Comparison of Platelet CD Markers between Normal Individuals and Untreated Patients with Type 2 Diabetes Mellitus

Muhammad Saboor, Moinuddin Moinuddin and Samina Ilyas
Baqai Institute of Hematology, Baqai Medical University, Karachi, Pakistan

Abstract

Objective: Micro and macro vascular complications are associated with diabetes mellitus. Purpose of this study was to evaluate and compare the platelet CD markers between normal individuals and untreated patients with type 2 diabetes mellitus using flow cytometry.

Methods: A total of 107 individuals [32 normal and 75 diabetics] were recruited in this study. Complete blood counts, random and fasting blood sugar, hemoglobin A1c, lipid profile and platelets flow cytometry were determined of all blood samples.

Results: In normal individuals and in patients with untreated type 2 diabetes mellitus, comparison of platelet markers CD41 and CD61 at rest and after activation with ADP did not show any statistical difference in their mean % fluorescence positivity. Number of CD63 and CD62p positive platelets increased with a p value of 0.001 in normal individuals and in patients with untreated type 2 diabetes after activation with ADP compared with their resting states.

Conclusion: Upon platelet activation with ADP, positivity of CD63 and CD62p was increased in normal individuals. CD63 and CD62p positivity was also increased in patients platelets after activation with ADP. Increase in the mean % positivity of CD63 and CD62p was however more in diabetics as compared with normal individuals.

Keywords: Diabetes mellitus; Platelets; CD markers

Introduction

Diabetes mellitus is associated with micro and macro vascular complications. Microvascular complications i.e. retinopathy, nephropathy and neuropathy result in increased morbidity in patients with diabetes mellitus [1]. Diabetes mellitus is a major risk factor for cardiovascular diseases [2]. Chronic hyperglycemia is not the only cause of these complications; these ischemic events are also associated with platelet hyper activation, abnormal activation of coagulation proteins, abnormal endothelial function and hypo-fibrinolysis [3,4]. More than 75% of patients with diabetes die due to cardiovascular complications [5].

Diabetic thrombocytopathy refers to differences in platelet functions between diabetic and non-diabetic individuals. A number of studies have documented several platelet function defects in patients with diabetes mellitus. Platelets hyper aggregation in response to glucose was recognized in 1965 [6]. Enhanced platelet aggregation in response to ADP, thrombin, collagen, arachidonic acid and epinephrine is seen in patients with diabetes mellitus as compared to non-diabetic individuals [7]. Under in vitro conditions these platelets after stimulation with platelet agonists show reduced threshold for platelet aggregation. Increased platelet aggregation is more apparent in patients with diabetes associated with macro vascular disease. Platelets from diabetic subjects show an increased adhesiveness and increased spontaneous aggregation as well as aggregation on extra cellular matrix [8,9].

CD61 and CD41 are glycoproteins present on platelets and megakaryocytes. These glycoproteins act as receptors for fibrinogen, von Willebrand factor [vWF], fibronectin and vitronectin [10] CD62p is a glycoprotein of 140 kDa present in α-granules of resting platelets. It is translocated to the plasma membrane after activation. Expression of CD62p on the surface of circulating platelets indicates in vivo activation of platelets [11] CD63 is a lysosomal membrane protein detected on the surface of activated platelets after release reaction normally not found on non-stimulated platelets [12].

Vericel et al. [13] found that platelets at rest show increased activity in patients with type 1 and type 2 diabetes patients. They further reported that hyper active platelets were detectable in metabolically controlled patients with diabetes without cardiovascular complications.

Eibl et al. [14] found that platelets of patients with type 2 diabetes mellitus show increased expression of platelet activation markers CD31, CD36, CD49b, CD62p and CD63 as compared to normal individuals. Newly diagnosed type 1 diabetic patients also show increased expression of activation dependent platelets surface glycoprotein receptors. Increased CD62 positive platelets were also found in normal first-degree relatives of type 1 diabetic patients [15].

One major cause of atherosclerosis and thrombosis in diabetes is enhanced platelet activation, aggregation and increased expression of CD63 and CD62. Enhanced platelet activation and aggregation leads to the development of vascular complications in diabetes in the early stage of the disease. Flow cytometric detection of platelets with increased surface expression indicates enhanced platelet activation a hallmark of thrombosis [16].

The purpose of this study was to compare the platelet CD markers between normal individuals and untreated patients with type 2 diabetes mellitus by flow cytometry. It was postulated that platelets in patients with untreated type 2 diabetes mellitus circulate in the blood in hyperactive state and are causative of thrombotic complications so frequently encountered in these patients.

*Corresponding author: Muhammad Saboor, Baqai Institute of Hematology, Baqai Medical University, Karachi, Pakistan, Tel: 0333-2279927; E-mail: msaboors@gmail.com

Received November 23, 2013; Accepted December 26, 2013; Published December 28, 2013

Citation: Saboor M, Moinuddin M, Ilyas S (2013) Comparison of Platelet CD Markers between Normal Individuals and Untreated Patients with Type 2 Diabetes Mellitus. J Hematol Thromb Dis 2: 123 doi: 10.4172/2329-8790.1000123

Copyright: © 2013 Saboor M, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
Material and Methods

A total of 107 individuals [32 normal and 75 diabetics] were recruited in this study. Criteria followed for normal individuals were no history of illness and anti-platelet drugs intake. Criteria for inclusion in the study for patients with diabetes mellitus were: untreated patients type 2 diabetes mellitus, no history of drug intake particularly the antidiabetic or anti-platelet drugs, no evidence of diabetic complications and non-smokers. Patients with type 2 diabetes mellitus were selected from Baqai University Hospital and Baqai Institute of Diabetes and Endocrinology Nazimabad. This study was approved by the ethics committee of Baqai Medical University, Karachi. Written informed consent was taken from all individuals.

All individuals were initially tested for random blood glucose level for exclusion or inclusion in the study. Whole blood samples were collected after overnight fasting by venipuncture technique under minimal tourniquet pressure. Blood was collected in vacutainers [BD Biosciences]. 3 ml of blood was collected in EDTA tube for CBC and HbA1c; 2.7 ml of blood was added to sodium citrate tube for platelet flow cytometry while 5 ml of blood was collected in a gel tube for biochemistry. Flow chart of the study design is shown below.

Complete blood counts of all samples were determined by automated cell analyzer. Fasting blood glucose level, urea, creatinine and lipid profile were determined using automated biochemistry analyzer [Hitachi 900 Roche].

Flow cytometric analysis of platelets was done within one hour after the collection of blood samples. Immediately after blood collection, the citrated tube was centrifuged for 10 minutes at 2000 rpm. Platelet rich plasma was separated into another polystyrene tube. Polystyrene test tubes were labeled as tubes 1, 2, 3, 4 and 5.10 µl of IgG isotype, anti CD41, anti CD63, anti CD61 and anti CD63 [BD Biosciences USA] were added to the tubes as follows:

Tube 1: IgG isotype control. Tube 2: Anti CD41 FITC and anti CD63 PE. Tube 3: Anti CD61 PE and anti CD 62P FITC. Tube 4: Anti CD41 FITC and anti CD63 PE. Tube 5: Anti CD61 PE and anti CD 62P FITC.

Tubes 2 and 3 were considered as resting platelets while tubes 4 and 5 were activated platelets. 50 µl of platelet rich plasma was added to each tube. 25 µl of ADP was added to tube 4 and 5. Each tube was incubated in the dark for 20 minutes. After incubation, 1 ml of phosphate buffer saline was added to each tube for dilution. The tubes were analyzed using flow cytometer FACSCalibur [BD Biosciences]. Forward scatter and side scatter signals were acquired with log amplification. A region was drawn on platelet population in the scatter plot. CD41, CD61, CD62P and CD63 expression was analyzed on the gated population. Results were recorded as % of the positive gated platelets.

Student t test was used for comparison of inter-group variables while independent t test was used for the comparison of groups using SPSS version 16 (Figure 1).

Results

Mean age of the normal individuals was 45 ± 6 years while the in diabetics it was 51 ± 8. Normal group comprised of 15 male and 17 females, while diabetic group included 28 men and 47 females. Mean fasting blood sugar of normal subjects was 81.27 ± 7.94 mg/dl while that of diabetic group was 140.54 ± 9.63 mg/dl. Comparison of both groups showed statistically significant difference [p<0.001]. Random blood

![Figure 1: Flow chart of the study design.](image-url)
stable markers are not affected by activation with ADP. This finding is inherent in patients with untreated type 2 diabetes mellitus [12].

Activation and thus forewarn the risk of thromboembolism which is the hallmark of such disorders. This finding shows that platelets of patients with diabetes mellitus are more responsive to agonists and are more prone to activation after exposure to the agonists. This observation constitutes an important and first step in the evaluation of the state of platelet reactivity in disorders associated with thrombo-atheromatus complications, type 2 diabetes mellitus being the prototype of such disorders. This finding shows that platelets of patients with diabetes are in activated state as compared to normal subjects. Our findings are consistent with that of Matzdorff et al. [11] and Ostermann et al. [18] Matzdorff et al. [11] reported that activation of platelets with agonists like ADP or epinephrine results in increased expression of CD62 and CD63 on the surface of the platelets. Nomura et al. [19] used CD62 for the identification of platelet functional status in diabetes.

Tschöpe et al. [15] showed that platelets with increased surface glycoprotein expression continue to circulate in the blood before consumption during platelet aggregation. It is a well-established fact that patients with diabetes mellitus have large platelets in their blood with enhanced aggregation potential [20]. Activated platelets in patients with diabetes suggest increased consumption and as well as production of platelets [21]. Young platelets are more reactive and become easily activated in vivo [22].

Glycation of platelet membrane proteins leads to changes in the protein structure, conformation and alterations in membrane lipids [23]. Altered structure of lipids in the platelet membrane causes increased expression of CD62, CD63, fibrinogen receptors and von Willebrand factor receptors [24]. Increase in the surface expression of these receptors results in the increased tendency of ligand binding [25].

In the light of the above results and discussion it is concluded that; CD41 and CD61 markers do not show any change after treatment with ADP. These markers therefore are of no value in the diagnosis and treatment of various thromboembolic episodes. Increased CD63 and CD62p expression in newly diagnosed untreated patients with diabetes is the hallmark of in vivo platelet activation and can be used as marker of activated platelets.

### References

1. American Diabetes Association (2012) Diagnosis and classification of diabetes mellitus. Diabetes Care 35 Suppl 1: S64-71.
2. Blake DR, Meigs JB, Muller DC, Najjar SS, Andres R, et al. (2004) Nathan DM. Impaired glucose tolerance, but not impaired fasting glucose, is associated with increased levels of coronary heart disease risk factors: Results from the Baltimore longitudinal study on aging. Diabetes 53: 2095-2100.
3. Matadamas-Zárate C, Hernández-Jetónimo J, Pérez-Campos E, Majluf-Cruz A (2009) [Platelet abnormalities in type 2 diabetes mellitus]. Arch Cardiol Mex 79 Suppl 2: 102-108.
4. Drouet L, Bai d’ollier C, Henry P (2010) [Antiplatelet agents and diabetes mellitus]. Ann Cardiol Angeiol (Paris) 59 Suppl 2: S56-64.
5. Haffner SM, Lehto S, Rönnemaa T, Pyörälä K, Laakso M (1998) Mortality from coronary heart disease in subjects with type 2 diabetes and in nondiabetic subjects with and without prior myocardial infarction. N Engl J Med 339: 229-234.
6. Bridges JM, Dalby AM, Millar JH, Weaver JA (1965) An Effect Of D-Glucose On Platelet Stickiness. Lancet 1: 75-77.
7. Leoncini G, Signorelli MG, Piana A, Carmuca M, Armani U (1997) Hyperactivity

### Parameters

| Parameters   | Normal | Diabetics | p value |
|--------------|--------|-----------|---------|
| FBS (mg/dl)  | 81.27 ± 7.94 | 153.54 ± 9.63 | 0.001 |
| RBS (mg/dl)  | 110.73 ± 8.93 | 198.79 ± 26.72 | 0.001 |
| HbA1c (%)    | 4.14 ± 0.61 | 7.43 ± 0.41 | 0.001 |
| Urea (mg/dl)| 18 ± 5 | 21 ± 7 | 0.132 |
| Creatinine (mg/dl) | 0.7 ± 0.2 | 0.8 ± 0.2 | 0.115 |
| Cholesterol (mg/dl) | 164 ± 18 | 176.45 ± 20.85 | 0.09 |
| Triglycerides (mg/dl) | 85 ± 14 | 154.87 ± 23.89 | 0.001 |
| HDL (mg/dl)  | 49 ± 09 | 42.86 ± 11.58 | 0.001 |
| LDL (mg/dl)  | 102 ± 05 | 110 ± 12 | 0.123 |

**Table 1:** Biochemical parameters in normal subjects and patients with untreated type 2 diabetes mellitus.

**Markers**

| Markers | Resting platelets | Activated platelets | p value |
|---------|-------------------|---------------------|---------|
| CD41    | 89.58 ± 4.61      | 89.33 ± 4.95        | 0.749   |
| CD61    | 90.38 ± 4.83      | 90.25 ± 4.81        | 0.870   |
| CD63    | 36.32 ± 5.97      | 85.26 ± 5.63        | 0.001   |
| CD62P   | 32.09 ± 5.08      | 83.81 ± 5.12        | 0.001   |

**Table 2:** Comparison of resting and activated platelets of patients with diabetes.

Paired t-test was used for comparison.
and increased hydrogen peroxide formation in platelets of NIDDM patients. Thromb Res 86: 153-160.

8. Knobler H, Savion N, Shenkman B, Kotev-Emeth S, Varon D (1998) Shear-induced platelet adhesion and aggregation on subendothelium are increased in diabetic patients. Thromb Res 90: 181-190.

9. Coppola L, Verrazzo G, La Marca C, Ziccardi GP, Grassia A, et al. (1997) Effect of insulin on blood rheology in non-diabetic subjects and in patients with type 2 diabetes mellitus. Diabet Med 14: 959-963.

10. Cox D (1998) Methods for monitoring platelet function. Am Heart J 135: S160-169.

11. Matzdorff A (2005) Platelet function tests and flow cytometry to monitor antiplatelet therapy. Semin Thromb Hemost 31: 393-399.

12. Michelson AD, Furman MI (1999) Laboratory markers of platelet activation and their clinical significance. Curr Opin Hematol 6: 342-348.

13. Vérice E, Januel C, Carreras M, Moulin P, Lagarde M (2004) Diabetic patients without vascular complications display enhanced basal platelet activation and decreased antioxidant status. Diabetes 53: 1046-1051.

14. Eibl N, Krugluger W, Streit G, Schrattbauer K, Hopmeier P, et al. (2004) Improved metabolic control decreases platelet activation markers in patients with type-2 diabetes. Eur J Clin Invest 34: 205-209.

15. Tschoepe D, Driesch E, Schwippert B, Lampeter EF (1997) Activated platelets in subjects at increased risk of IDDM. DENIS Study Group. Deutsche Nikolnamin Interventionsstudie. Diabetologia 40: 573-577.

16. Tschoepe D, Schultheiss HP, Kolarov P, Schwippert B, Dannehl K, et al. (1993) Platelet membrane activation markers are predictive for increased risk of acute ischemic events after PTCA. Circulation 88: 37-42.

17. Saboor M, Moinuddin M, Ilyas S (2012) Flow cytometry based chronologic analysis of surface glycoproteins of resting and stimulated platelets in normal individuals. Pak J Med Sci 28: 625-629.

18. Ostermann H, van de Loo J (1986) Factors of the hemostatic system in diabetic patients. A survey of controlled studies. Haemostasis 16: 386-416.

19. Nomura S, Shouzu A, Omoto S, Nishikawa M, Fukuhara S (2000) Significance of chemokines and activated platelets in patients with diabetes. Clin Exp Immunol 121: 437-443.

20. Tschoepe D, Roesen P, Eisser J, Schwippert B, Nieuwenhuis HK, et al. (1991) Large platelets circulate in an activated state in diabetes mellitus. Semin Thromb Hemost 17: 433-438.

21. Winocour PD (1994) Platelet turnover in advanced diabetes. Eur J Clin Invest 24 Suppl 1: 34-37.

22. Watala C (2005) Blood platelet reactivity and its pharmacological modulation in diabetes mellitus. Curr Pharm Des 11: 2331-2365.

23. Winocour PD, Watala C, Perry DW, Kinlough-Rathbone RL (1992) Decreased platelet membrane fluidity due to glycation or acetylation of membrane proteins. Thromb Haemost 68: 577-582.

24. Mazzanti L, Rabini RA, Fumelli P, Martarelli D, Staffolani R, et al. (1997) Altered platelet membrane dynamic properties in type 1 diabetes. Diabetes 46: 2069-2074.

25. Watala C, Gwoździnski K, Pluskota E, Pietrucha T, Walkowiak B, et al. (1996) Diabetes mellitus alters the effect of peptide and protein ligands on membrane fluidity of blood platelets. Thromb Haemost 75: 147-153.