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RESEARCH ARTICLE

ESTIMATION OF SOME HEMOSTATIC PARAMETERS IN DIABETES MELLITUS TYPE 2 AMONG SUDANESE PATIENTS

***,[1]Nihad Elsadig Babekir Ismail, [1,3]Alsadig Gassoum and [2]MohiEldein Abass Abdalla**

[1]National Center of Neurological Sciences
[2]Sudan University of Science and Technology
[3]Nahda College, Khartoum, Sudan

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ABSTRACT

Background: Patients with diabetes mellitus have a high risk of atherothrombotic events. Many studies have shown a variety of diabetes mellitus related abnormalities in hemostasis and thrombosis. The diabetic condition contributes for initiation and progression of microvascular and macrovascular complication.

Materials and Methods: This is a cross sectional study that had been conducted at Alrakah Hospital during May 2012 to June 2012. The study included samples from type 2 diabetic patients diagnosed clinically at Alrakah Hospital, during the above mentioned period. The study was conducted in accordance with the guidelines of the local ethical committee. The aim of this study is to investigate the coagulation parameters in diabetic type 11 patients, these parameters included (PT, APTT, TT, Fibrinogen and D- dimer). Citrated venous blood samples were collected from50 patients of diabetes mellitus type II, and thirty from non-diabetes individuals as control. Data were analyzed by using statistical package of social science (SPSS).

Results: The most affected age group was 20-45years which encountered in 44%of the patients. According to the duration of the disease 78% of the patients were found within the period of 5-10 years p =0.000.

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INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder characterized by chronic hyperglycemia due to disturbance of carbohydrate, fat, and protein metabolism that are associated with absolute or relative deficiencies in insulin secretion, insulin action or both (Chales, 1998). Classification of diabetes mellitus is based on itsetiology and clinical presentation. There are four types or classes of diabetes mellitus; type 1 diabetes, type 2 diabetes, gestational diabetes, and other specific types. Type 1 diabetes is said to account for only a minority of the total burden of diabetes in a population although it is the major type of the diabetes in younger age groups at majority of well developed countries (Sicree *et al*., 2006). 85 to 95% of all diabetes in high-income countries is of type 2, accounting for an even higher dominance in developing countries. It is intimately associated with improper utilization of insulin by target cells and tissues. It is currently a common and serious health concern globally. According to WHO, (1994), this problem has been aggravated by rapid cultural and social dynamics, ageing populations, increasing urbanization, dietary changes, reduced physical activity and other unhealthy lifestyle and behavioural patterns (World Health Organization, 1994).

Diabetes mellitus and lesser forms of glucose intolerance, particularly impaired glucose tolerance, can now be found in almost every population in the world and epidemiological evidence suggests that, without effective prevention and control programs, diabetes will likely continue to increase globally World Health Organization, 1994) .The debilitating effects of diabetes mellitus include various organ failures, progressive metabolic complications such as retinopathy, nephropathy, and/or neuropathy (Piero, 2006). Diabetics are accompanied by risk of cardiovascular, peripheral vascular and cerebrovascular diseases. Several pathogenesis processes are involved in the development of diabetes, including destruction of pancreatic β-cells that lead to lowered sensitivity of insulin action (Piero, 2006; World Health Organization, 1999). Body of evidence suggest that certain haematological indices are altered in patients with diabetes mellitus (Dallatu *et al*., 2010). In patient with diabetes mellitus, persistent hyperglycaemia exposes red

***Corresponding author: Nihad Elsadig Babekir Ismail,**
National Center of Neurological Sciences

blood cells (RBCs) to elevated glucose concentration, thus resulting in glycalation of haemoglobin, prothrombin, fibrinogen and other proteins involved inclotting mechanisms (Selvin *et al*., 2010). Glycation of intrinsic and extrinsic clotting proteins will decrease the availability of these proteins whichaffect the clotting capacity (Selvin *et al*., 2010).

Coagulation Mechanism

Is a complex process by which blood clots, it is an important part of hemostasis (the cessation of blood loss from damage vessel), where in damage blood vessel wall is covered by platelet and fibrin –containing clot to stop bleeding and begin repair of damaged vessel. Disorders of coagulation can lead to an increased risk of bleeding (hemorrhage) or obstructive clotting (thrombosis) (Furie and Furie, 2005). Coagulation is highly conserved throughout biology; in all mammals coagulation involves both a cellular (platelet) and a protein (coagulation factor) component. The system in humans has been the most extensively researched and is there for the best understand (Furie and Furie, 2005). Coagulation begins almost instantly after an injury to the blood vessel has damaged the endothelium (lining of vessel) exposure of the blood to proteins such as tissue factor initiates changes to blood platelet and the plasma protein fibrinogen, a clotting factor. Platelet immediately form aplug at the site of injury; this is called primary hemostasis. Secondary hemostasis occurs simultaneously ;protein in the blood plasma, called coagulation factors or clotting factors, respond in a complex cascade to form fibrin strands, which strengthen the platelet plug (Furie and Furie, 2005).

MATERIALS AND METHODS

This is across sectional study, that had been performed at Alrakah hospital during the period from May 2012 to June 2012. All diabetic type 11 patients were included. Thirty of non-diabetic individuals were selected as control. The aim of the present study is to investigate coagulation parameters in diabetic type 11 patients. Fifty of venous blood samples were collected from diabetic type II patients, and thirty venous blood samples were collected from healthy individuals as control. In sterile container that contains 0.5 ml of sodium citrate anticoagulant, 4.5 ml of venous blood was added from each patient and control. Then the all samples were centrifuged at 3000R.P.M for 15 minutes. A written consent was obtained from each participant in this study.

Data collection

Data was collected using predesigned structural questionnaire, clinical and demographic data, were obtained from data registry, andincluded (age, gender, and duration of thedisease).

Methodology

Prothrombin time and activated partial thromboplastin time were measured by using coagulometer machine.

Prothrombin Time

From each sample 0.1 ml of plasma was placed into coagulometer cuvette and then incubated at 37 °C for 3-5 minutes, then 0.2 ml of thromboplastin with calcium chloride was added to the sample, the result obtained was recorded as time/ second.

Activated Partial Thromboplastin Time (APTT)

Other terms of APTT test are known as partial thromboplastin time with kaolin (PTTK), and the kaolin cephalin clotting time (KCCT), reflecting the method used to perform the test.

Test procedure

0.1ml of tested plasma was added to the 0.1 ml of APTT reagent, and then mixed well and incubated at 37 °C for 3-5 min. Then 0.1 ml of pre warmed $CaCl_2$ was added, and then the result obtained was recorded as time/ second.

Thrombin Time Assay

100µl of thrombin solution was added to 200µl of tested plasma in the coagulometer cuvette, and then incubated at 37 °C for 2 minutes. The result obtained was recorded as time/ second.

Fibrinogen Assays

Fibrinogen is a large dimeric protein each half consist of three polypeptide chains A, B, and C. Held together by 12 disulphide bonds. The two monomers are joined together by a further three disulphide bonds. Fibrin is formed from fibrinogen by thrombin cleavage of the A and B peptides from fibrinogen this result in fibrin monomers that associate to form apolymer that is the visible clot. The central E domain exposed by thrombin cleavage then binds with a complementary region on the outer or D domain of another monomer. The monomers thus assemble into a staggered overlapping two stranded fibril (Lammle and Griffin, 1985; Marder, 1982) all fibrinogen molecules are capable of participating in clot formation. There for antigenic assay and clot – based assay may return different results depending upon the composition of fibrinogen molecules in a specific patient samples. Only clottable fibrinogen is of interest for the purpose of hemostasis screening fibrinogen assay available on current automated coagulation analyzers include the Clauss and PT – derived method.

Test procedure

The patients plasma was diluted with buffer (1:10 in buffer solution) and then incubated at 37°C, phospholiped and thrombin were added followed by calcium, the time taken for the clot to form is compared to calibration carve and fibrinogen concentration deduced.

D-dimer test

D-dimer, is a degradation product of cross- linked fibrin formed during activation of the coagulation system, commonly used to exclude thromboembolic disease

i CHROMA device was used to estimate D-Dimer, 75 µl of plasma was added in to the tube containing detection buffer,

and then mixed well then the sample mixture was immediately loaded into the well of disposable test device and leaved at room temperature for 5 minutes. The result was obtained as µg/L.

Data analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS) 13 software with reference P.value of 0.05 considered statistically significant.

RESULTS

A total of 50 diabetic type 2 patients were included in the present study, male were 22 constituting 44% and female were 28 constituting 56%. The most affected age group was ranging from 20-45years, which encountered in 22 of the patients (44%) Table (1). According to the duration of the disease 78%of the patients were found within the age group of 5-10 years (p value0.000) Table (2). In this study the coagulation tests where significantly correlated with the duration of the disease (p value0.000). results of PT, APTT, TT D-dimer and Fibrinogen level where displayed in tables (3, 4, 5,6 and 7). Cross tabulation of age group and coagulation parameters showed that, the highest score of TT, D-dimer, Fibrinogen where detected within the age group of 20-45 years in 44% ,36% and 44% of the patients respectively. Cross tabulation results of coagulation parameters were displayed in Fig. (1, 2, 3, 4, 5, 6). The results of non-diabetics individuals showed normal PT, APTT, TT, Fibrinogen and D-Dimers.

Table 1. The frequency of age in group

Age group	Frequancy	Percent
20-45	**22**	**44%**
46-56	16	**32%**
56-65	**12**	**24%**

Table 2. The frequency of the duration of disease

Duration	Frequency	Percent
5-10	**39**	78%
11-20	**10**	20%
21-30	1	2%

Table 3. The frequency of PT

PT	Frequency	Percent
<15 sec	**39**	78%
15 sec	5	10%
>15 sec	6	12%

Table 4. The frequency of APTT

APTT	Frequency	Percent
<30 sec	**11**	22%
30 sec	**6**	12%
>30 sec	**33**	66%

Table 5. The frequency of TT

TT	Frequency	Percent
10-15 sec	1	2%
16-20 sec	20	40%
21-25 sec	21	42%
26-30 sec	8	16%

Table 6. The frequency of D-dimer

D-dimer	Frequancy	Percent
450-500 µg/l	8	16%
550-600 µg/l	15	30%
>600 µg/l	27	54%

Table 7. The frequency of Fibrinogen level

Fibrinogen	Frequency	Percent
200-400 mg/l	2	4%
450-500 mg/l	9	18%
550-600 mg/l	17	34%
650-700 mg/l	9	18%
750-800 mg/l	4	8%
850-900 mg/l	7	14%
>900 mg/l	2	4%

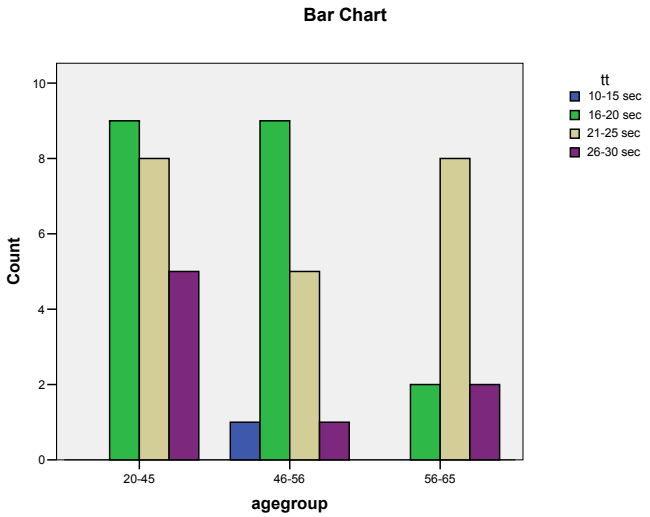

Fig. 1. The correlation between the TT and age group

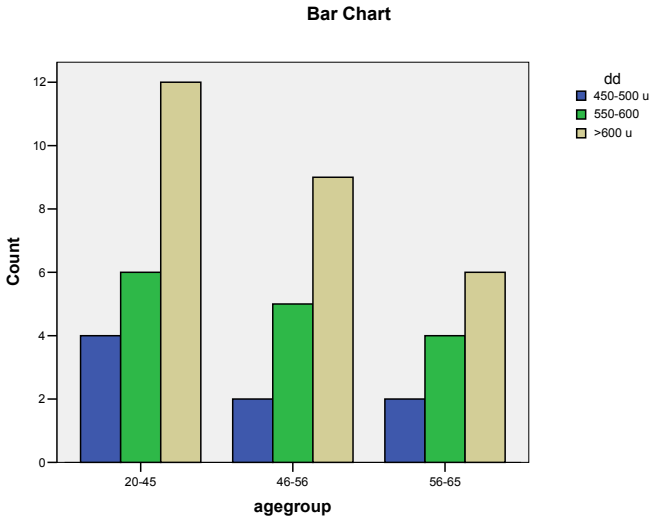

Fig. 2. The correlation between D-dimer and age group

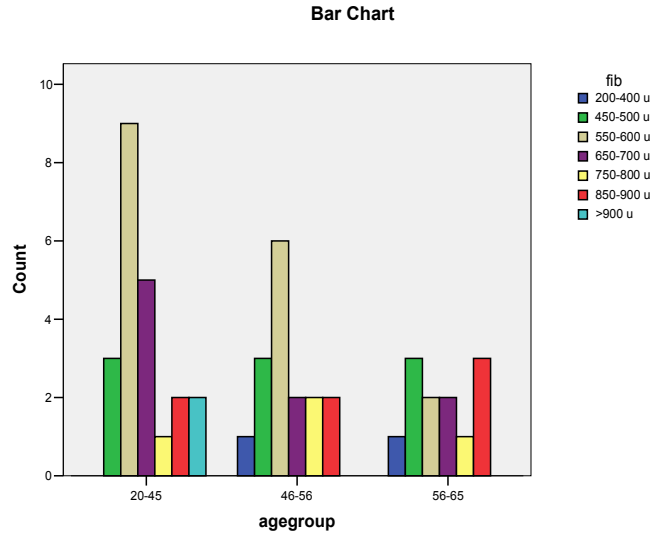

Fig.3. The correlation between fibrinogen and age group

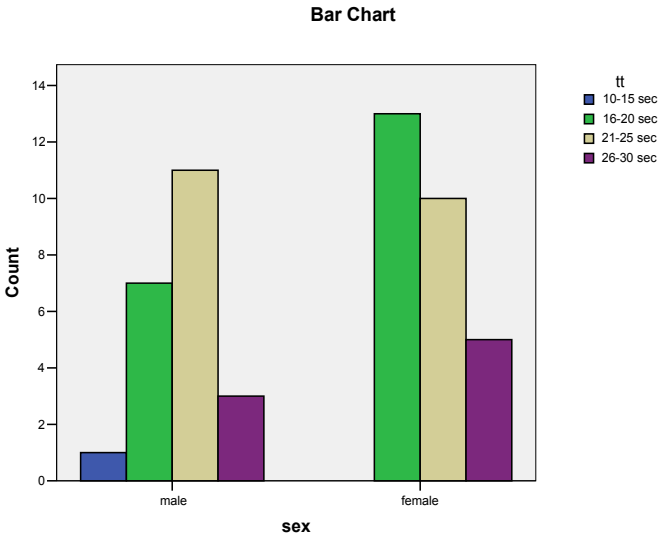

Fig. 4. The correlation between the TT and sex

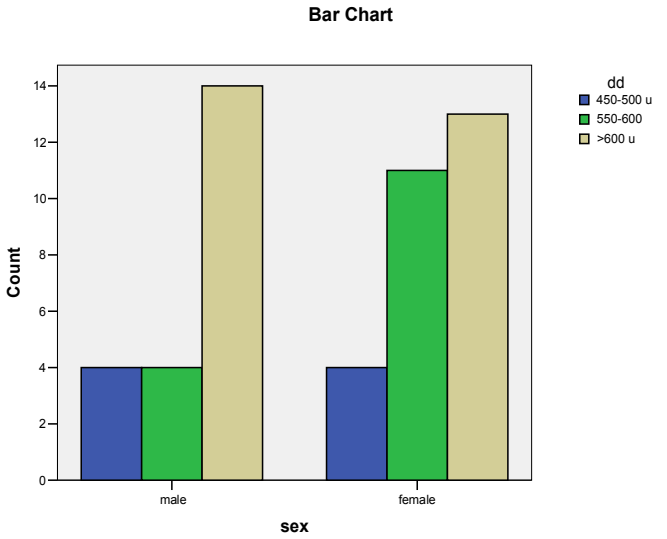

Fig. 5. The correlation between the D-dimer and sex

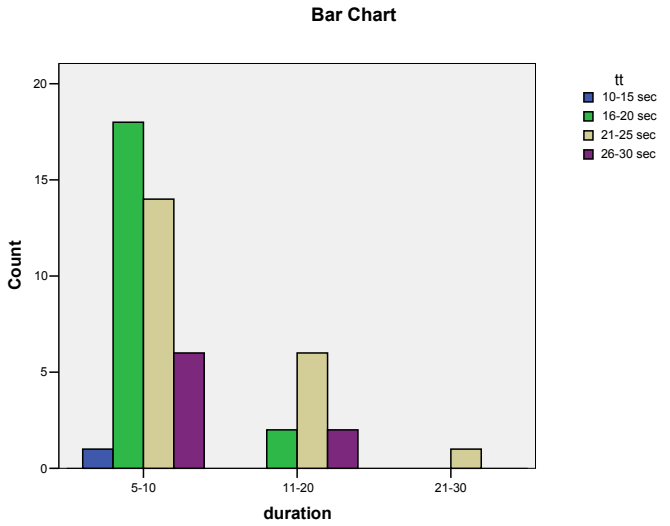

Fig. 6. The correlation between the TT and duration

DISCUSSION

In the present study our findings revealed that, the duration of PT and APTT results in diabetic patients type 2 and control group are similar. A study done by Abderlrahman and dallatu, soltaani and Mads *et al*. showed similar findings to our results (Abdulrahaman and Daiiatu, 2012; Madan *et al*., 2010). A study done byjapi (Japi, 2010) found that, results of diabetic patients type II and control group are similar. However another study done by Hassan, showed a significant prolongation of PT (p value =0.002), (Hassan, 2009). Another study done by Alao *et al*. (2010) in Nigeria revealed a significant prolongation of PT and APTT in diabetic patients type 2 compared with control group. The findings of this study revealed that, results of PT and APTT were not correlated with age group and sex with p value≥0.005. The findings of study done by Banin and Salus were agreed with our results (Sauls *et al*., 2007). Results of this study showed that, the disease duration of 5-10 years, was strongly associated with the prolongation of PT and APTT, P value 0.000. Hassan in (2009), reported that 40% of diabetic patients type 2 in Saudi Arabia were associated with disease duration less than 5 years. In the present study thrombin time was significantly associated with the duration, age and sex of the patients in type 2 diabetes when compared with control group p value=0.005. This findings agreed with study reporting in medical college of Virginia. A study done by Jones and Peterson (1981), showed that the results of thrombin time did not differ between patients and control group. Our findings in this study showed a significant difference of fibrinogen level in diabetic patients when compared to control group. Another study done by Binageetal was agreed with our findings p value=0.005. Study from USA and British, showed similar results to our findings in the duration of the disease, (Japi, 2010). The findings of D-dimer in this study correlated with the duration of the disease in diabetic patients type 2 when compared with control group, p value=0.005

Conclusion

In this study we concluded that, thrombin time, fibrinogen level and D –dimer might be useful hemostatic marker in diabetic

patients, these parameters can be used as screening test for hypocoagulable and hypercoagulable state for diabetes.

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