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Assessment of some coagulation parameters (PT, APTT, INR, PLTS COUNT and PLT indices) in Sudanese Patients with Diabetic Type 2 January 2016

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Abstract:

Increase in incidence of diabetes mellitus (DM) is a major health concern. In patients with diabetes, cardiovascular disease (CVD) remains the main cause of morbidity and mortality. These individuals have been shown to be in procoagulant state. Coagulation tests like prothrombin time (PT), the activated partial thromboplastic time (APTT) Platelets count and platelets indices are global tests used to assess the coagulation system in clinical settings. The present study was planned to assess and compare these coagulation tests in patients with type 2 diabetes (T2DM) and healthy individuals. We analyzed coagulation tests PT , APTT, platelet count and platelets indices of 90 individuals (both males and females) of which 60 were type T2DM and 30 were healthy individuals. T2DM individuals were selected on the basis of Diagnostic criteria for Diabetes mellitus issued by the National Diabetes Data Group and WHO. The Mean PT in T2DM individuals was found out to be 13.60 seconds as compared to13.57 seconds in healthy individuals. The values were analyzed by using unpaired t test; and we found no statistically significant difference in PT of T2DM and PT of healthy individuals (p value 0153). Mean APTT DM individuals was found out to be 24.90seconds as compared 38.19 seconds in healthy individuals. The value were analyzed by

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using unpair T test and I founded that there was statistically significant decreased value of APTT among T2DM than healthy individuals (p<0.0002). The results showed that, the APTT was shorter in T2DM individuals than healthy individuals. This Laboratory evidence of shorter APTT in diabetic patients supports the clinical observation that T2DM is a hypercoagulable.

Key words: PT, APTT, INR,PLTS COUNT and PLT indices

1. INTRODUCTION:

1.1 Diabetes

Diabetes is a long-term condition that causes high blood sugar levels. In 2013 it was estimated that over 382 million people throughout the world had diabetes. Type 1 Diabetes - the body does not produce insulin, approximately 10% of all diabetes cases are type 1. Type 2 Diabetes - the body does not produce enough insulin for proper function. Approximately 90% of all cases of diabetes worldwide are of this type. Gestational Diabetes - this type affects females during pregnancy.

The most common diabetes symptoms include frequent urination, intense thirst and hunger, weight gain, unusual weight loss, fatigue, cuts and bruises that do not heal, male sexual dysfunction, numbness and tingling in hands and feet.

80% of patients with diabetes mellitus die of a thrombotic death. 75% of these deaths are due to cardiovascular complications and remainder due to cerebrovascular events and peripheral vascular complications Patients with diabetes mellitus have a high risk of atherothrombotic events. Many studies have shown a variety of diabetes mellitus related abnormalities in homeostasis and thrombosis[1,2] The diabetic condition contributes for initiation and progression of microvascular and macrovascular complications [3]

Although modern coagulation diagnostic tests are becoming more sophisticated, standard coagulation screening tests, such as activated partial thromboplastin time (aPTT) and prothrombin time (PT) are still important basic examinations in clinical laboratories. Shortened aPTT values may reflect a hypercoaguable state, which is potentially associated with increased thrombotic risk and adverse cardiovascular events[4,5]. Shortened aPTT may result from an accumulation of circulating activated coagulation factors in plasma caused by enhanced coagulation activation in vivo[4,6]. Therefore, aPTT can be used to assess the risk of thromboembolic complications in patients with diabetes mellitus [4,7].

Acang and Jalil reported shorter PTs and aPTT s in diabetics [13]. Conversely, Collier et al found normal PTs in patients with type 2 diabetes[14]. Erem et al reported normal PTs and aPTTs in diabetes , Platelet counts have been found to be normal in some Studies [15]or decreased in others[16]

Plasma fibrinogen levels influence thrombogenesis, blood rheology, blood viscosity and platelet aggregation. Epidemiological studies have found a significant association between fibrinogen levels and insulin levels [8,9]. Markers of fibrinolysis are abnormal in people with metabolic syndrome and fibrinolytic dysfunction is markedly increased in subjects with diabetes mellitus and abdominal obesity [8,10]. In addition, chronic hyperglycemia and tissue glycation have marked effects on fibrin structure, clot generation and resistance to fibrinolysis [8]. Increased level of fibrinogen is common in non-insulin dependent diabetes mellitus (NIDDM) patients. In diabetic patients there is an increased rate of fibrinogen clearance, with shorter fibrinogen circulating half life[11]. Since free radicals activate thrombin formation in diabetics, oxidative stress may represent a possible link between the diabetic state and hyperfibrinogenaemia[12]. This suggests that the high level of fibrinogen in plasma will consequently shorten aPTT and might

be a risk marker for cardiovascular disease because it reflects increased thrombin formation and therefore a greater possibility that a thrombotic event will occur.

Although aPTT, PT measured for identifying abnormalities in the factor XII, prekallikrein and high molecular weight kininogen, intrinsic factors XI, VIII, IX and common factors X, V, prothrombin and fibrinogen pathways of coagulation, the present study has been conducted to study the platelets , PT , APTT and fibrinogen levels in patients with type II diabetes mellitus and complicated type 2 populations .

It is well known that the vascular endothelium plays an essential role in the regulation of (local) hemostatic processes. Endothelial dysfunction has also been shown to occur in T2DM. 191 Multiple mechanisms are found to be involved in it; but most likely mechanism is that of the insulin resistance syndrome may be central to the development of diabetic endothelial dysfunction. The hemostatic abnormality and endothelial dysfunction are responsible for the generation of hyper-coagulable state in T2DM individuals. Coagulation tests like prothrombin time (PT) and the activated partial thromboplastin time (APTT) are global tests used to assess the coagulation system in a clinical setting.

Since DM worsens various biological processes and fibrinolytic system. the present study was planned to assess and compare the coagulation tests in patients with T2DM and healthy individuals.

MATERIALS AND METHODS

Study design: A prospective case control study, comparative in nature, was conducted over a period of 4 month from January to April 2016.

Ethics Committee approval: The study was initiated following administrative and ethical approval from local ethical committee for human research. Informed consent was obtained from every individual prior to enrolment in the study.

Study Population: The study was conducted on 90 individuals of whom 60 were T2DM individuals and 30were healthy individuals.

Selection of subject:

For this study, diabetic individuals who attended diabetic clinic at tertiary care center; and were diagnosed previously as T2DM were selected.

Diagnostic criteria for DM issued by the National Diabetes Data Group and WHO was applied as:

1) Symptom of diabetes plus random blood glucose> 200mg% OR
2) Fasting plasma glucose >120mg%

All individuals were maintained on anti-diabetic treatment like oral hypoglycemic drugs, diet and exercise. None of the patients were on injectable insulin. Healthy individuals for this study were selected as control from the working staff. Controls had no DM (as per above diagnostic criteria), hypertension or cardiovascular diseases.

Inclusion Criteria:

I. T2DM individuals of either sex (male and female) between the age group of 30to 65 years.

2. Healthy individuals of either sex (male and female) between the age group of 30 to 65years.

Exclusion criteria:

1. H/o clinical conditions associated with increased risk of thromboembolic diseases, like venous thrombosis, cerebrovascular diseases, peripheral vascular diseases.
2. Past H/o predisposition to hypercoagability like known inherited coagulative disorders, cancer, recent surgery, pregnancy, hyperthyroidism etc.
3. H/o heart diseases like myocardial infarction, hypertension, angina pectoris, positive electrocardiographic changes etc.
4. H/o drugs that affect coagulation, fibrinolytic system such as anticoagulants and antiplatelet drugs or oral contraceptives, hormone replacement therapy or hypolipidemic drugs etc.
5. H/o liver diseases, liver dysfunction, hepatitis, hepatotoxic drugs like anti-tubercular drugs etc.
6. H/o of alcohol intake or cigarette smoking.

The above exclusion criteria were applied to avoid any direct as well as indirect effect on coagulation tests.

Study Procedure:

The objective and detailed procedure were explained to each individual, before collection of the blood sample.

Following coagulation parameters were studied,

a) Prothrombin time (PT)

b) Activated partial thromboplastin time (APTT)

c) Platelet count and Platelet indices (MPV, PDW, PLCR)

Method of collection of data:

For this study purpose, individuals were divided into two groups;

Group I: This group consisted of 60, T2DM individuals.

Group II: It consisted of 30, healthy individuals.

Collection of Blood sample:

Under all aseptic conditions blood samples were collected from Group 1 and Group II individuals from anti-cubital veins using 21g number needles.

Procedure for coagulation tests:

• Collected samples from both the patients and controls in two clean container or a tube having two type of anticoagulant 3.2% trisodiaum citrate and EDTA anticoagulant,

• Immediately mixed the blood with anticoagulant avoiding foam formation. Centrifuge the sample containing trisodiaum citrate for 15 mm at approximately 3000 rpm and collect the plasma in separate tube.

• Fresh plasma is preferred for testing as it performs best when tested immediately. Sample may be tested within 2 hours at 25 to 30° C and within 3 hour at 2 to 8° C.

• Take hemostatic reagent into a test tube or reaction cuvette; add patient plasma into test tube or reaction cuvette.

• Incubate the test tube or cuvette containing plasma and reagent, PT and APTT were measured on coagulometer model.

• Our laboratory reference ranges of coagulation tests were; PT (11-16 seconds) APTT(30-50seconds)platelet count (150-450x10(3) PDW (8.3-25fl) MPV (8.6-15.5fl) P-LCR (11.9-66.9%).

• The other tube containing EDTA anticoagulant after gentle mix analyze by sysmex KX21N

STATISTICAL ANALYSIS:

The data collected during the study from both patients as well as healthy individuals were arranged in a tabular form and analyzed statistically by using categorical variables. These variables are compared by using unpaired t test to determine the significance of different parameters by using SPSS package

data software. Data is presented as mean ± standard deviation (SD) with 95% confidence interval (CI). A p-value < 0.000 1 was considered significant.

RESULT:

This prospective case control study done January 2016.A total of 90 subject were included in this study 60 were patients known diagnosed by diabetes as a test group (50%) were female and (50%) were male; their mean age 43.38years .Others 30 subject were normal healthy individual as normal control group; their mean age and gender were similar with patients group.

A comparative study was performed in which some coagulation tests (PT, APTT, platelet count and platelet indices) were assessed in T2DM and healthy individuals. Statistics were performed using unpaired t-test to get the value (table 1 and table 2).

The mean value of prothrombin time (PT) among T2DM individuals was 13.60 seconds with standard deviation ±1.520seconds and the mean value of PT among healthy individuals was 13.57seconds with standard deviation ±1.478 seconds.

By applying unpaired t test, we found no significant difference in PT of T2DM individuals and PT of healthy individuals. (**Table 1)**

The mean value of APTT in T2DM individuals was24.90 seconds with standard deviation ±2.794 seconds while mean value with standard deviation in healthy individuals was38.17±4.594seconds.By applying unpaired t test, we found that, there were significantly decreased value of APTT among T2DM individuals than healthy individuals (p<O.0002). (**Table 1)**

The current study revealed that the activated partial thromboplastin (APTT) were statistically significantly lower in patients with diabetes type 2 compared with normal health control group, (The mean and SD(24.90±vs.2.794.) vs.(38.17±4.594mg/dl) *P value* 0.002) respectively. Table .1

This study showed that the activated partial thromboplastien(APTT) were statistically significantly lower in patients with diabetic type2 compared with normal health control group with (p value 0.002) Table**.**1

A corroding to the gender, age and duration this study found that there is no statistically significant different between Age, Gender, duration and levels of diabetes mellitus (p valu0.564, 0.556, 0.22 and5 a 0.550) respectively **Table1**

Table 1: Descriptive Statistics

Investigation	Patient		Normal		P. value
	Mean	Std. Deviation	Mean	Std. Deviation	
PT	13.60	1.520	13.57	1.478	0.153
PTT	24.90	2.794	38.17	4.594	0.002
INR	1.33	.225	1.34	.253	0.555
PLT	229.62	40.960	349.77	68.446	0.522
MPV	8.07	1.194	8.23	.880	0.564
PDW	8.32	1.467	8.33	1.373	0.369
PLCR	16.52	3.377	16.07	3.129	0.228

Table 2

	Patient		**Normal**		
	Mean	**Std. Deviation**	**Mean**	**Std. Deviation**	**P VALUE**
DM	275.72	55.041	101.50	1.478	0.550
DURATION	8.63	5.125	0	0	0.225
AGE	43.38	8.763	43.80	8.797	0.564
Gender	1.40	.494	1.40	.498	0.556

DISCUSSION

This study aimed to assess parameters of coagulation profile (PT ,APTT ,Platelet count and platelet indices) in the patient with diabetic type 2.

Many hypotheses suggest that a strong correlation between some coagulation factor in patient with diabetic and abnormal homeostasis.

The current study revealed that the APTTtest was statistically significantly lower in patients with diabetic compared with those normal healthy control groups. (P value 0.002). It was observed from table (1) that, the mean value of PT in T2DM was 13.60 seconds and in healthy individual it was 13.56 seconds. And no significant difference in PT was found in the T2DM and healthy individuals. Similar findings were observed in Soltani et al. and Madan et al. [12] studies in which the,' had reported normal PT in T2DM individuals. While Zhao et al. and Acang&Jalil [14) found shorter PT in T2DM individuals. And raised PT in T2DM individuals was found by Hassan 5j and Abdurrahman &Dallatu. [16]

From table (1) it was observed that the mean value of APTT in T2DM was 24.90 seconds while in healthy individuals it was38.17 seconds. We found statistically significant difference in p value as <0.0002, which shows that, T2DM is associated with shorter APTT. Our results are supported by similar findings of Zhao et al [13] and Chan et al. 7] Their results also showed that DM is associated with shortened activated partial thromboplastin time. In contrast, Abdurrahman &Dallatu [16) and Alao et al. [18] found increased APTT in their study results.

The most important factors for preventing clotting in the normal vascular system are the smoothness of the endothelial cell surface, which prevents contact activation of the intrinsic clotting system; a layer of glcocalyx on the endothelium

(glycocalyx is a mucopolysaccharide adsorbed to the surfaces of the endothelial cells), which repels clotting factors and platelets, thereby preventing activation of clotting and a protein bound with the endothelial membiane, thrombomodulin which binds thrombin. [20

Endothelial dysfunction is the earliest event that precedes the development and progression of diabetic vascular complications. The pathogenesis of endothelial dysfunction in diabetes is complex. Multiple cellular and molecular mechanisms are involved in the development of diabetic dysfunctional endothelium. Along with hyperglycemia insulin resistance, impaired lipid metabolism and lipoproteins, oxidative stress, all these factors lead to endothelial dysfunction in T2DM. [211 Hyperglycemia directly contributes to endothelial injury through irreversible glycation of collagen and other sub-endothelial structural proteins of the vessel, forming advanced glycation end products (AGEs). [22]

Auto-oxidation of glucose is a common consequence of high plasma levels of this sugar. This oxidative event and the release of free radicals from glycated proteins are thought to cause oxidative stress, which lead to endothelial dysfunction. In this way, the diabetic endothelium dysfunction occurs. One method to assess vascular integrity is by the measurement of plasma thrombomodulin (TM) since this is essentially a membrane protein. [23) Increased plasma level this TM shows laboratory evidence of endothelial injury and indirectly a reduction in the effectiveness of the protein C anticoagulant pathway. Particularly T2DM individuals have increased level of TM, this shows that there is vascular injury in T2D

CONCLUSION

APTT was significantly lower in patients with diabetic type 2. This study emphasizes the literature. This laboratory evidence

of shorter APTT in diabetic patients supports the clinical observation that T2DM is a hyper-coagulable state.

RECOMMENDATION:

Our study suggests that, thrombotic complication and hyper-coagulable state in DM are introduction to vascular and cardiovascular complications; as prognostic clues to the simultaneous measurement of APTT in T2DM. These can be initially prescribed to avoid further vascular complications.

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