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Platelets Abnormalities in Hemodialysis Type II Diabetic Patients

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Abstract: The aim of this study was to detect platelets abnormalities in haemodialysis type II diabetic patients. Twenty non-insulin dependent diabetic hemodialysis patients of both sexes (4 males & 5 females), their aged ranged from 40-84 years old were, participated in this study which was performed at King Abdulaziz University Hospital. Patients under aspirin treatment were excluded in this study. Twenty healthy non diabetic individuals participated in this study as a control group. Blood samples were collected from diabetic patients by a specialist phlebotomist using a butterfly needle. Whole blood was drawn and collected in 3 different test tubes containing different types of anti-coagulants, one containing sodium citrate for coagulation profile and the other two tubes containing EDTA for CBC and platelet activation study that has been assessed by flow Cytometry. Results indicated that there were significant differences between RBCs, platelet, MPV, Hgb, HbA_1C and CD markers expression on platelets of type II diabetes patients and control group. It was concluded that platelets obtained from diabetic subjects show hyperactivity increased adhesiveness and an exaggerated aggregation.

Key words: Platelets · Type II diabetes mellitus · Hemodialysis

INTRODUCTION

Type II diabetes mellitus (DM) is characterized mainly by tissue insulin resistance and impaired insulin secretion. Increased platelet activity due to abnormal insulin action is emphasized in the development of vascular complications of this disease [1]. Diabetes is associated with multiple disorders including metabolic, cellular and blood disturbances leading to vascular complications. Increased circulating levels of platelet-leukocyte aggregates (PLA) have been described in several thrombotic diseases [2].

The epidemiology of diabetes and diabetic nephropathy has changed substantially over recent years and continues to change. The developing world is increasingly involved showing a rising rates of diabetes, diabetic nephropathy and end-stage renal disease (ESRD) [3].

Diabetes mellitus (DM) is associated with increased cardiovascular morbidity and mortality, which in part may be due to a variety of abnormalities reported in diabetic platelets [4]. Accelerated atherosclerosis is commonly observed in diabetes mellitus and this may be partly due to platelet hyperactivity. Spontaneous platelet aggregation (SPA) is absent or rare in healthy subjects, while it has been described in adults with unstable angina, transient ischemic attack, myocardial infarction, diabetes mellitus, hyper-lipoproteinemia, emotional stress and strenuous exercise [5].

In general, platelets obtained from diabetic subjects show increased adhesiveness and an exaggerated aggregation, both spontaneous and in response to stimulating agents. The causes for this activation are manifold: altered exposure and/or abundance of glycoprotein receptors for agonists and adhesive proteins on the platelet surface, increased binding of fibrinogen, decreased membrane fluidity, altered platelet metabolism and changes in intra-platelet signaling pathways [6].

The importance of strict glycemic control in diabetic patients without nephropathy or end-stage renal disease (ESRD) has been well documented for the prevention of micro- and macro-vascular complications [7]. In monitoring glycemic control, the importance of hemoglobin A1C (HbA_1C) has been well established. In the advanced stage of renal disease, better glycemic control before hemodialysis has been suggested to be associated with reducing the risk of death from diabetic complication [8].

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The aim of this study was to detect platelets abnormalities in haemodialysis type II diabetic patients.

The activation of the coagulation system during haemodialysis is usually prevented by heparin. With respect to lipid and bone metabolism, poly-morphonuclear cell stimulation, induction of antibody mediated thrombocytopenia and aldosterone suppression, low molecular weight heparin (LMWH) is supposed to be advantageous compared with un-fractionated heparin (UFH) for chronic haemodialysis. Blood samples were obtained during early morning haemodialysis session from the arterial needle (before heparinization), from the predialyzer port.

MATERIALS AND METHODS

Subject: Twenty non-insulin dependent diabetic hemodialysis patients of both sexes (8 males & 12 females), their aged ranged from 40-84 years old, participated in this study which was performed at King Abdulaziz University Hospital. Patients under aspirin treatment were excluded in this study. Twenty healthy non diabetic individuals (age and sex matched group) participated in this study as a control group.

Sample Collection: Blood samples were collected from diabetic patients by a specialist phlebotomist using a butterfly needle. Whole blood was drawn and collected in 3 different test tubes containing different types of anti-coagulants, one containing sodium citrate for coagulation profile and the other two tubes containing EDTA for CBC and platelet activation study that has been assessed by flow Cytometry. The activation of the coagulation system during haemodialysis is usually prevented by heparin. With respect to lipid and bone metabolism, poly-morphonuclear cell stimulation, induction of antibody mediated thrombocytopenia and aldosterone suppression, low molecular weight heparin is supposed to be advantageous compared with un-fractionated heparin for chronic haemodialysis.

Blood samples were obtained during early morning haemodialysis session from the arterial needle (before heparinization), from the predialyzer port.

Sample Preparation: Whole blood drawn from diabetic subject was collected in EDTA test tube, Platelet-rich plasma (PRP) was prepared by centrifuging (Centra MP4R) the blood at 900 RPM for 10 minutes at room temperature.

Sample Procedure: 100µL of PRP was added to 20µL of fluorescent mono-clonal (mAb) (FITC-PE) for the detection of platelet expression, the samples were incubated in dark room for 20 min at room temperature, 1 ml of phosphate buffer solution (PBS) is added to the incubated samples, than centrifuged at 3500 RPM for 5 min at room temperature. The resultant platelets were washed twice with PBS and centrifuged after each wash. Analysis always took place within 8 hours of platelet preparation. For analyses of, CD41, CD42, CD61 and CD62p (P-selectin), IgG isotype controls were applied to detect non-specific staining (Becton Dickinson, Mountain View, USA) prior to flow cytometric analysis. Samples were analyzed on a (FACScan®) cytometer (Becton Dickinson). Fluorescent beads were applied daily to ensure the stability of the system (CaliBRITE™, Becton Dickinson). Following the setting of the appropriate threshold in the forward side scatter (FSC), 10,000 events were acquired in a life-gate. List mode data were acquired and analyzed using (CELLQuest®) software (Becton Dickinson). The results were expressed percentage of antibody-positive cells. Percentage of antibody-positive cells was defined as cells with specific fluorescence higher than the isotype and autofluorescence samples.

HbA1c: Blood samples were collected from diabetic patient in EDTA tube for HbA_1c analysis that was assessed in the clinical chemistry laboratory using Hitachi Modular P system, for the quantitative determination of percentage hemoglobinA_{1C} in anti-coagulated whole blood. Measurements of percentage hemoglobin A_{1C} are effective in monitoring long term glucose control in individuals with diabetes mellitus.

Complete Blood Count (CBC): Blood samples were collected from diabetic patient using EDTA tube. CBC was performed automatically by the coulter method (Beckman coulter, coulter LH 750 analyzer) in the hematology lab in king Abdulaziz University.

Coagulation Study: A fully automated instrument (Behring Coagulation Timer, BCT) is used for the measurements of coagulation profiles and other parameters that used to evaluate coagulation factors activity. Blood samples were centrifuged at 3500 RPM for 10 min in a refrigerated centrifuge (Centra MP4R) set to 4°C. PT, PTT, Fibrinogen measurement were performed automatically on BCS (Behring coagulation system) machine.

Table 1: Demonstrates the different haematological parameters such as, RBCs, platelet, MPV, Hgb and HbA_1C of type II diabetes patients and control group

Characteristics	Diabetic patients	Non-diabetic subjects	*p*-Value
RBCs	3.048 ± 0.467	4.83 ± 0.06	0.4560
Platelet	283.77 ± 111.69	310.1 ± 74.80	0.4080
MPV	8.74 ± 0.9900	8.73 ± 1.20	0.9700
Hgb	8.7 ± 1.13.00	15.02 ± 5.30	0.0001
HbA_1c%	10.6 ± 1.8.000	3.1 ± 0.434	0.0001

Table 2: Illustrates the relationship between the different CD markers expression on platelets of type II diabetes patients and control group

Characteristics	Diabetic patients	Non-diabetic subjects	*p*-Value
CD41	99.04 ± 6.6000	90.1 ± 4.20	0.0013
CD42	99.10 ± 0.4490	87.6 ± 9.50	0.0013
CD61	96.18 ± 4.7100	85.3 ± 10.8	0.0070
CD62	41.316 ± 21.07	24.3 ± 7.30	0.0001
Prothrombin Time	31.38 ± 12.550	11.2 ± 1.90	0.0001
Fibrinogen	317.85 ± 59.930	288.3 ± 42.2	0.1490

RESULTS

There were significant differences between RBCs, platelet, MPV, Hb, HbA_1C and CD markers expression on platelets of type II diabetes patients and control group (Tables 1 and 2).

DISCUSSION

Diabetes is strongly associated with early development of abnormal endothelial function, platelet hyper-reactivity, aggressive atherosclerosis and adverse arterial remodelling. Platelet activation occurs in several diseases as acute coronary syndrome, heart failure and, insulin resistance and diabetes. Activated platelets are essential for promoting leukocyte adhesion and determining the progression of atherosclerotic lesion formation the fibrinogen fragments in the blood of haemodialysis patients can occupy the fibrinogen receptor on the surface of platelets, preventing them from being activated. The platelet aggregation ability of haemodialysis patients is weaker than that in normal people.

Patients with diabetes mellitus have a variety of platelet and coagulation system alterations that can contribute to microvascular complications. Previous studies show that platelet adhesion and aggregation has been found to be enhanced in diabetic patients indicating platelet hyperactivity [9]. Platelet aggregation results from the combination of platelet secretion of dense and α-granules content such as ADP, serotonin and fibrinogen and rheologic changes resulting in collision of inactivated platelets with those that have undergone shape change, allows interplatelet contact and the formation of platelet aggregates. By this process, platelets interact with one another to form a haemostatic plug or thrombus. Many studies show that during thrombosis, an association of platelet-derived microparticles with fibrin can be involved in the haemostatic process [10]. Platelet-derived microparticles are small (<1 μm) vesicular structures that are released following platelet activation by different stimuli. These platelet-derived microparticles contain both pro- and anticoagulant proteins and the effect of these microparticles on blood coagulation could be related to the negative charge derived from exposed membrane phospholipids [11]. An increase of these platelet-derived microparticles was measured in some diseases such as diabetes mellitus [12].

The Expression levels of different monoclonal antibodies such as, CD62, CD41 and CD61 (especially the last two indexes) on the peripheral blood platelet surface were significantly increased in the patients with dialysis compared with those with normal kidney. Patients with diabetes are predisposed to atherosclerosis and have a two- to four-fold increased risk of cardiovascular morbidity and mortality compared with age-matched non-diabetic subject.

Numerous studies have shown that diabetes is associated with a significant increase of numerous markers of coagulation activation as well as plasma factors leading to a hypercoagulable state [13]. Recently, an increased level of inflammatory plasma parameters was reported in diabetic patients presenting vascular complications. Among these abnormalities, platelet dysfunction was well documented in diabetes [14].

Thus, platelets from diabetic patients exhibit an exacerbated response to classical agonists and a more important membrane expression of adhesive molecules such as thrombospondin, CD62 [15].

Type II diabetes in humans is associated with platelet abnormalities, alterations in coagulation factors and disturbances in endothelial cells that lead to a hypercoaguable state. People with obesity and diabetes die prematurely from thrombotic events and subsequent vascular disease secondary to this hypercoaguable state [16]. Mice fed a diet rich in animal fat become obese and diabetic, but do not exhibit the increased platelet aggregation, coagulation, or expression of platelet markers of activation observed in humans with obesity and type II diabetes [17].

Haemoglobin A_1c (HbA_1c) is the result of an irreversible non-enzymatic glycation of the β chain of haemoglobin A and is used as an objective measure of long-term blood glucose control in diabetic patients. AGE is formed by nonenzymatic glycation of proteins or lipids, HbA1c levels is elevated in diabetic patient who is thought to be implicated in the formation of micro- and macrovascular complications and increased in CHD. In this study we found increased HbA1c levels that show high significance between patient and control results but there is no direct correlation between HbA_1c and increased activity of platelet CD markers.

In several previous studies, HbA1C was also evaluated as a glycemic control index and higher HbA_1C levels in the pre-dialysis stage were associated with poor survival after haemodialysis initiation. In haemodialysis patients with diabetes, however, some have reported that HbA1C was significantly associated with survival, whereas others have reported that it was not [8, 18, 19]. Evaluation of HbA1C levels is important and valid in maintenance haemodialysis patients with diabetes and that good glycemic control is important for prevention of both cardiovascular and non-cardiovascular death in haemodialysis patients with diabetes [7].

Platelet function and activation, was significantly higher in patients with type 2 diabetes. Also, platelet activity was significantly higher in patients with HbA_1c levels >7% than in patients with HbA_1c levels =7%. Poor glycemic control (HbA_1c >7%), had a higher incidence of diabetic nephropathy and coronary artery diseases [20].

The fibrinogen fragments in the blood of uremic patients can occupy the fibrinogen receptor on the surface of platelets, preventing them from being activated. The platelet aggregation ability of uremic patients is weaker than that in normal people.

Diabetes mellitus markedly increases the risk of cardiovascular morbidity and mortality. Elevated levels of inflammatory cytokines, chemokines, adhesion molecules and platelet activation have been found both in type 1 and type II diabetes [2, 22].

Type II diabetes mellitus (DM), is a common ailment that accounts for about 90% of diabetes and affects more than 170 million individuals worldwide [23]. Type II DM leads to a number of cardiovascular disorders, including angiopathies, which are the major cause of morbidity and mortality in type 2 DM. Platelet hyperactivation and hyperaggregation play a pivotal role in the development of angiopathy in diabetic patients. Several factors are associated with platelet hyperactivation in type II DM, including an increased production of reactive oxygen species (ROS), an altered Ca2+ mobilization and an increased protein tyrosine phosphorylation [24]. Platelets from diabetic patients are more sensitive to agonists and show an enhanced adhesion and aggregation [25]. Platelet hyperactivation and hyperaggregation play a crucial role in thrombotic complications associated to type II DM [26].

It was concluded that platelets obtained from diabetic subjects show hyperactivity increased adhesiveness and an exaggerated aggregation.

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