula : fasting serum insulin (\(\mu\)U/ml) \(\times\) fasting plasma glucose (mmol/L) / 22.5, as described by Matthews and coworkers\((s_{120,22,34})\). With such a method, high HOMA scores denote low insulin sensitivity (insulin resistance). The other way of estimating insulin sensitivity was to calculate QUICKI (quantitative insulin sensitivity control index) \((s_{120,22,34})\).

\[\text{QUICKI} = \frac{1}{\log(\text{fasting serum insulin} (\mu\text{U/ml}))} \times \frac{1}{\log(\text{fasting serum glucose} (\text{mg/dl}))}.\]

**STATISTICAL ANALYSIS**

SPSS statistical software release 15.0 was used for statistical analysis. The differences between groups according to serum insulin levels, HOMA-IR, QUICKI, neutrophil \(\beta_2\) (CD18)
integrin expression, platelet indexes and other parameters were assessed by Mann Whitney U test. All obtained data were compared by Pearson correlation analysis. Differences were accepted as significant at p<0.05. All data are presented as means ± SD.

RESULTS
Mann Whitney U test showed that there were no statistically significant difference between study patients and healthy control subjects in accordance to age and mean body mass index (60.2 ±12.4 vs. 51.4 ±16.8 years and 29.5±5.1 kg/m² vs. 25.7±5.4 kg/m², p<0.05 respectively) (Table 1). There were statistically significant difference between study patients (type 2 diabetic patients) and healthy control subjects in accordance to serum concentration of insulin (12.2 ± 7.4 µU/mL vs 5.9 ± 2.7 µU/mL, p<0.01), HOMA scores (4.5 ± 3.0 vs 1.3 ± 0.6, p<0.001), QUICKI scores (0.55 ± 0.3 vs 0.69 ±0.12, p<0.01). There were no statistically significant difference between study patients (type 2 diabetic patients) and healthy control subjects in accordance to platelet count (260.9±84.5x10³/ml vs 243.6±39.6x10³/ml, p>0.05), mean platelet volume (8.3±0.8 fl vs 8.6±0.9 fl, p>0.05), platelet distribution width (16.2±0.7 vs 15.9±0.4, p>0.05). There were no statistically significant difference between study patients (type 2 diabetic patients) and healthy control subjects in accordance to CD18 expression (RFI) (2.60 ± 0.93 mg/dl vs 3.41 ± 1.33, p>0.05), platelet count (245.3±71.0x10³/ml vs 238.9±62.8x10³/ml, p>0.05), mean platelet volume (8.5±1.3 fl vs 8.6±0.9 fl, p>0.05), platelet distribution width (16.2±0.6 vs 16.1±0.7, p>0.05) (Table 1).

The correlation analyses (Pearson) have shown that in diabetic subjects, there was a statistically significant correlation between CD18 expression and MPV (r=-0,44, p<0.05). There was no statistically significant correlation between CD18 expression and platelet count (r=0,38, p>0.05), PDW (r=0,14, p>0.05), fasting serum insulin (r=0,21, p>0.05), HOMA scores (r=0,14, p>0.05), QUICKI scores (r=-0,13, p>0.05), hs-CRP levels (r=-0,14, p>0.05).

DISCUSSION
Clinically-apparent neutrophil-platelet interaction was reported in the early 1960s. The mechanisms responsible for the initiation and maintenance of this adhesive interaction are unclear. The activated polymorphonuclear leukocytes (PMN) induce platelet activation. PMN products released during PMN activation such as cathepsin G, a protease, is a potent platelet agonist. Thrombosis and inflammation involve complex platelet-leukocyte interaction, the details of which are not fully elucidated. There may be a cross talk between platelets and leukocytes in whole blood at baseline. Diabetes mellitus is associated with platelet and leukocyte dysfunction. Previous studies had supported that insulin may modulate thrombotic and inflammatory processes in vivo in a complex manner. Neutrophils and platelets interact both physically and metabolically during inflammation and thrombosis, but the mechanisms responsible for their adhesion remain incompletely understood. Neutrophil-platelet adhesion uses both platelet P-selectin and the neutrophil beta2 integrin. Diabetes mellitus and hyperglycaemia are associated with platelet activation. High glucose levels enhanced platelet reactivity to agonist stimulation. Platelet dysfunction plays a major role in the development of diabetic vascular complications. Thus, the assessment of platelet activation markers such as increased mean platelet volume reflects the prothrombotic state.
The Relationship Between Platelet Morphology and Neutrophil β2 (CD18) Integrins in Patients with Type 2 Diabetes

Leukocytes, and in particular polymorphonuclear cells, play a role in the organ injury that characterizes the progression of vascular atherosclerotic disease. Adhesion between platelets and polymorphonuclear leukocytes (PMN) is a key event in thrombosis and inflammation. Previous clinical studies had supported that circulating polymorphonuclear leukocyte activation occurred in patients with type 2 diabetes. Several authors had reported that PMN activation played an important role in increasing circulating PMN-platelet aggregates and polymorphonuclear leukocyte adhesion to activated platelets was important for the recruitment of PMN at sites of vascular damage and thrombus formation.

Evidence is increasing that platelets can initiate and propagate inflammatory processes by interacting with leucocytes and the vascular endothelium. Platelets have been shown to bind to neutrophils, existing as platelet/neutrophil complexes (PNC) within the circulation. Platelets also have an important pro-inflammatory role. Platelets normally circulate in a quiescent state. Upon activation, platelets can secrete and present various molecules, change their shape as well as the expression pattern of adhesion molecules. These changes are associated with the adhesion of platelets to leucocytes and the vessel wall. There is a growing interest in the use of platelet index changes for the diagnosis of platelet activation at clinical studies.

The present study demonstrated that there were significant correlation between CD18 and MPV. These results have shown that there was a relationship between platelet morphology changes and neutrophil activation. This relationship may be important for developing vascular complications in diabetic patients, and also gives an important data about the increased risk of cardiovascular and endothelial complication.

In conclusion, we found an altered pattern of neutrophil β2 (CD18) integrin in type 2 diabetic subjects at baseline, suggesting the presence of an increased inflammatory response and neutrophil activation in these individuals. These data may help in explaining the role of neutrophils in the evolution of diabetic vascular complications. PMN integrins and activated platelets are involved in the presence of diabetic vascular complications and may be potential targets for pharmacological intervention.

The results of this study clearly indicate that there may be a relationship between neutrophil and platelet activation in patients with type 2 diabetes. It must be planned the further investigations to evaluate the relationship between neutrophil surface integrin overexpression and platelet surface integrin expression changes by flow-cytometric analysis at the cellular level. Also, the further studies must be planned to evaluate the intracellular platelet and neutrophil signalling abnormalities, basic interactions between these cells in diabetic patients.

CORRESPONDENCE TO
Cihan TOP, M.D. Department of Internal Medicine, GATA Haydarpaşa Training Hospital, Tibbiye Cad. 34668 Haydarpaşa, İstanbul, Turkey e.mail address: tpcihan@hotmail.com

References
The Relationship Between Platelet Morphology and Neutrophil \( \beta_2 \) (CD18) Integrins in Patients with Type 2 Diabetes

The Relationship Between Platelet Morphology and Neutrophil β2 (CD18) Integrins in Patients with Type 2 Diabetes

Author Information

Cihan Top, M.D.
Department of Internal Medicine, Gülhane Military Medical Academy, Haydarpasa Training Hospital

Hakan Terekeci, M.D.
Department of Internal Medicine, Gülhane Military Medical Academy, Haydarpasa Training Hospital

Oral Öncü, M.D.
Department of Infectious Disease, Gülhane Military Medical Academy, Haydarpasa Training Hospital

Fevzi Demirel, M.D.
Department of Internal Medicine, Gülhane Military Medical Academy, Haydarpasa Training Hospital

Sezai Özkan, M.D.
Department of Anesthesiology, Gülhane Military Medical Academy, Haydarpasa Training Hospital