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Fibrinogen Plasma Level, Platelet Count and Mean Platelet Volume of Sudanese Diabetic Patients with Retinopathy

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Abstract

Diabetes mellitus is a carbohydrates disorder and is often associated with micro-vascular complications in particular which causes hemostatic abnormalities. This is a case control study conducted in Fedial Hospital Khartoum State during the period from June to October 2013. The study aimed to determine plasma fibrinogen level, platelet count and mean platelet volume (MPV) of diabetic patients (type 2) with retinopathy, 32 were males and 18 were females 150 were enrolled in the study with age ranged between 40-69 years, 50 of them were diabetic patients with retinopathy, mean age 52.7 ± 7.5 years, 50 were type 2 diabetes mellitus patients with mean age of 54.8 ± 8.5 years and 50 were non diabetic apparently healthy subjects with mean age was and 53.3 ± 7.9 years. Five ml venous blood was collected from each participant, 2.5 ml was collected in tri-sodium citrate container and poor platelet plasma was obtained. Stago coagulation analyzer was used to measure plasma fibrinogen level. The remaining 2.5 ml was collected in EDTA container and Sysmex autoanalyzer was used to measure platelet count and mean platelet volume. The results showed significant increase in plasma fibrinogen level in diabetic patients with retinopathy compared to both diabetic and control groups. Fibrinogen level increased from 278.3 ± 48.4 mg/dl in control group to 371.6 ± 56.6 mg/dl in diabetic patients with retinopathy. No significant difference in platelet count of diabetic patients with retinopathy and the other two groups. Mean platelet volume significantly increase from 10.1 ± 0.88 fl in diabetic patients to 11.2 ± 0.7 fl in diabetic patients with retinopathy. According to duration of diabetes mellitus, no significant difference was observed in fibrinogen level, blood platelet count or MPV. Elevation of fibrinogen plasma level and MPV indicated that diabetic patients are prone to hemostatic abnormalities irrespective of diabetes mellitus onset.

Keywords

Fibrinogen, Diabetes Mellitus, Retinopathy, Mean Platelet Volume

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1. Introduction

Diabetes mellitus is a global health problem associated with metabolic disorders. According to estimates of the World Health Organization there were 346 million individuals suffering from diabetes worldwide in 2011. [1]. Diabetes is one of the most common chronic hyperglycemic syndrome, affecting nearly 200 million people worldwide. If unchecked, by 2025, it is expected that diabetes will reach epidemic proportions, affecting 333 million people globally. Much of this increase is expected to occur in developing countries including India [2]. In Northern Africa prevalence of DM ranging from 18% to 75%, in Sudan diabetic prevalence was 2.6% and the prevalence of diabetic complications ranging from 8.1% to 41.5% for retinopathy [3]. The major complications resulting from T2 DM are related to micro-

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vascular and macro-vascular systems, the most common micro-vascular complications are nephropathy and retinopathy [4]. Macro and micro vascular complications are frequent in long standing patients with type 2 diabetes mellitus, thus metabolic syndrome and hyperfibrinogemia may contribute to early development of macro (ischemic heart disease) and micro (retinopathy) complications [2]. Platelets in DM have dys-regulatory signaling pathways that lead to an increased activation and aggregation in response to a given stimuli, platelets activation triggers thrombus formation causing micro-capillary embolization with the release of constrictive and oxidative and myogenic substances such as platelet – derived growth factor (PDGF) and vascular endothelial growth factor (VEGF) that accelerate progression of local vascular lesions such as neo-vascularization of lens in diabetic retinopathies. Hyperglycemia directly contributes to endothelial injury through irreversible glycation of collagen and other structural proteins of the blood vessels, forming advanced glycation end products [1] The osmotic effect of glucose together with hyperglycemia increases the propensity of platelets to aggregate and degranulate [4]. In addition**,** disorder of coagulation and fibrinolysis is significantly associated with diabetic retinopathy and nephropathy and exists at the early stage of microangiopathy [5]. Insulin resistance co-segregates with abnormalities in factors involved in coagulation, including platelet aggregability, platelet adhesion and levels of thrombaxane, von Willebrand factor, factor VIII tissue plasminogen activator (tPA) and fibrinogen [6] The entire coagulation cascade is dysfunction in diabetes, increased levels of fibrinogen and plasminogen activator inhibitor (PAI - 1) favors both thrombosis and defective dissolution of clots once formed, platelets in type 2 diabetic patients adhere to vascular endothelium and aggregate more than those in healthy individuals. Insulin is a natural antagonist of platelet hyperactivity, thus defects in insulin action in diabetics create disorders in platelet activity conductive to macro-vascular and micro-vascular events [6]. Platelet count was found to be decreased in diabetic patients but within normal range and did not significantly different between patients with and without complications [7]. Moreover, MPV is significantly higher in diabetic patients compared to healthy individuals, this indicate that elevated MPV (mean platelet volume) could be either the cause for or due to the effect of the vascular complications, hence platelet count and mean platelet volume can be used to assess the vascular events in diabetics [1] Thrombin – antithrombin III complex (TAT) and fibrinogen plasma levels were significantly higher in diabetic patients with retinopathy or nephropathy than in patients without diabetic complications [5]. An increased MPV has been associated with high incidence of proliferative diabetic retinopathy. Platelet count and MPV are simple, effective and cheap tests that may be used to predict angiopathy in type 2 DM. [8]

2. Materials and Methods

This is a case control study conducted in Fedail Hospital – Khartoum state during the period from June to October 2013. The study aimed to determine fibrinogen plasma level, platelet count and mean platelet volume (MPV) of Sudanese diabetic patients with retinopathy.

Sample size

One hundred and fifty individuals with matched age (between 40 and 69 years) were enrolled in the study, they were divided into 3 groups: 50 diabetic patients with retinopathy, 50 diabetic patients and 50 apparently healthy with matched aged subjects.

Inclusion criteria: subjects already diagnosed as diabetic patients and subjects with diabetes mellitus and retinopathy, both males and females were included.

Exclusion criteria: patients have diabetic complications such as nephropathy or CVD, or have other diseases that may affect hemostatic profile.

Demographic data was obtained via a designed questionnaire; clinical data of each patient was obtained from medical records. An informed consent was obtained from each participant before blood sample collection.

Sample analysis:

Fibrinogen plasma level was determined by Clauss Method using coagulation analyzer – Diagnostic Stago /

France, Commercial Kit Platelet count and MPV were determined by hematological analyzer

Sysmex KX -21.

Statistical analysis:

SPSS software program was used to obtain mean and SD of each parameter, ANOVA test was used for comparison of parameters of study population. Significance level was set at p- value ≤ 0.05.

3. Results

The study population with matched age which ranged between 40 to 69 years (Table 1). Male to female ratio in both diabetic patients and diabetic patients with retinopathy was 1: 1.1 (24:26) respectively.

Diabetic retinopathy was present among study group irrespective to age of patient. This indicated that glycemic control is a contributing factor for diabetic retinopathy on set regardless of age of patient.

Table 1. Distribution of study population according to age.

Age group	Control group N = 50		Diabetic patients N = 50		Diabetic patients with retinopathy N = 50	
Years	N	%	N	%	N	%
40 – 49	19	12.7	17	11.3	19	12.7
50- 59	19	12.7	16	10.7	16	10.6
60- 69	12	8.0	17	11.3	15	10.0
Total	50	33.4%	50	33.3%	50	33.3 %

Table 2. Fibrinogen plasma level of study population.

Study group	Fibrinogen level Mean ± SD (mg/ dl)	p- value
Control	278.3 ± 48.4	
Diabetic patients	300 ± 56.2	0.00
Diabetic patients with retinopathy	371.6 ± 56.6	

Fibrinogen plasma level significantly increased in diabetic patients with retinopathy compared to both diabetic patients and healthy individuals. Although diabetic patients and diabetic patients with retinopathy received anticoagulant medication, but fibrinogen plasma levels of diabetic patients and diabetic patients with retinopathy was significantly elevated compared to control subjects which strongly indicated that hyperfibrinogenemia is often associated with insulin resistance

Table 3. Blood platelet count of study population.

Study group	Blood platelet count Mean ± SD ($\times 10^3$)	p- value
Control	255.2 ± 74.6	
Diabetic patients	282.5 ± 68.4	P > 0.05
Diabetic patients with retinopathy	273.0 ± 69.3	

Platelet count of the study groups were within normal range ($p > 0.05$) This could be due to adequate glycemic control or due to falsely high platelet count of microcytic or fragmented red cells.

Table 4. Mean platelet volume of study population.

Study group	Mean platelet volume Mean ± SD (fl)	p- value
Control	10.1 ± 0.8	
Diabetic patients	10.2 ± 0.88	P < 0.05
Diabetic patients with retinopathy	11.2 ± 0.7	

Mean platelet volume significantly increased in diabetic patients with retinopathy compared to both diabetic patients without retinopathy complications and control. Accordingly, MPV could be considered as one of risk factors for micro-vascular complications in diabetic patients.

Table 5. Distribution of diabetic patients according to disease duration.

Duration of diabetes mellitus (years)		Patients no	
		Diabetic patients	Diabetic patients with retinopathy
Group I	4 ─ 9	22	22
Group II	10 ─ 19	18	18
Group III	20 ─ 25	10	10

22 out of 50 diabetic patients with retinopathy have diabetes mellitus less than 10 years

Table 6. Comparison of fibrinogen level of patients and duration of diabetes mellitus (Between groups).

Group	Fibrinogen level Mean ± SD (mg/dl)	p-value
I	379 ± 50	
II	371 ± 66	0.76
III	364 ± 40	

No significant difference in fibrinogen plasma level between the three groups. This is expected results since the three groups are subjected to inflammation which is common among diabetic patients and fibrinogen is an inflammatory marker which could be elevated irrespective of diabetes complications on set.

Table 7. Comparison of mean platelet volume of patients with retinopathy and duration of diabetes mellitus (between groups).

Group	Mean platelet volume Mean ± SD (fl)	p-value
I	11.1 ± 0.66	
II	11.4± 0.76	0.1
III	11.38 ± 0.44	

MPV of the three groups showed no statistical differences. Although MPV was elevated in diabetic patients with retinopathy compared to diabetic patients and control subjects, but duration of diabetes mellitus on set has no effect on MPV. Limitation of this study may be a contributing factor.

4. Discussion

Our results showed that fibrinogen plasma level significantly increased in diabetic patients with retinopathy compared to both diabetic and non-diabetic subjects. These results are confirmed by Azad *et al.,* [9] who stated that fibrinogen levels of patients with background retinopathy were significantly higher than those without retinopathy**.** However, hyperglycemia acts an fibrinolytic system by stimulating PAI – 1production, this condition favors the performance of fibrin clot, and consequently the development of thrombi [4]. Hyperfibrinogemia and mean fibrinogen level is significantly higher in diabetic patients with metabolic syndrome compared to diabetic patients without metabolic syndrome ($p < 0.001$) and the combination of metabolic syndrome and hyperfibrinogemia increase the risk of developing micro-vascular and macro-vascular complications [2]. In addition, PAI – 1, serum fibrinogen and vWF activity significantly increased in diabetic patients compared to healthy control subjects and PAI – 1 and vWF activity significantly increased in diabetic patients with micro-vascular complications compared to patients without complications and diabetic retinopathy was associated with decreased protein S levels and hypercoagulability as evidence by increased fibrinogen levels, decreased protein S levels and increased production of vWF by endothelium and hypofibrinolysis as evidence by increased PAI – 1 levels contribute to procoagulant state

observed in diabetics, this procoagulant state not only contributes to major vessel diseases but also to micro-vascular diseases [7]. In spite of elevation of plasma fibrinogen levels among diabetic patients with retinopathy but intensive control was associated with decreased progression of diabetic retinopathy in those with fibrinogen level < 296 mg/dl [9]. Furthermore, the prevalence of diabetic micro-vascular complications is higher in people with poor glycemic control, long duration of diabetes mellitus, hypertension and obesity [1].

The current study showed no significant difference in platelet count of diabetic patients with retinopathy compared to diabetic patients and control group (Table 3.) Similar findings was reported by Ritu *et al.,* [7] who reported that platelet count did not vary significantly between patients with diabetes with and without complications. Moreover, Ann *et al.,* [4] concluded that number of circulating blood platelets in diabetic patients is normal compared to non-diabetic individuals, but hyperglycemia, hypertension and dyslipidemia may independently cause vascular damage, endothelial dysfunction may be intrinsic to Type 2 diabetes mellitus and this condition may lead to activated state of blood platelets aggregation and adhesion. No significant difference in platelet count of diabetic patients with retinopathy compared to the other study groups. [9]. Other study showed conflicting results, some of them revealed higher platelet count in diabetic group compared to non-diabetic group, opposite findings was reported in other studies in which platelet count was lower in diabetic patients compared with non-diabetic healthy subjects, hence platelet counts in diabetic patients could be dependent on several variables, that is mean platelet survival, platelet production rate and turnover rate in DM [1].

Both diabetic patients with retinopathy and diabetic patients in this study were under aspirin therapy, and accordingly aspirin causes a reduction in the exposure of binding sites on both platelets from diabetic patients and normal subjects, in this respect platelets from diabetic patients become more like those from normal subjects [10]. Furthermore, aspirin in low doses inhibits thromboxane production by platelets, but has little or no effect on other sites of platelets reactivity [11]. Other studies showed hyperactivity of platelets associated with diabetes complications and platelets activation contributes to the pathology by triggering thrombus formation and causing micro- capillary embolization which accelerate progression of local vascular lesions like the neovascularization of lenses in diabetic patients with retinopathy. This could be due to quicker consumption of smaller platelets in the vascular events and compensatory production of reticulated platelets [1]. Some studies suggest an association between impaired platelets function and vascular complications in patients with diabetes mellitus and platelets accumulate in retinal vasculature and induce the release of local growth factors by causing inflammation and increased level of platelets derive growth factor in vitreous fluid of patients with proliferative diabetic retinopathy [12]. Platelet count decreased in diabetic patients but within normal range and did not vary between patients with or without complications [7]. Other study revealed that platelets hyperactivity and increased baseline activation in patients with diabetes mellitus is multifactorial, and different conflicting results were obtained and platelets count could be dependent on several variables, such as mean platelet survival, platelet production rate and platelets turn over in diabetes mellitus [1].

Mean platelets volume of diabetic patients with retinopathy increased significantly compared to diabetic patients without retinopathy and control groups (Table 4). Many studies confirmed these results [1, 12, 13, 14] Increased levels of MPV particularly in patients with proliferative retinopathy. The findings suggest the role for platelets in the pathogenesis of diabetic retinopathy [12]. Furthermore, the strong positive correlation with fasting blood glucose level and HbA IC levels and MPV elevation could be either a cause for or due to the effect of the vascular complications. Hence, platelets and MPV can be used as a simple parameter to asses vascular events in diabetic patients [1]. Increased MPV is closely associated with poor glycemic control, which may be a risk factor for diabetic retinopathy [13]. Significant difference (p value =.000) in MPV in diabetic patients with non-proliferative retinopathy compared to healthy subjects and diabetic patients, the risk of retinopathy development increases with Higher MPV values [14].

According to Table (5), 22 out of 50 of diabetic patients with retinopathy with duration of diabetes mellitus of less than 10 years, similar to a study in India where 28 out of 61 diabetic patients with retinopathy had diabetes less than 10 years which showed that plasma fibrinogen levels are significantly increased in patients with diabetic retinopathy and duration of diabetes and un controlled glucose levels are significant contributors to retinopathy development and duration of diabetes mellitus appears to be the most significant factor [15] this indicated that diabetes complications may start irrespective to diabetes mellitus onset, and this could be related to glucose control and diabetes mellitus management rather than duration of onset of the disease. However, the duration of diabetes was significantly longer in patients with any macroangiopathy than in patients without it [5].

The results of the current study showed no significant difference in fibrinogen plasma level and MPV of diabetic patients with retinopathy according to duration of diabetes (Tables 6 and 7). Many studies agreed with results of the

present study and complications of DM were not associated with duration of disease on set, no statistical correlation was seen between MPV, duration and the vascular complications of diabetes mellitus [1]. Accordingly, diabetic complications may be correlated to glycemic control and management of diabetes mellitus regardless to the duration of DM, this is confirmed by Azad *et al.,* [9], who stated that there was an interaction between glycemic treatment assignment and fibrinogen level at baseline and intensive control was associated with decreased progression of retinopathy in patients with fibrinogen < 290 mg/dl. However the exact duration of diabetes may sometimes be difficult to determine and patients do not present with diabetic retinopathy soon after diagnosis of diabetes [14]. MPV was significantly higher in diabetics with HbA 1c levels ≥ 6.5% than in diabetics with HbA 1c levels = 6.5% and there was a significant association between HbA 1c and MPV. The same author explained the increased number of diabetics in India may be due to poor dietary practices and lack of knowledge regarding the diet and exercise regimen which was also observed in many developing countries [1]. In addition, other studies reported positive relationship between the MPV and duration of diabetes which gives evidence that the risk of microvascular complications increases with duration of diabetes [8]. Furthermore, elevation of MPV of diabetic patients compared to control subjects was further documented by many studies, but MPV was not altered with the stage of diabetic retinopathy [16] which agreed with the findings of this study.

5. Conclusion

Plasma fibrinogen level and MPV were elevated in diabetic patients with retinopathy compared to diabetic patients and control subjects. Elevated fibrinogen plasma level and MPV reflects hypercoagulability associated with diabetic retinopathy irrespective of diabetes mellitus onset. Further studies are needed to confirm the current results.

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