SUST
Journal of Natural and Medical Sciences

Journal homepage: http://journals.sustech.edu/

Prothrombin Time (PT) and Activated Partial Thromblastin Time (APTT) In Sudanese Diabetic Patients – Khartoum State

Ayman H. Abdeen and Khalda M. Hamza*

Department of Haematology, College of Medical Laboratory Science, Sudan University of Science and Technology
*Corresponding author E-mail:khaladahamza@yahoo.com
Article history: Recieveed: 29.04.2014
Accepted: 25.09.2014

ABSTRACT

Diabetes Mellitus is a common endocrine disease of multiple etiology. It is characterized by chronic hyperglycemia with subsequent disturbance of carbohydrates, proteins and lipids metabolism. Type 2 diabetes Mellitus and insulin resistance syndromes are associated with an increased risk for cardiovascular diseases and thrombotic complications. PT and APTT are hematological indices that predict the coagulation status of patients This is a case control study aimed to determine Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) in patients with diabetes mellitus. This study was conducted at Jaber Abu Eleaz Center and Turiskh Teaching Hospital in 2010 . One hundred diabetic patients (84 with type 2 and 16 with type 1 diabetes mellitus) were enrolled in the study with age ranged between 5 and 75 years. Twenty apparently healthy non- diabetic subjects were selected as a control group.An informed consent was obtained from each participant before sample collection. A 2.5 ml blood specimen was collected from each participant in EDTA container from which platelet poor plasma specimens were obtained, then PT and APTT were determined using calibrated coagulometer (Bio-Bas). SPSS soft ware computer program was used for data analysis (t-tests and ANOVA tests).Significance level was set with p- value ≤ 0. 05. 28% of patients with age less than 36 years and 3 % with age less than 15 years. Insignificant prolongation of PT and APTT was observed in patients compared to control but within normal range. PT was ncreased from 14.14 ± 0.512 to 14.4 ± 1.18 seconds in control and patients respectively. APTT was increased from 25.95 ± 3.09 in control to 27.06 ± 3.92 seconds in diabetic patients. On the other hand, according to disease duration, PT of patients was within normal range, but APTT was prolonged progressively with increased period of disease on set. No statistical difference in PT of patients with type 1 compared to PT of type 2 diabetic patients. However, APTT was increased from 26.51± 3.4 seconds in patients with type 2 diabetes mellitus to 30.40 ± 5.2 seconds in patients with type1. In conclusion, some prolongation of PT and a true APTT was observed in diabetic patients compared to non diabetic control. APTT prolongation is related to period of disease onset. Diabetic patients were subjected to haemostatic abnormalities, accordingly routine coagulation tests are recommended for better management of diabetes mellitus.

المستخلص

مرض السكري من أمراض الغدد الصماء والذي يسبب إختلالاً في التمثيل الغذائي للمواد النشوية والبروتينية والدهنية. مرضى السكري من النوع الثاني أكثر عرضةَ للإصابة بأمراض القلب والأوعية الدموية والجلطات. تعتبر بعض عوامل الدم مثل زمن الثرومبين وزمن الثرمبوبلاستين الجزئي النشط مؤشراَ للتعرف على مضاعفات مرض السكري.تهدف هذه الدراسة لتحديد زمن البروثرمبين وزمن الثرمبوبلاستين الجزئي النشط لمرضى السكري . أجريت هذه الدراسة بمركز جابر أبوالعز والمستشفى التعليمي التركي . تم إختيار 100 مريضاً ، 84 منهم مصاباً بالنوع الثاني من مرض السكري و 16 منهم مصاباً بالنوع الأول. تم إختيار 20 من المتبرعين الأصحاء ظاهرياً كمجموعة ضبط . بعد موافقة المشاركين ، اخذت 2.5 مل من الدم في وعاء يحتوي على مادة مانعة للتجلط . بعد الحصول على بلازما الدم وبإستعمال جهاز قياس عوامل التجلط . 28% من المشاركين تقل أعمارهم عن 36 عاماً و 3 % تقل أعمارهم عن 15 عاماً. أظهرت الدراسة إرتفاعاً ذات دلالة غير إحصائية في زمن البروثرمبين وزمن الثرومبوبلاستين الجزئء النشط عند المرضى مقارنةً بمجموعة الضبط . حيث إرتفع زمن البرثرومبين من 0.512 ± 14.14 ثانية عند مجموعة الضبط الى 1.18 ± 14.40 ثانية عند مرضى السكري . لا توجد فروقات ذات دلالة إحصائية في زمن البروثرومبين وفترة الإصابة بمرض السكري ، اما زمن الثرومبوبلاستين الجزئي النشط فقد إرتفع كلما زادت فترة الإصابة . لا توجد فروقات ذات دلالة إحصائية في زمن الثرومبين عند مرضى السكري من النوع الأول والثاني أما وزمن الثرومبوبلاستين الجزئي النشط فقد إرتفع من 3.4 ±26.51 ثانية عند مرضى السكري من النوع الثاني الى 5.2 ±30.40 ثانية عند مرضى السكري من النوع الأول. خلصت الدراسة الى أن زمن الثرومبين وزمن الثرومبوبلاستين الجزئي النشط إرتفع عند مرضى السكري مقارنةً بمجموعة الضبط . إرتفع زمن الثرومبلاستين الجزئي النشط إرتفاعاً حقيقياً كلما إزدادت فترة الإصابة بمرض السكري.مرضى السكري عرضة لإختلال آليات تجلط الدم لذلك يجب ان يكون هنالك فحص روتيني لقياس عوامل التجلط مما يساعد في التحكم في مضاعفات المرض .

***KEYWORDS* :**Thrombosis, Diabetes Mellitus, Thrombin , coagulation.

INTRODUCTION

Homeostasis is one of a number of protective processes which are evolved in order to maintain a stable physiological state. The haemostatic system is a complex of activating or inhibitory feedback or feed- forward pathways, integrating its five major components (blood vessels, blood platelets, coagulation factors, coagulation inhibitors and fibrinolytic elements) [(1)].

Prothrombin Time (PT) reflects the activities of factors II, V and factor X which leads to formation of thrombin and fibrin polymerization takes place and a clot is formed. Therefore PT is sensitive to the activities of factors II, V, VII and X. International Normalized Ratio (INR) is a derivative of PT is useful to monitor anticoagulation with warfarin. INR =Patient PT/ control PT. Activated Partial Thrombin Time (APTT) is affected by coagulationfactors of intrinsic pathways (factors II, V, VIII, IX. X, XI and XII). APTT is a useful screening tests [(2)]**.** Diabetes Mellitus (DM) is a metabolic carbohydrates disorder in which glucose is underutilized producing hyperglycemia.

Chronic hyperglycaemia may be due to relative insulin deficiency, or resistance or both. Type 1 diabetes formally known as insulin dependent diabetes mellitus (IDDM), approximately 5%-10% of all individ-uals have type 1 diabetes mellitus. Type 2 diabetes mellitus formally known as non-insulin dependent diabetes mellitus (NIDDM). Type 2 diabetes comprises 90% of all individuals with diabetes mellitus **(3)**. In Sudan, diabetes prevalence is 2.6% (4).

Undiagnosed DM is common in Northern Africa with a prevalence ranging between 18% to 75%, In Nigeria and worldwide at large, diabetes mellitus is a major health problem with about 90% of diabetic patients with type 2 and only 10 % with type 1 diabetes mellitus. Increased plasma level of PT and APTT are consistent with abnormal coagulation mechanisms and may be interpreted as a tendency of bleeding and cardiovascular disorders**.**

Patients with diabetes mellitus with persistent hyperglycemia exposes RBC to elevated glucose concentration, resulting in glycalation of hemoglobin, prothrombin, fibrinogen and other proteins involved in clotting mechanisms. Glycalation of intrinsic and extrinsic clotting proteins will decrease the availability of these proteins which affect the clotting capacity **(5)**. Insulin resistance is the inability of insulin to stimulate glucose up – take. Approximately 50% of patients with Type 2 diabetes are insulin resistance and they are subjected to atherothrombotic risk factors including changes in platelets, coagulation and fibrinolytic pathway**(1)**.

Impaired fibrinolysis is found in impaired glucose tolerance and type 2 diabetes and the risk of stroke and myocardial infarction is considerably increased in subjects with diabetes mellitus **(6)**. Several studies of fibrinolytic system in diabetes have provided conflicting results , type 2 diabetes is often associated with profound depression of fibrinolysis(7)Endothelial abnormalities play a role in the enhanced activation of platelets and clotting factors are seen in diabetic patients, coagulation activation markers such as : fibrinogen, F VII, FXII and vWF are elevated in diabetes**.(8)**

MATERIALS and METHODS

This is a descriptive analytical case control study aimed to determine PT , APTT and INR of Sudanese Diabetic patients (type 1 and type 2). The study was conducted in Jaber Abu Eleaz Center and Turkish Teaching Hospital in 2010. One hundred diabetic patients of both sex and with different age group were enrolled in the study after fulfilling clinical diagnosis of type 1 and type 2 diabetes mellitus .

Twenty apparently healthy individuals were selected as a control group. Clinical data was collected through a designed questionnaire. A written consent was obtained from each participant before blood sample collection. 2.5 ml of venous blood was collected in aqueous trisodium citrate pentahydreate in 1:9 ratio of anticoagulant. Platelet poor plasma (PPP) was prepared by centrifugation of citrated blood at 3000rpm for 15 minutes.

Coagulom-eter (Bio- Bas1) was used for PT and APTT Determination following the instructions provided by Dia Med AG Company – Switzerland. The coagulometer was caliberated accord-ing to Standard Calibration Manual approved by Ministry of Health – Khartoum – Sudan. SPSS software program version 11.5 was used for T-test and one way ANOVA

tests, significance level was set at $p \leq 0.05$.

RESULTS

Out of 100 patients, 28% were less than 45 years of age and 3% showed age range of 5 – 15 years (table 1).

Table1: *Distribution of Diabetic Patients according to age*

Age group (years)	Percentage
5 – 15	3.0
16-25	9.0
25- 35	13.0
36- 45	28.0
46-55	25.0
56-66	16.0
66-75	6.0
Total	100

42% of the patients with disease on set between 1 to 5 years, 30% with disease on set between 6 to 10 years (Table 2).

Table 2: Distribution of Diabetic Patients according to duration of disease onset

Duration of DM (years)	Percentage %
1- 5	42
6-10	30
11- 15	11
16-20	11
21- 25	4
26-30	2
Total	100

PT showed no significant difference between diabetic patients and control. True increase of APTT with prolonged duration.

Table.3:PT and APTT of Diabetic Patients compared to control group

Study group	Diabetic patients N =100. mean± SD	Control N= 20 mean± SD	P- value
Parameter			
PT (seconds)	14.42± 1.18	14.14 ± 0.512	0.699
APTT (seconds)	27.06 ± 3.92	25.95 ± 3.09	0.237

Insignificant prolongation in PT and APTT of patients compared to control (table)($p = 0.699$ and 0.237) respectively. According to disease duration, PT was within normal range but APTT was prolonged progressi-vely with duration of disease onset (table 4).

Table 4: PT and APTT of diabetic patients with different disease duration

Duration of DM (years)		**N**	**Mean ± SD**	**P – value**
PT	1-5	42	14.22 ± 1.11	0.743
(seconds)	6-10	30	14.40 ± 1.46	0.448
	11-15	11	13.81 ± 1.12	0.279
	16-20	11	14.94 ± 0.68	0.114
	21-25	4	13.60 ± 0.68	0.080
	26-30	2	14.55 ± 0.92	0.318
control		20	14.14 ± 0.51	
APTT	1-5	42	26.38 ± 3.69	0.652
(seconds)	6-10	30	26.91 ± 3.34	0.311
	11-15	11	27.86 ± 4.23	0.162
	16-20	11	27.78 ± 5.09	0.224
	21-25	4	29.05 ± 6.20	0.138
	26-30	2	31.15 ± 1.48	0.032
	control	20	25.95 ± 3.09	

Insignificant difference in PT of patients with type 1 diabetes mellitus compared to PT of patients with type 2. However, APTT was significantly prolonged in patients with type 1 compared to APTT of patients with type 2 DM (Table 5).

Table 5: Comparison between PT and APTT of type 1 and type 2 diabetic patients

Type of DM		**N**	**Mean ± SD**	**P – value**
PT	: Type 1	14	14.64 ±1.8	0.234
(seconds)	: Type 2	86	14.17 ± 1.05	0.862
	: control	20	14.14 ± 0.521	
APTT	: Type 1	14	30.40 ± 5.2	0.004
(seconds)	: Type 2	86	26.51 ± 3.4	0.500
	: control	20	25.95 ± 3.09	

DISCUSSION

Our study showed that 42 % from total diabetic patients with disease duration of less than 5 years which reflects progressive increase in number of diabetic patients. Hassan in (2009 **)** [7] reported that 40% of diabetic patients in Saudia Arabia with disease duration of less than 5 years. The results of the present study showed insignificant prolongation of PT and APTT of diabetic patients compared to control. Similar findings were reported by Abdeulrahman and Dallatu[5], they stated that both PT and APTT of treated diabetic patients were insignificantly different compared to non diabetic group. However, Hassan study showed significant prolongation of PT (p value = 0.02) in Saudi patients which disagreed with our study which showed no significant results, but APTT was insignificantly prolonged which was in consistent with our findings . The same author stated that in various reports plasma PT levels were found to be either decreased or increased according to the type of diabetes, while reduced plasma APTT levels have been described in patients with type 1 diabetes mellitus and elevated in patients with type 2 diabetes mellitus and both coagulation and fibrinolysis are enhanced concomitantly in patients with diabetes mellitus. Furthermore Erem *et al.* [9]**,** concluded that PT was significantly prolonged in type 2 diabetic patients compared to healthy subjects which disagreed with the present results . Although a study by Alao *et al.*[10]**,** in Nigeria, revealed a significant

prolongation in PT and APTT in diabetic patients compared to non-diabetic control but the values were within normal limits, which disagreed with our findings. The same authors reported that prolongation of PT and APTT suggest that diabetic patients may be prone to hemorrhagic complications and hypercoagulable tendency resulting from a shift of thrombo – hemorrhagic balance in favor of thrombosis. According to Hassan [(7)], hyperglycemia has been considered to be the causative factor of the abnormalities of the anticoagulant pathway and enhanced activation of the clotting system has been previously reported in patients with type1 and 2 diabetes mellitus and patients with type 2 diabetes mellitus had hypercoagulable state and hypofibrinolysis thereby increasing the risk of CVD of type 2 diabetic patients. Abdeulrahman and Dallatu[(5)], reported that in patients with diabetes mellitus, abnormalities in coagulation hemostatsis, platelets dysfunction and reduced activity of fibrinolytic system collectively accelerate atherogensis, increase in tissue factor (TF) and subsequent conversion of inactive factor VII to active factor VII which triggers the extrinsic pathway which lead to activation in PT, and the increase in the intrinsic pathway proteins and activation of blood coagulation mechanisms are consistent in diabetic patients. Stegenga *et al.*[(11)],reported thatHyperglycemia selectively stimulates coagulation irrespective of insulin level, whereas hyperinsulinemia inhibits fibrinolysis by enhancing plasminogen activator inhibitor 1(PAI-1) secretion, hence the presence of both hyperglycemia and hyperinsulinemia such as in type2 diabetes mellitus has a strong procoagulant effect by enhancement of coagulation simultaneous inhibition of fibrinolysis.[(11)] In subjects with normal glucose tolerance, elevated levels of fasting insulin are associated with increased circulating of PAI-1levels, providing further evidence for a link between hyperinsulinemia and impaired fibrinolysis, also coagulation activation markers, including thrombin-antithrombin complexes (TATcs) have been found to be elevated in patients with type 2 diabetes[(11)]. Moreover, Meigs *et al.* [(12)], observed a strong positive association between levels of fasting insulin and levels of PAI -1 antigen, t-PA antigen, factor VII antigen, vWF anigen and fibrinogen, suggesting a mechanism for increased risk factor for CVD and acute thrombosis associated with hyperinsulinemia.Eilasson *et al* .[(6)], reported thatincreased plasma levels of PAI-1 were strongly related to the development of diabetes independent from insulin resistance, the possibility of elevated PAI-1 being a very early risk marker of diabetes. Furthermore , haemostatic variables related to endothelial function such as vWF and factor VIII also predicted diabetes especially in women. The results of the present study showed no significant difference of PT and APTT according to disease duration, but APTT prolongation was consistent with duration of disease , (21 – 30 years) - latest stage with expected complications - accordingly an association between and duration of disease onset and APTT may be present. Binaya *et al.* [(13)], concluded that association of APTT with duration of diabetes mellitus is not significant ($p > 0.05$). Insignificant difference in PT of patients with type 1 diabetes mellitus compared to patients with type 2. APTT of type 1 diabetes mellitus was significantly prolonged compared to APTT of patients with type 2 diabetes mellitus.($p < 0.004$). In an epidemiological study in Wisconsin and after ten years follow up of type 1

diabetic patients , for every one percent increase in glycated hemoglobin, the risk for CVD nearly doubled[14] .

According to the present results PT and APTT failed to detect hypercoagulable state of diabetes. Other factors deficiency or presence of coagulation inhibitors could interfere with APTT prolongation [15] . Furthermore Alao *et al.,* suggested that PT prolongation could be possibly alter in vivo- pathways that may occasionally tilt thrombotic-hemorrhagic balance in favor of hemorrhage in some diabetics.

CONCLUSIONS

PT and APTT of diabetic patients were prolonged compared to non diabetic control subjects. APPT was prolonged progressively with increased duration of disease on set. PT of type 1 and type 2 diabetic patients was within normal range, but APTT of type 1 diabetic patients was prolonged compared to patients with type 1 diabetes mellitus. Diabetes mellitus affects haemostatic mechanisms particularly with increase period of disease on set. Coagulation profile assay is recommended for better management of diabetes mellitus.

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