Original Article

Hemostatic Abnormalities in Diabetic Patients

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ABSTRACT

Objective: To evaluate hemostatic Abnormalities in Diabetic patients via deranged PT, APTT and D-dimer.
Study Design: Descriptive study
Place and Duration of Study: This study was conducted at the Pathology Department, Bacha Khan Medical College and MMC Teaching Hospital Mardan from September 2016 to June 2017.
Materials and Methods: In study 100 patients of Diabetes Mellitus (type-2) and 50 healthy cases as control were included. Sample was divided into Group A and Group B, each with 50 patients on the basis of glucose levels, 200-300 mg/dl and 300-400 mg/dl respectively. In all these patients Hemostatic markers were studied.
Results: In Group A, 25 patients had elevated D-dimer level. Among 20 of them D-dimer level was 250-500 ng/ml and 5 had 500-1000 ng/ml level, however 3 patients had shortened PT, mean value 12.562±0.432 seconds while APPT was normal. In Group B 27 patients had raised D-dimer level. Among 20 of them D-dimer value was 500-1000 ng/ml and 7 had 250-500 ng/ml. However 2 patients had shortened PT and 2 had prolonged APTT. Mean values of 13.562±0.232 and 43.562±0.262 seconds respectively. This study show that PT and APTT were not significantly altered, while D-dimer level was significantly elevated in both groups in comparison to control group, P<0.00326 and P<0.00322 respectively.
Conclusion The study concluded that Diabetes Mellitus is associated with significant hemostatic abnormalities and deranged hemostatic markers identify patients prone complications and helps in reducing morbidity and mortality in Diabetic patients.

Key Words Diabetes Mellitus, D-dimer, PT, APTT

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INTRODUCTION

Diabetes is the most important cause of vascular Morbidity, often associated with cardiovascular compilations, hypercholesterolemia, hypertension, obesity and increased markers of coagulation and inflammation.[1] cerebrovascular, peripheral vascular and Coronary heart disease has 80% prevalence in patient with Diabetes Mellitus.[2] Cerebrovascular disease and peripheral vascular disease is tenfold more common in Diabetic patients.[3] Increased risk of stroke has also been reported in diabetes mellitus.[4] Diabetic patients have a hyper-coagulable state, associated with high risk of thrombus formation and accelerated atherosclerosis. This is evident by increased serum level of fibrinogen, low levels of plasma proteins and increased formation of von-Willi brand factor by Endothelium.[5] Diabetes Mellitus (type 2) patients can have both micro-vascular and macro-vascular complications.[6]

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Common micro-vascular complications in these patients include neuropathy, nephropathy and retinopathy while coronary artery disease, peripheral arterial disease and strokes are macro-vascular complications.[7] Diabetes Mellitus has a hypercoagulable state and hemostatic abnormalities are commonly seen in this disease. Among diabetic patients 80% die of thrombotic complications and 70% deaths result from cardiovascular events.[8] Metabolic disturbances commonly occur in type 2. Diabetes Mellitus such as atherogenic dyslipidemia, hypertension, glucose intolerance and a pro-thrombotic state.[9] The pro-thrombotic state is caused by increased fibrinogen level, increased plasminogen activator inhibitors and many different abnormalities.[10] Diabetes Mellitus is a major public disease and involves a huge population of the world, and about 347 million individuals are affected. The aim of the study was to identify those diabetic patients who are at increased risk of hypercoagulable and pro-thrombotic states, by measuring coagulation marker like D-dimer PT and APPT which can provide immediate information regarding thromboembolic conditions and also useful to the clinician to reduce the complications of the disease.

MATERIALS AND METHODS

The study was done in the Medical unit of MMC teaching hospital Mardan and Pathology Laboratory of

Bacha Khan Mediacal College Mardan. Patients were divided into two groups, group A and group B and a control group C. In each group 50 cases were included. Group A had 50 patients of diabetes having glucose level at the range of 200-300mg/dl. Group B included patients having glucose level of 300-400 mg/dl. Group C had 50 normal healthy individual as control. Only diabetic patients both adults males and females were included in the study. The patients with Hypertension and history of DVT, pulmonary Embolism, and septicemia were excluded from the study.

Blood sample of 5 ml was collected from each patients in a tube containing sodium citrate, the plasma was then separated and this plasma was then utilized for determination of coagulation markers. Prothrombin time (PT), D-dimers and Activated partial thromboplastin time (APPT) were studied. D-dimer is a fragments of clot, it is formed due to proteolytic degradation of clot by enzyme plasmin. D-dimer fragments increases in any condition where both clot formation and subsequent fibrinolysis are increased so measurement of D-dimer identity both clot formation and its subsequent degradation. Its function is to determine the seventy of hypercoagulable state, as hypercoagulable state is more prone to thrombogenic state and this D-dimer is a reliable marker for systemic pro-thrombotic conditions or clot formation in the body. Minutex D-dimer is a latex agglutination test for semi-quantitative determination of D-dimer fragments. Minutex D-dimer contains a monoclonal antibody reacting with fibrin D-dimer fragments. In this method, 2 µl of sample is mixed with 20 µl of D-dimer reagent and agglutination is seen within 3 minutes. If agglutination is positive then serial dilution is done. Serial dilutions of test plasma are made with Buffer: for 1:2 dilution 100 µL test plasma, 100 µL Buffer solutions is added. For 1:4 dilutions, to100 µL of 1:2 diluted plasma add 100 µL Buffer solutions. For 1:8 dilution, to 100 µL 1:4 dilution plasma add 100 µL Buffer solution. Then with each dilution sample test is repeated to quantitatively measure D-dimer concentration. Normal level of D-dimer is less than 250 ng /ml in undiluted sample. When positive in undiluted sample its level is 250-500 ng/ml, if agglutination seen in 1:2 dilution its level is 500-1000 ng/ml if agglutination seen in 1:4 dilution D-dimer level is 1000-2000 ng/ml, if agglutination seen in 1:8 dilution D-dimer level is more than 2000 ng/ml. Their raised levels characterize thromboembolic condition in the body in any system and are therefore a useful hemostatic marker and help the physician to go further for other supportive investigation and treatment option. PT and APTT are also hemostatic investigation and measure the activity of both extrinsic and intrinsic pathway of coagulation cascade. Normally PT level is 10-14 seconds and APPT level is 30-40 seconds. Its increased or low level gives information to the clinician about the hemostatic states of the patients. These investigations were also performed according to standard method manually and both by coagulation analyzer for accuracy. Its prolongation signifies the coagulation factor deficiency or consumption, while its low level signifies a hypercoagulable state.

The data was statistically analyzed by Chi-Square and T-test, level of significance was set at $P<0.0005$.

RESULTS

In our study 100 patients were included, which were divided into two groups, group A and group B. Group A patients had glucose level at the range of 200-300 mg/ml and Group B patients had glucose level at the range of 300-400 mg/ml. Group C included healthy individual as a control group.

In Group A 25 out of 50 diabetic patients had raised D-dimer level, Mean D-dimer level was 250-500 ng/ml and 5 out 50 patients, D-dimer level was 500-100 ng/ml while rest of the patients had normal value. A result is shown in table 1.

In Group B 27 out 50 patients had elevated D-dimer level, 20 had D-dimer level of 500-1000 ng/ml and 7 had D-dimer level of 250-500 ng/ml. as shown in table 2.

In both the two groups D-dimer level was significantly raised as compared to control group, P value was significantly elevated in both the two groups $P<0.0032$ in Group A and $P<0.0042$ in Group B respectively. From study we concluded that D-dimer signifies coagulation activation in diabetic patients and give useful information to the clinician about the hypercoagulable state and subsequent thromboembolic condition.

Similarly in Group A, 5 out of 50 cases had shortened PT while in Group B, 3 out of 50 cases had shortened PT also 2 out of 50 patients had prolong APTT. As shown in Table 1 and Table 2. D-dimer, PT and APTT are haemostatic markers. D-dimer is the fibrin mediated proteolytic degeneration of fibrin clot and its increased level shows increased fibrin turn over, PT and APTT also haemostatic marker and its shortened and increased level also signify haemostatic abnormally in the coagulation system.

D-dimer assay were performed by agglutination method and a semi-quantitative procedure both in diluted and undiluted form according to stand operation method. PT and APTT were also performed both by manual and coagulation analyzer for accurate results. P values for D-dimer in Group A and Group B is $P<0.00326$ and $P<0.00322$ respectively.

Table No.1. Frequency of Abnormal coagulation markers in Group A Diabetes Mellitus

S.No	Test	Percentage (%)
1	D-dimer	25 out 50 (50%)
2	PT (shortened)	3 out 50 (6%)

Table No.2. Frequency of Abnormal coagulation markers in Group B Diabetes Mellitus

S. No.	Test	Percentage (%)
1	D-dimer	27 out 50 (54%)
2	PT shortened	2 out 50 (4%)
3	APTT Prolonged	2 out 50 (4%)

Table No.3. Mean Value of coagulation parameters / markers in Diabetes Mellitus in both Groups

Groups	D-dimer Level in Group A and B		PT	APTT
Group A 200 to 300 mg/dl	20 out of 50 patients	250-500 ng/ml	3 out of 50 12.562±0.432 seconds	
	5 out of 50 patients	500-1000 ng/ml		
Sugar level in Group B. 300-400 mg/dl	20 out of 50 patients	500-100 ng/ml	2 out of 50 13.65±0.23 seconds	2 out of 50 43.56±0.262 seconds
	7 out of 50 patients	250-500 ng/ml		
Control Group	5o patients	<250 ng/ml	14.562±0.246 seconds	40.325±0.262 seconds

DISCUSSION

Diabetes Mellitus Type 2 is a major public health disease and is a hypercoagulable and pro-thrombotic state, evident by many studies. Diabetes Mellitus type-2 is associated with both micro-vascular and macro-vascular complications which may result from disturbance in Hemostatic mechanism and reduced fibrinolytic activity. The entire coagulation cascade is dysfunctional in Diabetes Mellitus, which may result in a variety of complication. In this study we evaluated coagulation activation markers, D-dimer, PT and APTT. In the present study, in group A (who had glucose level at 200-300mg/dl), D-dimer level was raised in 25 out of 50 patients (50 %), Mean D-dimer level was raised to 250-500 ng/ml, in group B diabetic patients (whose glucose level were at the range of 300-400 mg/dl), D-dimer level was raised up to 500-1000 ng/ml in 27 out of 50 patients (54%) . A similar study had been conducted by Lentonja et al who reported elevated D-dimer level in their Diabetic patients.[2] Another study was conducted by Muhsin et al who reported elevated D-dimer, level in Diabetic patients Type 2.[12] The same observation had also been reported by Long Z et al that Diabetic Mellitus Type 2 is associated with elevated D-dimer level.[14]

Diabetes Mellitus has hypercoagulable state and is associated with increased risk of atherosclerosis and Hemostatic abnormalities and the development of Micro and Macro-vascular complications. The thrombogenic and atherogenic fibrinogen level and increased platelet aggregation contribute to fibrin clot formation.[15]

Premature atherosclerosis with more extensive vascular damage, platelet hyper-reactivity, increased activation of pro-thrombotic coagulation factors and depressed fibrinolysis all contribute to increased thrombosis in Diabetes.[16] Increased expression of tissue factor (TF), raised fibrinogen level are important in atherosclerosis complications.[17] Increased expressions of Tissue Factor (TF) and increased synthesis of thromboxane A2 potentiate thrombosis and increase fibrin deposition and responsible for pro-thrombotic state of Diabetic patients.[18]

In the present study in group A has 3 out of 50 patients had shortened PT while APTT were in normal range while in group B only 2 out of 50 patients had shortened PT while APPT 3 out of 5o were prolonged both of these values were not significantly changed as compare to control.

A study conducted by Sunita et al on Type 2 diabetic patients, also reported shortened PT and APPT which shows similar correlation to our study.[19] In studies conducted by Yang Zaho et al and Wolfarang Korte et al. They also reported shortened PT and APPT.[20,21]

But study performed by Abdul Rahman et al disagree with our study and they observed prolonged PT and APPT in their study of significant degree.[22] Various observation have been given by different authors regarding PT and APPT derangement in Diabetic patients. Some reported shortened and some prolonged PT and APPT the exact mechanism is not clear, but there is perturbation associated with anticoagulant system and glycemic status of diabetic patients. Hyperglycemia cause depression of the biological activity of the anticoagulant proteins such as Antithrombin-İİİ[23] and dysfunctional Antithrombin-İİİ leads to prolong PT and APPT, others reported that there is increased generation of thrombin and elevated level of both Thrombin and Prothrombin contribute to both thrombotic risk and shortened PT and APPT.[20] Diabetes Mellitus is also associated with Endothelium injury, platelet reactivity, elevated levels of coagulation factors, defects in natural anticoagulant and fibrinolytic system all these changes are caused directly or indirectly by hyperglycemia and as a whole Diabetes Mellitus is a state of hyper-coagulability and hypofibrinolysis which finally contribute to Hemostatic abnormalities and other complications.[24]

CONCLUSION

The study concluded that the Diabetic Mellitus is a hypercoagulable state and is more prone to cardiovascular, cerebrovascular, micro-vascular, macro-vascular and other thrombotic complications so these Hemostatic markers have both preventive and prognostic value to reduce Morbidity and mortality from Diabetic Mellitus.

Author's Contribution:

Concept & Design of Study:	Subhan Uddin
Drafting:	Shahtaj Khan
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Conflict of Interest: The study has no conflict of interest to declare by any author.

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