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Measurement of Platelet Parameters and D-Dimer Level in Sudanese Patients with Long Standing Diabetes Mellitus Type 2

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Abstract

Background: This is an observational analytical case control study that had been conducted among diabetic patients in Khartoum State, during the period from December to February 2016 - 2017, to compare platelets parameters and D-dimer level between diabetic patients attending Diabetic and Endocrine Hospital, Soba University Hospital and non-diabetic controls. 80 blood samples were collected 40 samples from diabetic patients and 40 samples from non-diabetic controls.

Objectives: To evaluate platelet parameters and D-Dimer level in long standing Diabetes mellitus type 2 in Sudanese patients.

Method: After obtaining informed consent from each patient and non-diabetic control, 4 ml of venous blood were collected and divided into two parts: 2.5 ml transferred into K2EDTA container for complete blood count, and 2.5 ml transferred into Tri Sodium Citrate container (9 volumes of blood is added to 1 volume of a 32 g/1 solution of TSC anticoagulant) and separated by centrifugation at 3000 rpm for 15 minutes, to obtain platelet poor plasma for measurement of D-Dimer level. The following tests were performed (platelets count, MPV, PDW, P-LCR and D-Dimer level) in both groups (case and control).

Result: Platelets count in both case and control was statistically insignificant ($p = 0.392$), while MPV ($P = 0.034$), PDW ($p = 0.020$), P-CLR ($p = 0.012$), and D-Dimer. Level ($p = 0.015$) were statistically significantly increased in patients compared to controls. There were no statistically significant differences in all measured parameters between gender, age and duration groups except platelets count which was significantly increased in patients under 50 years old. P-value < 0.05 was considered significant throughout the study.

Conclusion: Our study identified the altered platelets morphology and increased D-Dimer level among the long standing DM type 2 which are indicators of thrombotic state in diabetes mellitus. Based on these results we recommend the platelets indices and D-dimer level to the spectrum of thrombophilia which will be carried on long standing diabetes mellitus type 2 patients.

Keywords: *Platelets; D-Dimer; Diabetes Mellitus*

Introduction

Diabetes is a collection of metabolic illnesses recognized by hyperglycaemia resulting from defects in insulin secretion, insulin action, or both [1]. It is a chronic, lifelong state that affects the body's ability to use the energy found in food [2]. The chronic hyperglycaemia of it is connected with long-term damage, dysfunction, and failure of different organs, especially the eyes, kidneys, nerves, heart and blood vessels [1].

There are three main kinds of diabetes: type 1 diabetes mellitus, type 2 diabetes mellitus, and gestational diabetes.

It is also named insulin-dependent diabetes mellitus and juvenile-onset diabetes since it often begins in childhood, it is an autoimmune state caused when the body attaching its own pancreas with antibodies. The injured pancreas doesn't make insulin.

Blood glucose manage is challenged commonly for patients with diabetes mellitus, Illness, stress, and unplanned life events often make glycaemic control unstable, which puts the patient at risk for hypoglycemia or hyperglycemia. These acute consequences may be mild and managed independently by the patient, or they may be severe, thus threatening life and requiring intensive monitoring. Outcomes are improved with timely recognition of the patient at risk, their symptoms, and appropriate intervention [3].

The chronic consequences of diabetes mellitus comprise

Retinopathy with potential blindness, nephropathy leading to renal failure, peripheral neuropathy with risk of foot ulcers, amputations, Charcot joints and autonomic neuropathy leading to gastrointestinal, genitourinary, and cardiovascular symptoms and sexual dysfunction. Individuals with diabetes mellitus have raised incidence of atherosclerotic cardiovascular, peripheral arterial and cerebrovascular disease. Hypertension and abnormalities of lipoprotein metabolism are frequently found in persons with diabetes mellitus [4].

Internationally, estimated 422 million adults were living with diabetes in 2014, compared to 108 million in 1980. The global prevalence (age-standardized) of diabetes has nearly doubled since 1980, rising from 4.7% to 8.5% in the adult population.

This reveals increase in accompanied risk factors such as being overweight or obese. More than the past decade, diabetes prevalence has risen faster in low- and middle-income countries than in high-income countries.

Diabetes caused 1.5 million deaths in 2012. Higher-than-optimal blood glucose caused additional 2.2 million deaths, by increasing the risks of cardiovascular and other diseases. 43% of these 3.7 million deaths occur before the age of 70 years. The percentage of mortality referred to elevated blood glucose or diabetes that occurs prior to age 70 is higher in low-and middle-income countries than in high-income countries.

The bulk of people with diabetes are affected by type 2 diabetes. This used to occur nearly entirely among adults, but now occurs in children [5].

Sudan is one of 19 countries and territories of the IDF MENA region. Four hundred and fifteen million citizens have diabetes in the world and more than 35.4 million people in the MENA region. In 2040 this will increase to 72.1 million. There were over 1.4 million cases of diabetes in Sudan in 2015 [6].

Platelets are small fragments of cytoplasm derived from megakaryocytes. On average, they are 1.5 – 3.5 mm in diameter, with a mean volume 7 - 11 FL, but may be larger in some disease states. They do not contain a nucleus and are bounded by a typical lipid bilayer membrane.

Platelets are produced in the bone marrow by fragmentation of the cytoplasm of megakaryocytes, the megakaryocytes matures by endomitotic synchronous replication enlarging the cytoplasmic volume as the number of nuclear lobes increase in multiples of two. Very

early on invaginations of plasma membrane are seen, called the demarcation membrane, which evolves through the development of the megakaryocytes into a highly branched network. At a variable stage in development, most commonly at the eight nucleus stage, the cytoplasm becomes granular. Platelets form by fragmentation of megakaryocytes cytoplasm, approximately each megakaryocytes giving rise to 1000 - 5000 platelets. Thrombopoietin is the major regulator of platelet production and is constitutively produced by the liver and kidneys. The normal platelet count is approximately 250 x 10^9/L (range 150 - 400 x 10^9/L) and the normal platelet. Life span is 7 - 10 days.

Structure

Platelets are extremely small and discoid, platelets surface coated by glycoprotein (GP1a, GP1b and GP11b\111a), membrane phospholipids (platelet factor 3). Platelet contains 3 types of granules (dense, alpha, lysosomes). The more frequent specific alpha granules contain clotting factors, VWF, PDGF and others. Dense granules less common and contain ADP, ATP, serotonin, calcium and lysosomes contain hydrolytic enzymes. Platelet also so rich in signalling and cytoskeleton proteins.

The main platelets function is formation of mechanical plugs during haemostatic response to vascular injury. There are three major platelet functions: adhesion, aggregation and release reaction and amplification [7].

Alterations in the endothelium can activate inflammatory processes, which together with other factors such as hypertension and dyslipidaemia, cause atherosclerotic plaques. These plaques may remain asymptomatic for years and not cause any clinical changes in diabetic patients.

Hyperglycemia frankly participates to endothelial damage through irreversible glycation of collagen and other subendothelial structural proteins of the vessel, formed advanced glycation end products (AGEs). AGEs accumulate in the sub endothelium over time influenced by increases in blood sugar levels and are directly related to atherosclerosis and renal failure.

A platelet plug forms after endothelial injury. The dynamics of platelet plug formation involves several steps including: adhesion, change in shape, aggregation, and platelet granule secretion. Platelets adhere to the sub endothelium then change its shape and release its granules contents. The secretion of granules signals the recruitment of other platelets to the vessel wall resulting in platelet plug formation. Although hyperglycemia, dyslipidaemia, and hypertension may independently cause vascular damage, endothelial dysfunction may be intrinsic to T2DM. This condition can lead to an activated site characterized, in part, by platelet adhesion and increased aggregation. The osmotic defect of glucose consists in a mechanism by which hyperglycemia increases the propensity of platelets to aggregate and degranulate.

In vivo studies have shown evidence of increased platelet activation in patients with metabolic syndrome and T2DM due to increased levels of the beta-thromboglobulin and platelet factor IV in the plasma [8].

D-Dimer levels had raised in diabetic patients. In normal conditions, when there is hypercoagulable state, there is consequently a state of hyperfibrinolysis. As hypercoagulability and hypo fibrinolysis states are present in diabetic patients, the expression of markers such as D-Dimer may be under estimated. Moreover, the more rigid fibrin from glycated fibrinogen may also contribute to relatively low values as this fibrin is more difficult to break down [8].

Change of blood coagulation and fibrinolysis along with deprived glycaemic control in diabetes has also been concerned in the progress of diabetic vascular consequences. Plasma level of D-Dimer reveals the quantity lyses of cross-linked fibrin and hence is an accepted marker of hypercoagulability [9].

There are close relationship between thrombosis and the developments of atherosclerotic vascular disease. There are at least three ways which blood component may contribute to the development of atherosclerosis and its complication:

- By hemodynamic factors and platelets-leukocyte interactions with vessel wall which may lead to endothelial injury and consequent smooth migration and proliferation.
- By the formation of persistent mural thrombi which are organized and incorporated into sub endothelium, potentiating vessel wall damage.
- By formation of thrombi in association with advanced atherosclerosis.

There is a strong possibility that vascular spasm (for example, of the coronary arteries) may be caused by thromboxane A_2, serotonin, or other vaso-active substance released as a consequence of platelet activation. Such spasm may cause ischemic symptom, particularly if the circulation is already compromised by proximal atheroma [10].

Diabetes is associated with accelerated rate of atherosclerosis and circulatory dysfunction [10]. Atherosclerosis is characterized by intimal lesion called atheroma's (also called atheromatous atherosclerotic plaques), that protrude into vascular lamina.

Besides obstructing blood flow, atherosclerotic plaques weaken the underline media and can they rupture, causing acute catastrophic vessel thrombosis [11].

Mean platelet volume (MPV) is part of the Complete Blood Count (CBC) tests and it identifies the average size of platelets found in the blood of an individual [23]. Normal MPV range is approximately from 7 to 11 fl [12].

Elevated MPV levels have been identified as an independent risk factor for myocardial infarction in patients with coronary heart disease and for death or recurrent vascular event after myocardial infarction. Moreover, increased platelets size has been reported in patients with vascular risk factor such as diabetes, and smoking. Previous studies have demonstrated higher levels of MPV in patients with acute ischemic stroke than in control subject. In contrast, data regarding the association between MPV and stroke severity or stroke outcome have been controversial [13].

The Mean platelet volume can be an indication of platelet turnover because younger platelets tend to be larger [14]. A spectrum of platelet size is seen in patient with rapid turnover [24].

MPV is recently taken as a determinant for platelet function, as it is positively associated with platelets reactivity function [15].

The measure of platelet anisocytosis and the plateletcrit which is the product of MPV and platelet count and by analog with the haematocrit may be seen as individual of the quantity of circulating platelets in a unit volume of blood [16]. Normal PDW is approximately 9 - 13 fl [17].

Causes of increased P-LCR (Platelet-large cell ratio):

- Autoimmune thrombocytopenic purpura.
- Essential thrombocythemia [7].
- Acute myocardial infarction and coronary heart disease [18].
- Idiopathic thrombocytopenic purpura [19].

D-Dimer is a fibrin degradation products (FDPs), a small protein fragment present in the blood after a blood clot is degraded by fibrinolysis. It is so named because it contains two D fragments of fibrin protein joined by a cross-link.

D-Dimer concentration may be determined by a blood test to help diagnosis of thrombosis. Since its introduction in 1990s, it has become an important test performed in patients with suspected thrombotic disorders. A negative result practically rules out thrombosis,

while a positive result may indicate thrombosis but does not rule out other potential causes. Its main use, therefore, is to exclude thrombo-embolic disease where the probability is low. Moreover, it has been used in the diagnosis of the blood disorder Disseminated Intravascular Coagulation (DIC) [20].

Hekimsoy Z., *et al*. at 2004 may-Jun: they measured mean platelet volume in type 2 diabetes patients and found that the altered platelet morphology and function have been reported in patients with diabetes mellitus. They were likely to be associated with the pathological processes and increased risk of vascular disease seen in diabetic patients.

The results showed that the mean platelet volume was significantly higher and the mean platelet counts were significantly lower in diabetics compared to age and sex matched non-diabetic healthy control [21].

Kodiatte AT., *et al.* J lab physicians at 2012. Found that an increasing in platelet activity may play role in the development of vascular complications of this metabolic disorder. The mean platelet volume (MPV) is an indicator of the average size and activity of platelet. Bigger platelets are younger and reveal more activity. The results of this study had been shown that: the mean platelet counts and MPV were higher in diabetics when compared to non-diabetic subjects [22].

In 2014 Ulutas KT., *et al.* evaluated the mean platelet volume in patients with type2 diabetes mellitus and blood glucose regulation and found that: platelets play an important role in atherosclerosis and arterial thrombosis. Cardiovascular complications prevalence of type 2 diabetes mellitus (type2DM) may be associated with measurements of glycosylated hemoglobin (HbA1c), and mean platelet volume (MPV) [23].

A study to evaluate MPV in type 2 diabetic patients was conducted at University of Khartoum reported higher MPV in diabetics ($p = 0.015$) than non-diabetic subjects [24]. Other study showed higher MPV in type 2 diabetic patients versus non- diabetic controls ($p = 0.01$) [25]. Another study in platelet parameters (MPV, PDW) reported significantly higher MPV and PDW in diabetics ($p < 0.05$ for all) than non- diabetic subjects [26].

Yoshimasa Aso, Sachiko Matsumoto., *et al.* found that plasma concentration of D-dimer was higher in diabetic patients than in control subjects [27].

Refik Demirtune A, Dursun Dunan*., et al.* found that the MPV was significantly higher in patients with Diabetes Mellitus than in control. ($8.7 \pm .8$fl vs $8.2 \pm .7$, $p = .002$) [28].

PN Papanas A, G Symeonidisb., *et al.* found that MPV was significantly higher ($p = .01$) in patients with diabetes mellitus (14.2 ± 2.2 fl) than in controls (7.1 ± 1.2 fl) [29].

The elevation of D-dimer in diabetic patients is attributed to inhibition of fibrinolytic system which is the means of removing clots due to abnormal colt structure that are more resistant to degradation and an increase in plasminogen activator inhibitor type 1 (PAI-1) [30]. Nwose EU., *et al.* reported that D-dimer level was significantly increased ($p < 0.002$) and changes in D-dimer level may indicate diabetes disease progression to macrovascular complications [31].

Another study conducted at 2013 by Sid Ahmed, Dalia Dafa Allah Osman, on Sudanese diabetic patients in Ibrahim Shams Alden hospital in Khartoum north on 60 patients of both sexes with type 2 diabetes mellitus. The result showed significantly increased platelet indices (MPV, PDW, and P-LC) ($p \leq 0.01$) and lower values for platelet count in diabetic patients compared with non-diabetic controls [32].

Rationale

Diabetes mellitus is an extremely common disorder which carries a risk of vascular impairment. DM type 2 can be characterized by dysfunction on haemostasis manifesting by stimulated coagulation process. Altered platelets morphology and increased D-dimer levels are risk factors for microvascular and macrovascular complications [33].

So our study had been made to evaluate the changes in platelets morphology and D-dimer level in diabetes mellitus type 2 and to correlate with hypercoagulable states that result in the thrombotic disorders. To what extent changes in coagulation process occur in Sudanese individuals with type 2 diabetes mellitus has had limited study. To clarify the association between the clinical and haemostatic findings that may occur in these individuals, we examined a group of type 2 diabetes mellitus patients to determine to what extend these changes in haemostatic process occur. These provide evidence for clinicians to manage their diabetic patients probably and thus reduce the risk of serious complications and improve the health care services for type 2 diabetes mellitus patients in the future.

Objectives of the Study

General objective

To measurement the platelet parameters and D-dimer level in long standing diabetes mellitus type 2 patients attending to Diabetic and Endocrine Hospital, Soba University Hospital from December to February 2016 - 2017.

Specific objectives

- To measure platelets count.
- To assess the mean platelet volume (MPV).
- To estimate platelet distribution width (PDW).
- To measure platelet- large cell ratio (P-LCR).
- To measure D-dimer level.
- To determine if gender, age and duration are effects platelet count, MPV, PDW, P-LCR and D-dimer in Sudanese diabetic patients or not.

Materials and Methods

Study design

An observational analytical case control study.

Study area

The study was conducted in Khartoum state (Diabetic and Endocrine Hospital, Soba University Hospital), Sudan.

Study period

From December 2017 to February 2018.

Study population

Total of 40 Longstanding type 2 diabetes mellitus patients as cases more than 3 years and 40 non-diabetic individuals as controls were included in this study. The selected diabetic and non-diabetic subjects has included both genders with age group 25 - 80 years old. The total subjects (both case and control) were 37 males and 43 females. The case group was 9 males and 31 female.

Inclusion criteria

This study included adult longstanding type 2 diabetes mellitus with more than 3 years diabetes mellitus.

Exclusion criteria

Diabetes mellitus type 1 and other types, short standing less than 3 years (recent diabetic patients) and children.

Sample size

80 Participants divided equally between patients and normal (control) group.

Data analysis

Data analysed using SPSS statistical package, descriptive statistics, mean and standard deviation, were determined.

Data collection

The data were collected by using a direct interviewing questionnaire. The questionnaire was used to collect data regarding name, age, gender, weight, tribe, residence, and duration of disease, complications, medications, occupation and other diseases.

Ethical considerations

This study was posed no physical risk participants through an interview of 5 minutes. Neither the participant name nor his institution will used in any of study material. Ethical approval for this study will be obtained from the ethical board Ministry of Health as well as informed written consent from patient or their guardians.

Method

Samples collection

4 ml of venous blood collected from cases and controls, samples divided into two part: 2.2 ml transferred into K_2EDTA container for CBC, and 1.8 ml had been transferred into Tri Sodium Citrate container (9 volumes of blood is added to 1 volume of a 32g/1 solution of TSC anticoagulant) and separated by centrifuge 3000 rpm for 15 min, to obtain platelet poor plasma for D-dimer test.

Specimen processing

Tests performed on (PPP), which is prepared by centrifugation at 3000 rpm for 15 minutes for D-Dimer. Whole blood in EDTA for blood count (plts count, MPV, PDW, and P-LCR).

Measurement of platelet parameters

A blood cell counter Sysmex KX-21 was used to measure platelets parameter. All measurements (platelet count, MPV, PDW and P-LCR) were measured in whole blood anticoagulant with EDTA.

All measurements were made between half and one and half hour of collection. The whole blood mode (WB) was selected to analyse the whole blood sample without pre-dilution. The sample number was entered before each sample. This procedure was followed:

- A well-mixed anticoagulant sample was set to sample probe, and the start switch was pressed till the aspirating process was finished (volume aspirate approximately 50 µl).
- The sample was removed straight down and the sample probe was automatically cleaned.
- The aspirate sample was then automatically suspended into different blocks and different parameters were measured.
- The results of parameters were then viewed on the screen and subsequently printed out.

D-Dimer

Nycocard was used to estimate the concentrate of D-Dimer fragments in the sample applied 50 µl of washing solution to the test devise, and then applied 50 µl from sample. The sample was absorbed into membrane in less than 50 seconds. Then applied 50 µl of conjugate to test devise. The conjugate was absorbed into membrane in less than 50 seconds, finally applied 50 µl of washing solution to devise. Test response should preferably be measured as soon as the washing solution has soaked completely into devise. The Nycocard D-Dimer single test result has to be measured within 2 minutes.

Results

The distribution of all compared groups and the result of each comparison are mentioned below.

Platelets count in diabetic and non-diabetic subjects

Platelets count in diabetic patients was lower (mean = 235.5 x 10^3 µl) than non-diabetic subjects (mean = 248 x 10^3 µl) this difference was statistically insignificant (P. value = .392).

MPV in diabetic and non-diabetic subjects

MPV in diabetic patients was higher (mean =10.5 fl) than non-diabetic subjects (mean = 10.07 fl) this difference was statistically significant (P. value = 0.034).

PDW in diabetic and non-diabetic subjects

PDW in diabetic patients was higher (mean = 14.5fl) than non-diabetic subjects (mean = 12.7 fl) this difference was statistically significant (P. value = 0.020).

P-LCR in diabetic and non-diabetic subjects

P-LCR in diabetic patients was higher (mean = 30.3%) than non-diabetic subjects (mean = 26%) this difference was statistically significant (P. value = 0.012).

D-dimer in diabetic and non-diabetic subjects

D-dimer in diabetic patients was higher (mean = 0.5008) than non-diabetic subjects (mean = 0.06) this difference was statistically significant (P. value = 0.015).

Platelets count in diabetic males and diabetic females

The difference in platelet count between both diabetic males (mean = 229.7 x 10^3 µl) and females (mean = 237.5 x 10^3 µl) was statistically insignificant (P. value = 0.767).

MPV in diabetic males and diabetic females

The difference in MPV between diabetic males (mean = 10.43fl) and diabetic females (mean = 10.6fl) was statistically insignificant (P. value = .653).

PDW in diabetic males and diabetic females

The difference in PDW between diabetic males (mean = 14fl) and diabetic females (mean = 14.7fl) was statistically insignificant (P. value = .469).

P-LCR in diabetic males and diabetic females

The difference in P-LCR between diabetic males (mean = 28.9%) and diabetic females (mean = 30.7%) was statistically insignificant (P. value = 0.531).

D-dimer in diabetic males and diabetic females

The difference in D-dimer between diabetic males (mean = 0.318) and diabetic females (mean = .1003) was statistically insignificant (P. value = 0.318).

Platelets count among diabetics according to age

The difference in platelet count was statistically significant (P. value = 0.036) between diabetic over 50 years old (mean = 219.3 x 10^3/µl) and diabetic under 50 years old (mean = 269.23 x 10^3/µl).

MPV among diabetics according to age

The difference in MPV was statistically insignificant (P. value = 0.375) between diabetic over 50 years old (mean = 10.66fl) and diabetic under 50 years old (mean = 10.34fl).

PDW among diabetics according to age

The difference in PDW was statistically insignificant (P. value = 0.381) between diabetic over 50 years old (mean = 14.79fl) and diabetic under 50 years old (mean = 14fl).

P-LCR among diabetics according to age

The difference in P-LCR was statistically insignificant (P. value = 0.371) between diabetic over 50 years old (mean = 31.09%) and diabetic under 50 years old (mean = 28.67%).

D-dimer among diabetics according to age

The difference in D-dimer was statistically insignificant (P. value = 0.353) between diabetics over 50 years old (mean = .37) and diabetics under 50 years old (mean = .692).

Platelet count among diabetics according to duration

The difference in Platelet count was statistically insignificant (P. value = 0.353) between diabetic group 1 - 10 years (mean = 236.3 x 10^3/µl), group 11 - 20 years µl (mean = 211.09 x 10^3/µl), group 21 - 30 years (mean = 267 x 10^3/µl),

MPV among diabetics according to duration

The difference in MPV was statistically insignificant (P. value = 0.845) between diabetic group 1 - 10 years (mean = 10.33fl), group 11 - 20 years (mean = 10.49fl), group 21 - 30 years (mean = 10fl).

PDW among diabetics according to duration

The difference in PDW was statistically insignificant (P. value = 0.489) between diabetic group 1 - 10 years (mean = 13.86fl), group 11 - 20 years (mean = 13.96fl), group 21 - 30 years (mean = 12.4fl).

P-LCR among diabetics according to duration

The difference in P-LCR was statistically insignificant (P .value = 0.759) between diabetic group 1 - 10 years (mean = 28.32%), group 11 - 20 years (mean = 29.6%), group 21 - 30 years (mean = 26.1%).

D-dimer among diabetics according to duration

The difference in D-dimer was statistically insignificant (P. value = 0.804) between diabetic group 1 - 10 years (mean = 0.167), group 11 - 20 years (mean = 0.150), group 21 - 30 years (mean = 0.13).

Discussion

Diabetes is associated with increased risk of atherosclerosis; enhanced activation of the clotting system has been implicated as an important contributing factor for the occurrence of vascular complications in diabetes mellitus.

The results of our study showed that no statistically significant difference in platelets count, correlated with study on platelets count in patients with diabetes mellitus has been found and They reported normal platelets count. while other platelet parameters (MPV, PDW and P- LCR) has been significantly increased in diabetic patients, correlated with study done by O.A Widaa at university of Khartoum reported that higher MPV in type 2 diabetic patients ($p = 0.015$) than non-diabetic subjects. Papanas M, Symeonidis G., *et al.* study showed higher MPV in type 2 diabetic patients versus non-diabetic controls ($p = 0.01$). Jindal S., *et al.* Studied platelet parameters (MPV, PDW) and reported that: there is statistically significantly higher MPV and PDW in diabetic patients ($p < 0.05$ for all) than non-diabetic subjects. Refik Demirtune A, Dursun Dunan., *et al.* also found that MPV was significantly higher in patients with diabetes mellitus than in control subjects (8.7 ± .8fl vs 8.2 ± .7, $p = .002$). PN Papanas A, G Symeonidisb., *et al.* found that MPV was significantly higher ($p = 0.01$) in patients with diabetes mellitus (14.2 ± 2.2 fl) than in control subjects (7.1 ± 1.2 fl).

D-dimer of our study showed significantly increased in case group compared with control. Yoshimasa Aso, Sachiko Matsumoto., *et al.* also found that plasma concentration of D-Dimer was higher in diabetic patients than in control subjects. Nwose EU., *et al.* also reported that D-Dimer level was significantly increased in diabetic patients ($p < 0.002$), but it differs from the results obtained by, Wieczor R., *et al*. and Marumo M., *et al.* whom their studies showed that; No significant impact was observed of diabetes on D-dimer [34,35].

Our study is supported by study done by Aberer F., *et al.* that showed, D-dimer was significantly increased after formation of hypoglycemic clamp in patients with type 2 diabetes [36].

In addition to platelet parameters and D-Dimer level we looked also into the association between age of type 2 diabetes mellitus and age of control group, their ages between 25 - 80 years old (mean = 46 years old) the result showed that: statistically higher platelets count in diabetic patients < 50 years old than diabetic patients > 50 years old. Most of the patients are NIDDM takes other medications about 21 patients (58%), while others are IDDM about 13 patients (32%) and about 6 patients (10%) do not takes any medications.

We also looked into the association between the measured parameters in the study with age and gender: we compared all studied males (9 diabetic males) to all studied females (31 diabetic females) in the case group, and we found that there are no statistically significant differences in all measured parameters.

In addition to gender and age we looked into the association between the measured parameters and duration of diabetes mellitus (duration classified into 3 groups) (1 - 10 years), (11 - 20 years) and (21 - 30 years). We found that no statistically significant difference in all measured parameters.

Conclusion

In the present study, consisted of 80 subjects, 40 are diabetes mellitus type 2 patients long standing more than 3 years, and 40 are healthy subjects, in the age of 25 - 80 years old. The study showed statistically significantly higher platelet parameters (MPV, PDW and P-LCR) and D-Dimer level in diabetic patients. Platelet count significantly increased in diabetic under 50 years old than diabetic over 50 years old, while age and duration have not any effect on changes of platelet count, MPV, PDW P-LCR and D-Dimer level.

Gender has not any effect on changes of platelet count, MPV, PDW, P-LCR and D-Dimer level. D-Dimer can serve as a novel marker for prediction of the risk of micro and macrovascular complications. D-Dimer if employed as an additional test may improve the risk assessment for early diagnosis of coronary artery disease and other vascular diseases in DM patients. In our study there is a higher concentration of D-Dimer in type 2 diabetic patients, it indicate increased procoagulant activity in type 2 diabetic patients. It is reasonable to assume that the higher level of D-Dimer is primarily the result of increased fibrin clot formation and subsequent breakdown. The increased thrombogenic state may be related to increased susceptibility to vascular diseases in these patients, D-Dimer and platelet parameters, hence, can be used as additional, novel biomarker of diabetic complications.

Recommendations

Based on this study the following recommendations are to be considered:

1. Further study should be done in larger sample size.
2. Another study should be conducted to correlate between changes in platelet parameters (platelet counts, MPV, PDW, and P-LCR) and platelet activity in diabetic patients.
3. Association between platelet parameters, coagulation abnormality in well controlled diabetic patients and uncontrolled diabetic patients (HbA1c) should be confirmed by other studies.
4. Similar studies on two groups of diabetic patients, one group with type 1 diabetes mellitus while the other group with type 2 diabetes mellitus.
5. Platelet parameters and D-Dimer level should be included in the spectrum of thrombotic tests carried on diabetes mellitus type 2 patients.
6. Additional studies should be conducted to confirm our findings, and more tests should be included to become routine tests for well follow up long standing diabetes mellitus type 2 patients in the future.

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