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COLLEGE OF MEDICINE AND HEALTH SCIENCES

SCHOOL OF BIOMEDICAL AND LABORATORY SCIENCES

DEPARTMENT OF HEMATOLOGY AND IMMUNOHEMATOLOGY

DETERMINATION OF PROTHROMBIN TIME, ACTIVATED PARTIAL THROMBOPLASTIN TIME AND PLATELET COUNT OF TYPE II DIABETES MELLITUS PATIENTS ATTENDING FELEGEHIWOT REFERRAL HOSPITAL, BAHIR DAR, NORTHWEST ETHIOPIA

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ABREVATIONS

AGE	Advanced Glycation End Product
aPTT	Activated Partial Thromboplastin Time
BM	Bone Marrow
BMI	Body Mass Index
CVD	Cardiovascular disease
DKA	Diabetic Ketoacidosis
DM	Diabetes Mellitus
DNA	Deoxyribonucleic Acid
FBS	Fasting blood sugar
IDDM	Insulin dependent diabetes mellitus
IDF	International Diabetic Federation
NIDDM	Non-Insulin Dependent Diabetes Mellitus
NKHS	Non Ketotic Hyper Osmolar State
PPP	Platelet poor plasma
PT	Prothrombine Time
RBC	Red blood cells
RBS	Random blood sugar
TYPE II DM	Type II Diabetes Mellitus
WHO	World Health Organization

LIST OF TABLE

ABSTRACT

Introduction: Diabetes mellitus is a metabolic disorder characterized with chronic hyperglycemia which results in polyuria, polydipsia and polyphagia. The incidence of cardiovascular disease (CVD) due to thrombosis is 2-4 folds greater in diabetic patients than the general population. Prothrombine time, activated partial thromboplastin time and platelet count are hematological indices that give an insight about the coagulation status of diabetic mellitus patients.

Objective: -To compare Prothrombin time, activated partial thromboplastin time and platelet count of type II diabetes mellitus patients attending Felegehiwot referral hospital, Bahir Dar, Northwest Ethiopia.

Method: A comparative cross-sectional study from March to April 2015 was carried out on a total of 40 treated type II diabetic, 40 untreated diabetics and 40 non-diabetic controls with similar age range of 30-60years.Simple random sampling technique was used to select study subjects of untreated DM and systematic sampling technique was used to select study subjects of both treated type II DM and non-diabetic individuals. Demographic data were collected through face to face interview and about 4ml of blood was collected aseptically by Felegehiwot referral hospital laboratory staff. The collected data was entered and analyzed through SPSS ver 16.One-way Anova and independent t-test were used to compare means of PT, aPTT and platelet count. $P < 0.05$ was considered as statically significant.

Result: The Mean aPTT in second was 34.44±5.35, 25.42±8.46, and 32.79±4.12; mean PT in second was14.65±2.50, 13.54±3.44, 14.28±1.50 and mean Platelet count was 254,000±95,077, 250,000±75,546, and 251,000±71,964 in treated type II diabetes mellitus, untreated and non-diabetic individuals, respectively. There was statically significant shortening of aPTT in untreated diabetes mellitus patients when compared with both treated diabetics and non-diabetic individuals ($P < 0.05$). However there was no significant difference between treated and non-diabetic controls. There was no statistical significance difference of PT and platelet count between all groups ($P > 0.05$).

Conclusion and recommendation: There was a shortening of aPTT in untreated type II diabetic patients. Therefore, monitoring the aPTT in newly diagnosis diabetic patients is important.

Key words: Prothrombine time, activated partialthromboplastin time, platelet count and type II diabetes mellitus.

1. INTRODUCTION

1.1. Background

Diabetes mellitus (DM) is metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action, or both. Etiopathogeneticaly diabetes classified into two, type1 and type 2 diabetes mellitus**.** Type I Diabetes called insulin-dependent diabetes mellitus (IDDM) is characterized by pancreatic Beta-cell loss eventually producing insulin deficiency within the affected individual [1].

The other most prevalent DM is type II diabetes mellitus, previously called non-insulin dependent diabetes mellitus (NIDDM) or adult onset diabetes. It is most commonly seen in adults but it can occur at any age. It's characterized by decreased insulin sensitivity which can subsequently provoke decreased insulin secretion as a result of Beta-cell loss. Its etiology is idiopathic although it is thought to result from a combination of genetic and environmental factors [2].

The International Diabetes Federation (IDF) has predicted that the number of individuals with diabetes will increase from 387 million in 2014 to 592 million in 2035, with 77% of the disease burden in lowland middle-income countries [3].Similarly the IDF have also reported that in Ethiopia about 2 million people are expected to live with DM with the national prevalence of 4.84%[3]. Type II DM is far more common than type 1 DM and accounts for about 90% of all cases of DM. The incidence of cardiovascular disease (CVD) due to thrombosis is 2-4 fold greater in diabetic patients than the general population. In patients with DM, CVD remains the main cause of morbidity and mortality and approximately 80% of patients die as a result of cardiovascular complications [4].

Furthermore, Prothrombine time (PT), activated partial thromboplastin time (aPTT) and platelet are hematological indices that give an insight into the coagulation status of patients. (PT) is a screening test used to detect disorders involving the activity of clotting factors (proteins) such as I, II, V, VII, and X of the extrinsic and common pathways while activated partial thromboplastin time (aPTT) is used to screen for abnormalities of the intrinsic and common clotting systems and to monitor the anticoagulant effect of circulating heparin. It measures the activities of factors I, II, V, VIII, IX, X, XI, and XII of the intrinsic and common pathways [5].

1.2. Statement of the problem

Persistence hyperglycemia may affect different biological process such as hemodynamic disturbances (increased blood flow, increased plasma viscosity, decreased red-cell deformability, increased platelet aggregability), and Structural abnormalities (Leakage of glaciated plasma proteins, Extracellular matrix is increased, bone marrow (BM) is thickened) [6]. Diabetes mellitus complication divided into acute and chronic complication. The acute complications include diabetic ketoacidosis (DKA) and non-ketoetic hyper osmolar state (NKHS) [7].Whereas, the chronic complications (long term effects) of diabetes includes progressive development of retinopathy, nephropathy, and neuropathy and atherosclerosis disease [8].

Body evidence also suggested that certain hematological indices are altered in patients with DM (9). In patient with diabetes mellitus, persistent hyperglycemia exposes red blood cells (RBCs) to elevated glucose concentration, thus resulting in glycalation of hemoglobin, Prothrombine, fibrinogen and other proteins involved in clotting mechanisms [10]. Glycation of intrinsic and extrinsic clotting proteins will change the normal functioning of these proteins which affect the clotting capacity [11].Therefore,glacyation of these proteins either may favor the development of hyper-coagulable and pro-thrombotic state or may also prone to develop hemorrhagic complication which results prolongation of aPTT [12,19].

Auto-oxidation of glucose is a common consequence of persistent hyperglycemia. This oxidative event and the release of free radicals from glaciated proteins are thought to cause oxidative stress, which lead to endothelial dysfunction. In this way, the diabetic endothelium dysfunction occurs [13]. When the endothelial wall is damaged, its smoothness and its glycocalyx – thrombomodulin layer are lost, which activate both factor XII and the platelets. Thus setting the intrinsic pathway of coagulation. If Factor XII and the platelets come in contact with the sub endothelial collagen the activation is even more powerful [14]. Thus, the presence of endothelial dysfunction and elevated level of clotting factors in Type II DM enhances the intrinsic pathway of clotting process [15].

2. LITERATURE REVIEW

The comparative cross-sectional study conducted in 2014, reported that ADP induced platelet aggregation was significantly higher while PT and aPTT were significantly shorter in diabetic group when compared with control group ($P<0.05$). The study concluded that type II diabetic patients are more prone to diseases such as coronary artery disease, cerebrovascular disease and peripheral vascular disease [16].However, other cross-sectional comparative study conducted in America using 87 normal control and 55 diabetes patients have got significant difference for platelet count between both group ($P<0.005$) whereas there was no significant difference for PT ($P>0.005$) [17].Similarly the research conducted in New Delhi, India using 60 type II DM patients and 30 non-diabetic individuals have reported that platelet count showed significant decrease in DM patients ($P =0.002$) Whereas, there was no significant difference of PT and aPTT values [18].

The other research conducted in 2011 by Zhao and his colleques on 1300 patients grouped based on their HbA1c level have got statistically significant shortening of aPTT, PT and increasing of fibrinogen of diabetic group with the mean value of aPTT <22 s ($p = 0.001$) , PT <10.5 s ($p = 0.012$), and fibrinogen >4.0 g/L ($p = 0.004$ respectively. They finally concluded that shorter aPTT and increased fibrinogen levels might be useful hemostatic markers in patients with diabetes and in patients at high risk for diabetes [19].

However the research conducted in Tehran, Iran by using 40 type II diabetic and 21 health individual with similar study design have got significant prolonged of aPTT value for diabetic type II group ($p <001$) whereas there was no significant difference of PT between groups. Finally they concluded that this prolonged of aPTT may be due to the presence of inhibitors of the intrinsic and common pathway [20].On the other hand, in contrast to the above, the study conducted in 2010 in Saudi Arabia science college using 114 diabetic and 23 non-diabetic individual have got significant difference due to shortening of PT in diabetic type II group while aPTT and platelet count didn't show significant difference between the two groups [21].Similarly the cross-sectional comparative study conducted at Saudi Arabia, Riyadh have reported that the mean level of prothrombintime (PT) was significantly decrease in diabetic group ($P = 0.02$), whereas the mean level activated partial thromboplastin time (aPTT) was not significant ($P = 0.826$). Finally he concluded that type II diabetes mellitus had hypercoagulable state and hypofibrinolysis indicating that the activation of

coagulation and reduced fibrinolytic activity may contribute to the increased risk of vascular disease in type II diabetic patients [22].

Another Cross-sectional comparative study conducted in Nigeria by using 50 treated, 50 untreated and 50 apparently health individual have reported that the PT and aPTT value were statistically significant differences between the untreated and treated diabetics as well as between untreated and non-diabetic controls due to the elongation of PT and aPTT in untreated individuals ($p<0.05$). Whereas, the aPTT Value of treated and controls are not significantly difference ($p>0.05$). The mean value of PT was 20.620± 2.849, 16.720±2.339, and 14. 920±1.209 in untreated, treated and in non-diabetic controls respectively. The mean value of aPTT was 58.460±4.146, 43.260±5.587 and 41.380±4.295 in untreated, treated and in non-diabetic controls respectively. Finally they concluded that there was an abnormal PT and aPTT in untreated patients with diabetes mellitus [23]. However, similar research conducted by using 50 apparently health and 50 type II diabetic patients have reported that the mean of PT of the two groups were not significant ($p>0.05$) however the mean aPTT was significant due to shortened aPTT in diabetic type II patients ($p<0.001$) and they concluded that aPTT was shorter in Type II DM than healthy individuals. This laboratory evidence of shorter aPTT in diabetic patients supports the clinical observation that Type II DM is a hyper-coagulable state [24].

On the other hand the cross-sectional comparative study conducted in Nigeria using 30 confirmed Type II DM and 30 non-diabetics individuals have got the significant prolongation in mean value of aPTT and the significant increasing weight of fibrinogen relative to the non-diabetic group ($p<0.05$) but there was no significant difference for PT and TT ($P>0.05$) [25].However, the other similar study conducted in South Asia, Nepal also reported that statistically significant shortening of aPTT (p value 0.000) and increasing of fibrinogen ($p = 0.000$) of the diabetics group. The Mean value of aPTT for the diabetic patients and non-diabetic individuals were 29.88 ± 4.89 seconds and 32.44 ± 2.25 seconds respectively. They finally concluded that Diabetics have an increased level of fibrinogen and relatively shortened aPTT as compared to the non-diabetic patient [26].In contrast to the above, Crosse-sectional study conducted in Nigeria using 50 diabetic type2 and 50 non-diabetic individuals have got PT and APTT showed significant prolongation in the diabetic group when compared with the control ($P < 0.05$) even though values were still within the normal limits. They finally concluded that despite the popular notion of a prothrombotic tendency in diabetes, diabetics may also be prone to developing hemorrhagic complications [12]. Similarly other cross-sectional

conducted in Sudan, Khartoum using 100 diabetic and 20 apparently healthy individual have got insignificant prolongation of PT and aPTT in diabetic group. Similarly aPTT was also increased from 25.95±3.95sec in control and 27.06±3.92sec in diabetic group. They finally concluded that some prolongation of PT and APTT was observed in diabetic group due to the presence of hemostatic abnormality in DM patients [27].

3. SIGNIFICANCE OF THE STUDY

Type II diabetic patients are more risk for thrombosis and bleeding disorder. About 80% of type II DM patients are die due to CVD associated with vascular thrombosis. Therefore, my plan was to assess the coagulation status of treated and untreated diabetic type II patients of Felegehiwot referral hospital. There was no previous study had been conducted concerning on evaluating the normal functioning of intrinsic and extrinsic factor of diabetes type II patients in Amhara. The current study has reported that those untreated DM patients have shortened aPTT which may indicate their hypercoagulabity state or procoagulant state. Therefore, the study was given important information about coagulation status of treated type II DM patients and newly diagnosis diabetic (untreated) patients of Felegehiwot referral hospital.

4. **OBJECTIVE**

4.1. General Objective

- To assess PT, aPTT and platelet count of type II DM patients as compared to healthy controls attending Felegehiwot referral hospital Bahir Dar, Northwest Ethiopia from March to April 2015.

4.2. Specific Objectives

- To measure PT, aPTT and platelet count of treated type II diabetic and untreated diabetic patients.
- To Compare PT, aPTT and platelet count of treated type II DM and untreated DM patients and healthy individuals

5. MATERIALS AND METHODS

5.1. Study area and period

The study was conducted at Felegehiwot referral hospital laboratory. This hospital is found in Bahir Dar which is 546km from Addis Ababa in the Northwest Ethiopia. This hospital has delivered service for more than 800 new out patients and 5 – 10 follow-up diabetes patients per day. The study was conducted from March to April in 2015.

5.2. Study design

A comparative cross-sectional study design was used to determine and compare PT, aPTT and PLT count of treated type II DM, untreated DM and healthy individuals.

5.3. Population

5.3.1. Source population

All type II diabetic patients of Felegehiwot referral hospital who have already started their non-insulin hypoglycemic treatment and newly diagnosis diabetic patients (untreated DM) at Felegehiwot referral hospital, Bahir Dar. Apparently Health individual coming to hospital for medical checkup.

5.3.2. Study Population

All type II diabetic patients of Felegehiwot referral hospital who have already started their non-insulin hypoglycemic treatment with the age of 30-60years old and newly diagnosis diabetic patients (untreated DM) with similar age range and who fulfill the inclusion and exclusion criteria..Similarly, health individuals with the age range of 30-60 and with FBS is between 70-110mg/dl was also selected as a study subject.

5.4. Inclusion criteria

Diabetic type II patients who stabilized with non-insulin hypoglycemic medicines such as metformin, glibenclamide, and newly diagnosed diabetic patients with the age of 30-60years and FBS>126mg/dl, RBS>180mg/dl were included for the study. Apparently healthy individuals with similar age group of 30-60 and their FBS(Fasting blood sugar) level is between 70-110mg/dl were also included for the comparison.

5.5. Exclusion criteria

Treated type II diabetic and newly diagnosis DM patients, on warfarin or heparin or any other anticoagulation therapy such as aspirin, and with other complications such as history of liver diseases, liver dysfunction, on hepatotoxic drugs, history of alcohol intake or cigarette smoking, hypertensive and psychic patients were excluded from the study. Apparently healthy individuals with any of DM symptom or who had taken any anticoagulant drug or who have a history of hypertensive were also excluded from the study.

5.6. Variables

5.6.1. Dependent Variable

- Prothrombin time
- Activated partial Thromboplastintime
- Platelet count

5.6.2. Independent Variable

- Treated diabetic
- Untreated diabetic
- Non-Diabetes

5.7. Operational Definition

Apparently healthy individual: Individuals who haven't had a diabetic history, symptoms, any anticoagulant therapy, and hypertensive, clinically proven liver dysfunction and their fasting blood glucose level is between 70mg/dl and110 mg/dl.

Untreated diabetic patients: Those newly diagnosis diabetic patients who have had symptoms of diabetic which was confirmed by physician and their FBS>126mg/dl or RBS>180mg/dl.

Treated Patients: Those type II DM patients who have been treated with non-insulin hypoglycemic drug with age range of 30-60 years old at Felegehiwot referral hospital**.**

5.8. Sample Size and Sampling Technique

The total of 120 study subject out of which 40 Diabetic type II individuals who already on treatment and 40 non-diabetic individuals with the age of 30-60 years old were selected using systematic sampling techniques from laboratory staffs and clients came to hospital for medical checkup whereas the other 40 untreated (new diagnosed diabetic individuals) with similar age range 30-60years were selected using simple random sampling technique at Bahir Dar Felegehiwot referral hospital. The sample size was determined conveniently by considering the previous similar studies and costs.

5.9. Sample collection

Standardized questionnaire was used to collect socio-demographic data of study subjects. The questionnaire contains sex, age, marital status, education background, residence and duration of treatment. The data was collected with face to face interview. Following interview **a**bout 4 ml of fasting blood sample was collected with a sterile disposable syringe. From this 1.8 ml blood sample was delivered into a test tube containing 0.2ml trisodium citrate anticoagulant (9:1blood to anticoagulant ratio) for PT and aPTT determination. The remaining 2ml blood was delivered into EDTA test tube for platelet count.

5.10. Laboratory analysis

PT was determined with the one step procedure using Huma clot-junior semi-automated instrument by measuring optical density. PT measures the clotting time of plasma after adding a source of tissue factor (thromboplastin) and calcium. The recalcification of plasma in the presence of tissue factor generates activated factor Xa, with the consequent formation of thrombin and ultimately an insoluble fibrin clot.The aPTT was also determined with aPTT reagent containing a plasma activator and phospholipid to the test specimen, the phospholipid serves as a substitute for a platelets. The mixture is incubated for activation, then recalcified with calcium chloride and clot formation is termed. The reference range of PT and aPTT was 10-15sec and 26-45sec respectively.

On the other hand platelet count was determined with the three part Huma-Count hematology analyzer based on electrical impedance method (coulter method) which counts and sizes cells by detecting and measuring changes in electrical impedance when a particle in a conductive liquid passes through a small aperture. There is a constant direct current flowing between the external and internal electrodes. When each cell passing through the aperture, there is current resistance. These

changes are recorded as a pulse. The number of pulses is proportional to the number of particles whereas the intensity of each pulse is proportional to the volume of that particle. The reference range of platelet count was 150,000-450,000/µl.

5.11. Quality Assurance

Pre analytical: Proper labeling, patient identification and sample collection procedure, utilizing appropriate test tube and safe transportation of sample to the testing area was performed.

Analytical: Calibration and validation of the instrument was done by laboratory quality officer timely using standard and a quality control reagent respectively.

Post analytical: Appropriate recording, result interpretation using their reference range and cross check the result with its correspondence labeling was performed**.**

5.12. Statistical analysis

Data was checked for completeness, cleaned and entered in to SPSS version 16 for analysis. In order to compare means one-way ANOVA and independent t-test were used. The result was expressed as mean ±SD.The independent t-test was also used to determine the specific significant groups. $P<0.05$ was considered as statically significant. The result was presented with tables.

5.13. Ethical considerations

Ethical approval was obtained from School of Biomedical and Laboratory Sciences Research and Ethical Committee, University of Gondar, College of Medicine and Health Sciences. Then permission was given from Felegehiwot referral hospital authorities. Written informed consent was taken from each study participants. The purpose and objectives of the study was clearly explained and participation was also in volunteer. Confidentiality was maintained throughout the research work. The respondent was allowed to quit if he/she doesn't want to participate in the research. Informed consent obtained from every individual prior to enrolment in the study.

6. RESULTS

Characteristics of study participants: A total of 120 study participants were included in this study. From these 40 (25 males and 15 females) were untreated DM patients, 40 (25males and 15 females) were treated type II DM patients and the remaining 40 (24 males and 16 females) were healthy controls. The minimum and maximum age was 30and 56 with the mean age of 40.57±7.47 in untreated, 37.80±5.9 in treated and39.27±6.59 in healthy controls, respectively. Among study subjects 57.5%, 75% and 65% of untreated, treated type II DM and non- diabetic individuals were lived in urban area respectively. About 30%, 42.5% and 20% of untreated DM,treated type II DM and non-diabetic control were illiterate respectively on the other hand 27.5%,20% and 37.5% of untreated DM,treated type II DM and non-diabetic control were university graduated respectively. Similarly, 62.5%, 75 % and 62.5% of untreated DM, treated type II DM and non-diabetic controls were married respectively. Furthermore 70% of treated type II DM patients were treated using non-insulin hypoglycemic drug for less than 5 years and only 2% of treated type II DM were treated for more than 10 years with similar drug (Table 1).

Table 1: Demographic data of treated, untreated diabetic and non-diabetic patients attending Felegehiwot hospital, Bahir Dar, North west Ethiopia from March to April 2015.

Parameters		Untreated DM		Treated type II DM		Healthy control	
		Frequency	**Percentage**	**Frequency**	**Percentage**	**Frequency**	**Percentage**
Sex	Male	25	62.5%	25	62.5%	24	60%
	Female	15	37.5%	15	37.5%	16	40%
Age in years	30-40	23	57.5%	30	75%	26	65%
	41-50	12	30%	9	22.5%	13	32.5%
	51-60	5	12.5%	1	2.5%	1	2.5%
Residence	Urban	23	57.5%	28	70%	29	72.5%
	Rural	17	42.5%	12	30%	11	27.5%
Educational status	illiterate	12	30%	17	42.5%	8	20%
	elementary	6	15%	6	15%	7	17.5%
	high school	11	27.5%	9	22.5%	10	25%
	University graduated	11	27.5%	8	20%	15	37.5%
Duration of treatment	0-5years	NA	NA	28	70%	NA	NA
	6-10years	NA	NA	10	25%	NA	NA
	>10	NA	NA	2	5%	NA	NA
Marital status	married	25	62.5%	30	75%	25	62.5%
	Not married	15	37.5%	10	25%	15	37.5%

N.B.: NA= Not applicable

Laboratory Findings: Untreated diabetic patients had 62.5%, 10%, and 92.5% of normal PT, normal aPTT and normal platelet count respectively whereas treated diabetic had 80%, 95%, and 82.5% of normal PT, normal aPTT and normal platelet count respectively. However, treated diabetic had 20%, 2.5% and 7.5% of prolonged PT, aPTT and platelet count respectively. Untreated diabetic patients had 25%, 5% and 0% of prolonged PT, aPTT and platelet count respectively. Similarly treated diabetic patients had 0%, 2.5% and 10%of a decreased PT, aPTT and platelet count respectively. Whereas, untreated diabetic patients had 12.5%, 85% and 7.5% of decreased PT, aPTT and platelet count respectively (Table 2).

Table 2. Percent of normal and abnormal PT, aPTT and platelet count in treated and untreated diabetic patients attending Felegehiwot hospital, Bahir Dar, North west Ethiopia from March to April 2015.

Parameters		Untreated DM		Treated DM		Health	
		Frequency	Percentage	Frequency	Percentage	frequency	percentage
PT(sec)	Decreased	5	12.5%	0	0%	0	0
	Normal	25	62.5%	32	80%	40	100
	Prolonged	10	25%	8	20%	0	0
aPTT(sec)	Decreased	34	85%	1	2.5%	0	0
	Normal	4	10%	38	95%	40	100
	Prolonged	2	5%	1	2.5%	0	0
Platelet(per µl)	Thrombocytopenia	3	7.5%	4	10%	0	0
	Normal	37	92.5%	33	82.5%	40	100
	Thrombocytosis	0	0%	7	17.5%	0	0

The mean aPTT of non-diabetic, treated and untreated type II diabetic patient was 32.8±4.12 seconds, 34.4±5.3 seconds, and 25.42±8.46seconds, respectively. Similarly, the mean PT of non-diabetic controls, treated type II DM and untreated DM were 14.28±1.50 seconds, 14.65±2.50seconds and 13.54±3.44seconds, respectively. Moreover, the platelet count of non-diabetic control, treated type II DM, and untreated DM were 251,000± 71,964, 254,000± 95,077 and 250,000± 75,546, respectively. There was a significant shortening of aPTT in untreated diabetic when compared with both treated and untreated diabetic patients ($P < 0.05$). However, the mean

aPTT was not significant between treated and non-diabetic individuals (P >0.05). Prothrombine time and platelet count was not significant between all paired groups (P >0.05(Table3).

Table 3: Mean of **PT**, aPTT and platelet count of treated and untreated diabetic patients attending Felegehiwot hospital, Bahir Dar, North west Ethiopia from March to April 2015.

Parameters	**Untreated DM Mean ± SD**	**Treated type II DM Mean ± SD**	**P Value**
Prothrombintime(Sec)	13.54±3.44	14.65±2.50	0.103
Activated partial thromboplastin time(sec)	25.42±8.46	34.4±5.35	**0.000**
Platelet count (per µl)	250,000±75,546	254,000±95,077	0.84

	Untreated DM Mean ± SD	**Non-diabetic Mean ± SD**	**P value**
Prothrombin time(sec)	13.54±3.44	14.28±1.50	0.22
Activated partial thromboplastin time(sec)	25.42±8.46	32.79±4.12	**0.000**
Platelet count(per µl)	250,000±75,546	251,000±71,964	0.94

	Treated Type II DM Mean ±SD	**Non-diabetic Mean ±SD**	**P value**
Prothrombin time(sec)	14.65±2.50	14.28±1.50	0.41
Activated partial thromboplastin time(sec)	34.4±5.35	32.79±4.12	0.13
Platelet count(per µl)	254,000±95,077	251,000±71,964	0.86

N.B: P value is derived from independent t test, numeric numbers in bold show significant association.

7. DISCUSSION

In the current study, non-diabetic, treated and untreated diabetic have a mean aPTT of 32.79±4.12 seconds, 34.45±5.35 seconds, and 25.42±8.46seconds respectively. There was a significant shortening of aPTT in untreated diabetic patients when compared with both treated and non-diabetic individuals (P <0.05).Similar findings were found byzhaos.et.al in 2011[19] and chava.et.al in 2014[24].They have concluded that the shortening of aPTT in type II DM patients showed that those type II DM patients are high risk for hypercogulabile state. In contrary to this, the study conducted by Soltani et.al in 2011 and Abdeen A et al in 2014 have got the prolonged aPTT in diabetic patients and they concluded that the presence of inhibitors of the intrinsic and common pathway in DM patients [20,27].

Similarly, the current study also showed that the presence of significant shortening of aPTT in untreated DM patients which may be due to the glycation of intrinsic clotting factors caused by the presence of persistent hyperglycemia. Persistent hyperglycemia may result the glycation of intracellular and extracellular protein. Glaciations of intrinsic and extrinsic clotting proteins will change the normal functioning of these proteins which affect their clotting capacity [10].Thus, glycation of clotting factors may results the activation of inactive intrinsic factors which finally results the shortening of aPTT [11]. The current study also showed that there was no significant difference of the mean PT, aPTT and platelet count between treated and non-diabetic individuals. This is may be the effect of non- insulin hypoglycemic drug on glucose level which in turn prevents glycation process in treated DM patients.

Furthermore, the mean PT of treated, untreated diabetic patients and non-diabetic individuals were 14.65±2.50seconds, 13.54±3.44 seconds and 14.28±1.50 seconds, respectively. They are almost within the normal range and there was no significant difference between groups which was similar finding with a study conducted by Soltani.et.al in 2011[20] and Ifeany E. et.al in 2014 [25].

Similarly, the platelet count of treated, untreated and non-diabetic individuals were 254,000± 75,077;250,000± 75,546 and251,000±71,964 respectively and their mean platelet count was within the normal range. This finding was similar with a study conducted by Mohamed S, 2010[21]. The current study showed that the PT and platelet count was no significant difference between groups; this may be due to the absence of endothelial damage in untreated diabetic patients. Endothelial

damage due to persistent hyperglycemia may results the activation of coagulation factor and the adherence of platelet with the exposed collagen. Thus, platelets adhere to the site of the damage and become partially activated. Factor VII (FVII) binds to exposed tissue factor (TF) and forms a complex, which subsequently activates FIX and FX [14]. Therefore, this may shortened the PT time and decrease platelet number.

Moreover, the current study finding also indicates 95% of treated type II diabetic patients have normal aPTT whereas only 10% of untreated diabetic had normal aPTT therefore aPTT abnormality was dominant in untreated diabetic patients. Similarly 12.5% of untreated diabetic had a decreased PT whereas there was no decreased PT in treated diabetic patients. Similarly about 85% of untreated diabetic had a decreased aPTT whereas only 2.5% of treated diabetic had a decreased aPTT. Therefore there is a higher PT and aPTT abnormality in untreated diabetic than treated diabetic. This indicated that those treated diabetic patients had properly control their blood glucose than untreated individuals so that glycation associated with the persistence hyperglycemia may not exist. Since glycation is non-enzymatic binding of glucose on protein which could change the normal function of the intrinsic factors (aPTT) and the extrinsic factors (PT) [10].

8. CONCLUSION

There was a significant shortening of aPTT in untreated diabetic as compared with treated and non-diabetic groups. Therefore, shortening of aPTT might be caused by the activation of intrinsic factor through glycation associated with the presence of persistent hyperglycemia. However PT and platelet count was not show significant difference between the groups. This might be due to the absence of endothelial damage and exposed tissue in untreated diabetic patients.

9. Recommendation

The study showed a significant shortening of aPTT in untreated diabetic patients. Therefore, the intrinsic pathway better to be part of test requested by the clinicians when there is presentation of diabetes mellitus.

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11. Annexes

Annex 1: Questionnaires

1. Questionnaires for Non-diabetic patient and untreated diabetic patient (newly diagnosis)

NAME…….AGE….SEX) M OR F

1. Where is your residence? A) Urban B) rural

2. Marital status A) married B) not-married

3. What is your educational background? A) Illiterate B) Elementary C) High School D) Higher Education (University or College)

4. Have you had smoking? A) YES B) NO

5. Have you had drinking alcohol? A) YES B) NO

If yes, are you chronic or occasional drunker? A) Chronic B) occasional

6. Have you had liver disease? A) YES B) NO

7. Are you pregnant? A) YES B) NO

8. Have you had hypertensive? A) YES B) NO

9.Have you had taken any anticoagulant drug? A) Yes B) NO

Name of data

collector…….sign……date…….tell.n

LABORATORY TEST RESULT

PT……

aPTT……..

CBC (PLATELET COUNT)……

FBS (RBS)……

Reported by…….sign…….date……tell no……

2. Questionnaire for Non-insulin hypoglycemic drug user diabetic patients (type2 DM)

NAME…….AGE….SEX) M or F

1. Which type of drug you has been used? A) Insulin B) Non-insulin or oral hypoglycemic drug? .
2. Where is your residence? A) Urban B) rural

3. Marital status? A) Married B) not married

4. What is your educational background? A) Illiterate B) Elementary C) High School D) Higher Education (University or College).

5. Have you had smoking? A) YES B) NO

6. Have you had drinking alcohol? A) YES B) NO

If yes, are you chronic or occasional drunker? A) Chronic B) occasional

7. Have you had liver disease? A) YES B) NO C) I DON'T KNOW

8. Are you pregnant? A) YES B) NO

9. Have you had hypertensive? A) YES B) NO

10. Have you had taken any anticoagulant drug? A) Yes B) NO

11. For how long you have had taken your non-insulin hypoglycemic drug? A) 0-5years B) 6-10years C)>10years (only for treated diabetic patients).

Name of data collector…….sign……date…….tell.n

LABORATORY TEST RESULT

PT……

aPTT……..

CBC (PLATELET COUNT)……

Reported by…….sign…….date……tell no……..

መጠይቅ በአማርኛ

1. **ለጤነኞች ና ላልታከሙ የስኪር በሽተኞች መጠይቅ**

ስም ---------- እድሜ ---------- ጾታ ------- ሴት/ወንድ/

1. **የመኖሪያ አድራሻ የት ነው ?**

   ሀ. ከተማ ለ.ገጠር

2. **አግብተሀል /አግብተሻል/?**

   ሀ. አወ ለ. የለም

3. **የትምህርት ደረጃ ህ/ሽ/?**

   ሀ. ያልተማረ ለ. የመጀመሪያ ደረጃ ከፍተኛ ደረጃ . መ. የዩኒቨርሲቲ ምሩቅ

4. **ታጨሳለህ/ሽ/?**

   ሀ. አዎ ለ. የለም

5. **ትጠጣለህ/ሽ/?**

   ሀ. አዎ ለ. የለም

   አወ ከሆነ መልሱ መቼ?

   ሀ. ሁልጊዜህ ለ. አልፎ አልፎ

6. **የታወቀ የጉበት በሽት አለብህ/ሽ/?**

   ሀ. አዎ ለ. የለም

7. **ነፍሰ ጡር ነሽ?**

   ሀ. አዎ .ለ የለም

8. **የደም ግፊት አለብህ/ሽ?**

   ሀ. አዎ ህ. የለም

9 .**እየወሰድከው ያለ የደም ማቅጠኛ መድሀኒት አለ?**

   ሀ. አዎ ለ. የለም

   መጠየቁን የሞላው ሰው ---------- ፊርማ ---------- ቀን------

የላቡራቶሪዉጤት

PT _____

aPTT _____

CBC (platlatecount)

FBS (RBS)_____

Reported By ---------- sign ---------- Date ----------

2. **ከኢንሱሊን ውጭ የሆነ የደም የስኳር መጠን መቆጣጠሪያ መድሀኒት ለሚወስዱ መጠይቅ**

ስም ---------- እድሜ ---------- ጾታ ------- ሴት/ወንድ/

1. **የትኛውን አይነት የደም የስኳር መቆጣጠሪያ መድሀኒት ነው የምትወስደው/ጅው/?**

   ሀ. ኢንሱሊን ን. ለ. ከኢንሱሊን ውጭ

2. **የመኖሪያ አድራሻ የት ነው ?**

   ሀ. ከተማ ለ. ገጠር

3. **አግብተሀል/አግብተሻል/?**

   ሀ. አወ ወ. የለም

4. **የትምህርት ደረጃህ/ሽ/?**

   ሀ. ያልተማረ ለ. የመጀመሪያ ደረጃ ሐ. ከፍተኛደረጃ መ. የዩኒቨርሲቲ ምሩቅ

5. **ታጨሳለህ/ሽ/?**

   ሀ. አዎ ለ. የለም

6. **ትጠጣለህ/ሽ/?**

   ሀ. አዎ ህ. የለም

   አወ ከሆነ መልሱ መቼ?

   ሀ. ሁልጊዜ ዜ. አልፎአልፎ

7. **የታወቀ የጉበት በሽት አለብህ/ሽ/?**

   ሀ. አዎ ል. የለም

8. **ነፍሰ ጡር ነሽ?**

ሀ. አዎ ጡ. የለም

9. የደም ግፊት አለብህ/ሽ?

ሀ. አዎ ህ. የለም

10. እየወሰድከው ያለ የደም ማቅጠኛ መድሀኒትአለ?

ሀ. አዎ ኒ. የለም

11. የምትወስደውን የስኳር መድኒት ለምን ያህል ጊዜ ወሰድክ?

ሀ. ከ0-5 ዓመት ለ. ከ5-10 ዓመት ሐ. ከ10 ዓመትበላይ

መጠየቁን የሞላው ስም ---------- ፊርማ ---------- ቀን----

የላቡራቶሪ ዉጤት

PT _____

aPTT _____

CBC (platlate count)

Reported By ---------- sign ---------- Date ----------

Annex II: Informed consent

Hello! My name is Yitayal Amogne I am here to collect blood sample for the purpose of research from university of Gondar. The purpose of this study is to evaluate PT, aPTT and platelet count. I am requesting your permission to give your blood specimen for analysis purpose. Your participation in this research is voluntary; you have the right to withdraw at any point of the study, for any reason, and without any prejudice. You will be under no obligation to complete the interview and to give blood sample if you choose to start it. This study will help you to know the status of your PT, aPTT and platelet count and I also assure you that laboratory result will not bring any harm and I will inform to you and your physician if there is any abnormal lab result. If you are interested in participation, please sign and give me the consent form.

Thank you for your time and for agreeing to participate in this study.

I (The participant) have read and understand the information above, and any questions I have asked have been answered to my satisfaction. I understand that my participation is voluntary and I agree to participate in this research, knowing that I can withdraw at any time.

Participants

Name :----------(block letters)

Participant's signature: ----------Date:----------

Means of contact (Tel number or address) ----------

Annex III: Materials

- ✓ 3.2% SODIUM CITRATED TEST TUBE(BLUE TOP)
- ✓ EDTA TEST TUBE
- ✓ 5ML SYRINGE
- ✓ GLOVE
- ✓ BINDER
- ✓ QUETIONER
- ✓ SAMPLE TRANSPORTING RUCK
- ✓ PT AND APTT REAGENT
- ✓ MICROPIPETTE
- ✓ YELLOW TIPS

Annex VI: Data Collection Format

Ser. No	Name	Sex	AGE	PT	aPTT	platelet count	Diabetes type2		Non-diabetes	Duration of treatment
							Treated	Untreated		
1										
2										

Annex V: Laboratory Procedures

1. **Laboratory Procedure for Prothrombine time(PT)**

- I was collected samples from both the patients and controls in clean container or a tube having 3.2% trisodium citrate.

- Immediately, I have mixed the blood with anticoagulant avoiding foam formation. Centrifuge the sample for 15 min at approximately 3000 rpm and collect the plasma in separate tube.

- I used Fresh plasma which is preferred for testing as it performs best when tested immediately.

- I dispensed properly 100µl of platelet poor plasma (PPP) into a cuvette and incubate for 3 minute.

- I placed the curette into measuring position or optical area carefully.

- I vertically dispensed 200µl of thromboplastin reagent into the cuvette.

- Start timer with addition of RGT and record time required for clot formation. It's counting and record times in seconds when clotting occur. Normal range 10_15second [28].

2. **Laboratory Procedure for activated partial thromboplastine time(aPTT) [28]**
   - I Properly Dispensed 50µl pre warmed platelet poor plasma (ppp) into cuvette and then incurve for 3 minutes after adding RGT1.

   - I Carefully Dispensed 50µl aPTT RGT2 into cuvette
   - Immediately Start timer with addition of reagent and record time required for clot formation.

   - Record the time in second.

Normal range 26_45second

3. **Procedure for platelet count [28]**
   - I Collected 2ml of blood with EDTA test tube.
   - I Mixed gently
   - I placed the well mixed EDTA tube to sample position of HUMA CONT hematology analyzer.

Press start and then the 18 CBC profile including platelet will be measured and displayed on the screen within a minute. The reference range of the laboratory was 150,000-450,000/µl

DECLARATION

I, the undersigned, Hematology student declare that this thesis entitled **"determination of prothrombin time, activated partial thromboplastin time and platelet count of type ii diabetes mellitus patients attending Felegehiwot referral hospital, Bahir Dar, northwest Ethiopia"**is my original work in partial fulfillment of the requirement for the degree of Master of Science in Hematology.

Name: YitayalAmogne

Signature: _____

Place of submission: Department of Hematology, School of Biomedical and Laboratory sciences, University of Gondar.

Date of Submission: _____

This thesis work has been submitted for examination with our approval as university advisor(s).

Advisors' Name Signature

Mr. Bamlaku Enawgaw _____

Mr. Molla Abebe _____

Examiners' Name Signature

_____ _____

_____ _____