CHAPTER ONE

1. Introduction. Literature review & objectives.

1.1 Introduction :

1.1.1Coagulation mechanism :

Blood coagulation is a series of reactions in which plasma zymogens are converted into active enzymes. The final event of these reactions is the formation of an insoluble fibrin clot. These coagulant reactions are regulated by a number of stimulatory and inhibitory mechanisms. Thus, coagulation is a finely regulated system that maintains blood in a fluid phase, but can rapidly respond to injury for the formation of clots. Factor VII is a vitamin K–dependent serine protease glycoprotein (also known as stable factor or proconvertin) with a pivotal role in haemostasis and coagulation. Other vitamin K–dependent factors include prothrombin, factors IX and X, and proteins C and S. Tissue factor is an intrinsic membrane glycoprotein that is normally not exposed on the surface of intact blood vessels. When the vascular lumen is damaged, tissue factor is exposed and then binds to the small amounts of circulating factors VIIa and VII. This facilitates conversion of factor VII to factor VIIa. Factor VIIa bound to tissue factor in the presence of calcium and phospholipids facilitates the conversion of factor IX to factors IXa and X to factor Xa. Coagulation has traditionally been considered to occur via extrinsic and intrinsic pathways. (Gene, 2011)

Although this division is useful for understanding in vitro laboratory coagulation tests, no such division occurs in vivo because the tissue factor VIIa complex is a potent activator of factor IX and factor X. (Dugdale et al,.2001)

Several observations showed that vascular damage and endothelial dysfunction occurs early in the course diabetic microangiopathy. Increased endothelin-1 (ET-1) levels, increased plasma concentrations of tissue plasminogen activator (tPA) inhibitor, von Willebrand factor, fibrinogen, activated factor VII (FVII:c) and decreased concentrations of endothelium derived relaxing factor, prostacyclin, tPA and reduced fibrinolytic potential of vascular endothelium. The net effect of all these changes is to convert

the endothelium from a thrombo-resistant to a thrombogenic surface and consequently, impairment of coagulation and of anticoagulant pathways. Coagulation and fibrinolytic abnormalities are stronger determinants of the presence of diabetic vascular complications. Coagulation profiles can be used to evaluate the incidence and assess the severity of diabetic retinopathy and other microangiopathies. Endothelin-1 (ET-1), a novel 21-amino acid vasoconstrictive peptide secreted by endothelial cells, has been thought to play a role in various forms of vascular disease(Heit, 2005).

1.1.2 Diabetes mellitus:

is a syndrome characterized by chronic hyperglycaemia, disturbed carbohydrate, fat, protein, water, electrolytes and acid base metabolism. It is actually a group of metabolic diseases resulting from defects in insulin secretion, insulin action or both. (Rochester 2015)When viewed in the context of coexisting insulin resistance; lack of insulin effect plays a primary role in the metabolic derangements linked to diabetes and hyperglycaemia in turn plays an important role in disease-related complications. Diabetes affects more than 135 million people. This figure is projected to reach 300 million cases by 2025 (Inzucchi et al 2010).

`1.2 Literature review:

1.2.1 Overview of NormalHaemostasis:

Haemostasis refers more widely to the process whereby blood coagulation is initiated and terminated in a tightly regulated fashion,together with the removal (orfibrinolysis) of the clot as part of vascular remodeling. Hemostasis depends on a system of checks and balances between thrombosis and hemorrhage that includes both procoagulants and anticoagulants. This scale needs to be kept in balance.

Blood coagulation is a complex process by which blood clot is formed. It is an important part of haemostasis where the blood clot occludes the blood vessel injury and hence stopping the bleeding. And thereafter repair of the damaged vessel begins. Disorders of coagulation can lead to hemorrhage or thrombosis. Coagulation begins almost instantly after an injury to the blood vessel endothelial lining (Gene, 2011). Platelets immediately form a plug at the site of injury; this is called primary haemeostasis Secondary haemostasis occurs simultaneously where the coagulation factors respond in a complex cascade to form fibrin strands, which strengthen the platelet plug (Gene, 2011). The coagulation cascade in secondary homeostasis has two pathways which convert fibrinogen, a soluble protein, to insoluble strands of fibrin, which, together with platelets, form a stable thrombus (Heit, 2005).

The intrinsic and extrinsic pathway model divides the initiation of is thought to be responsible for the initial generation of activated Factor X (Xa), where as the extrinsic pathway leads to amplification of coagulation into two distinct parts. The extrinsic pathway Factor Xa generation. Factor Xa plays a central role in the coagulation cascade because it occupies a pointwhere the intrinsic and extrinsic pathways converge

(Colman et al., 2002). The other mechanism that occurs in equilibrium withclot formation is fibrinolysis, which is a process thatprevents blood clots from growing and becomingproblematic.

This process has two types:

Primaryfibrinolysis and secondary fibrinolysis. The primary type isa normal body process, whereas secondary fibrinolysis isthe breakdown of clots due to a medicine, a medicaldisorder, or some other cause (Dugdale et al. (2011).

In fibrinolysis, a fibrin clot is broken down by theenzyme plasmin that cuts the fibrin mesh at variousplaces, leading to the production of circulating fragmentsthat are cleared by other proteases or by the kidney andliver (Cesarman-Maus and Hajjar, 2005).

1.2.1.1Coagulation cascade:

A cascade is a mechanism in which enzymes activate other enzymessequentially usuallyleading to an amplification of an initial signal. (Heit, 2005)

_ Each of these pathwaysleads to the conversion offactor X (inactive) tofactor Xa(active).

The intrinsic and extrinsic coagulation pathways are a series of reactions involve coagulation factors known as:

1. Enzyme precursors (zymogens)
2. Non-enzymatic (cofactors)
3. Calcium (Ca ++)
4. Phospholipids (PL).

_ All coagulation factors normally are present in the plasma, with PL being provided by platelets

.Cagulation factors:

Zymogens:

_ Factors II, VII, IX, X, XI, XII, and prekallikrein

_ NO biologic activity until converted by enzymes to active enzymes called serine proteases. (Cesarman-Maus and Hajjar, 2005).

_ Cofactors:

_ Factors V, VIII, tissue factor, and HMWK.

1.2.1.2 Intrinsic Pathway:

Activated in vivo by endothelial injury, in vitro by glass or other contact A foreign surface such as _ collagen activates factor XII (Hageman factor). _ Acting as catalysts are high MW Kininogen(HMWK) and kallikrein in the contact phase . (Colman et al., 2002)

1.2.1.2.1.Contact Group:

XI, XII, HMWK, PK _ The contact group is adsorbed by contact witha negatively charged surface such as collagen or the sub endothelium in vivo_ Not Vitamin K dependent.

1.2.1.2.2 Contact Activation Pathway:

Calcium is involved in three steps: the activation ofFIX, X and prothrombin.

cofa_ Cofactor VIII interacts in the activation of factor X andctor V reacts with prothrombin.

_ The platelet phospholipid surface acts as template in the activation of FX and prothrombin. (Colman et al., 2002)

1.2.1.3 Extrinsic pathway:

Is initiated by the release of tissue thromboplastin

(Factor III) which is exposed to the blood when there is damage to the blood vessel.

Factor VII which is a circulation coagulation _ factor, forms a complex with tissue thromboplastin and calcium.

_ This complex rapidly converts Factor X to the enzyme form Factor XaFactor catalyzes the prothrombin (Factor II) to thrombin (Factor IIa) reaction which is needed to convert fibrinogen (Factor I) to fibrin.

_ XIIIa and Ca++ stabilize fibrin clot(Cesarman-Maus and Hajjar, 2005).

_ Formation of blood clot causes more clotting to occur—positive feedback.

1.2.1.4Activation of Prothrombin into Thrombin:

Prothrombin is soluble single chain glycoprotein (72kDa)synthesized in liver.

_ Thrombin is produced by the enzymatic cleavage of two sites on prothrombin by activated Factor X (Xa) and generate active 2 chain thrombin molecule which is then released from platelet surface.

_ The A and B chains of thrombin are held together by a dissulfide bond. The activation of prothrombin occurs on the surface of activated platelets and requires assembly of prothrombinase complex consisting of platelet, anionic PLs, Ca2+, factor Xa and prothrombin.

_ This complex is termed factor Vawhich is activated bytraces of thrombin

_ Factor Vais subsequently inactivated by further action of thrombin to limit activation of prothrombin tothrombin. (Colman et al., 2002)

1.2.1.5 Coagulation Tests:

Coagulation tests measure ability to clot and the amount of time it takes , testing can help to assess risk of bleeding or thrombosis in blood vessels(Colman et al., 2002)

1.2.1.6 Prothrombin Time:

Prothrombin time (PT) is a blood test that measures how long it takes blood to clot. A prothrombin time test can be used to check for bleeding problems. PT is also used to check whether medicine to prevent blood clots is working.

the PT test measures the clotting time of recalcified plasma in the presence of an optimal concentration of tissue extract tthromboplastin and indicate the overall efficiency of the extrinsic clotting system. Altough originally thought to measure prothrombin, the test is now known to depend also on reactions with factors V, VII, X and on the fibrinogen concentration in the plasma. A PT test may also be called an INR test. INR (international normalized ratio) stands for a way of standardizing the results of prothrombin time tests, no matter the testing method. It lets doctor understand results in the same way even when they come from different labs and different test methods. In some labs, only the INR is reported and the PT is not reported

Blood clotting factors are needed for blood to clot (coagulation). (Cesarman-Maus and Hajjar, 2005). Prothrombin, or factor II, is one of the clotting factors made by the liver. Vitamin K is needed to make prothrombin and other clotting factors. Prothrombin time is an important test because it checks to see if five different blood clotting factors (factors I, II, V, VII, and X) are present.

The prothrombin time is made longer by:

- Blood-thinning medicine, such as warfarin.
- Low levels of blood clotting factors.
- A change in the activity of any of the clotting factors.

- The absence of any of the clotting factors.
- Other substances, called inhibitors, that affect the clotting factors.
- An increase in the use of the clotting factors.

The normal range of Prothrombin time (PT) is 11-13 second.

An abnormal prothrombin time is often caused by liver disease or injury or by treatment with blood thinners. (Colman et al., 2002)

1.2.1.7Partial Thromboplastin Time:

Partial thromboplastin time (PTT) is a blood test that measures the time it takes your blood to clot. A PTT test can be used to check for bleeding problems.

Blood clotting factors are needed for blood to clot (coagulation).:

Normal range of Activated partial thromboplastin time (APTT) is 30-40 seconds

Abnormal Activated partial thromboplastin time may be due to:

- A longer-than-normal PTT or APTT can mean a lack of or low level of one of the blood clotting factors or another substance needed to clot blood. This can be caused by bleeding disorders, such as hemophilia or von Willebrand's disease.
- A longer-than-normal PTT or APTT can be caused by liver disease, kidney disease (such as nephrotic syndrome), or treatment with blood thinners, such as heparin or warfarin (Coumadin).

- A longer-than-normal PTT may be caused by conditions such as antiphospholipid antibody syndrome or lupus anticoagulant syndrome. These conditions happen when the immune system makes antibodiesthat attack blood clotting factors. This can cause the blood to clot easily in veins and arteries .The test measure the clotting time of plasma after activation of ccontacts factors and the addition of phospholipid and CaCl2 and so detect the overall efficiency of the intrinsic pathway(Colman et al., 2002). To standardize the activation of contact factors the plasma is first preincubated for a set period with a contact activator such as kaolin , silica or ellagic acid. During this phase of test, factor XII is produced, which cleaves factor XI , but coagulation does not proceed in the absence of calcium. After recalcification factor XI activates factor IX and coagulation follows. A standardized phospholipid is provided to allow the test to be performed in PPP. The test does not depend on contact factors and factor VIII, IX but also on the reaction with factors X , V, prothrombin and fibrinogen. It also sensitive to the presence of circulating anticoagulants**.** (Cesarman-Maus and Hajjar, 2005).

1.2.2 Diabetes Mellitus

1.2.2.1 Back ground

Diabetes mellitus is a complex metabolic disorder characterized by persistent hyperglycaemia (higher than normal blood glucose levels) resulting from defects in insulin secretion, insulin action or both. The two main types of diabetes are type 1 (formerly known as insulin-dependent diabetes) and type 2 (formerly known as non–insulin-dependent

diabetes). Type 1 diabetes is caused predominantly by the autoimmune destruction of the insulinproducingβ-cells of the pancreatic islets, while type2 diabetes results from both impaired insulin secretion and resistance to the action of insulin. Diabetes is a major global health problem and in 2010 was estimated to affect 285 million individuals worldwide; this figure is projected to rise to more than 400 million over the next two decades as a result of changing population demographics, such as ageing and urbanization, and changes in lifestyle, such as diet an exercise, and the associated increase in obesity (Richard and Neil 2012).

1.2.2.2 Prevalence of diabetes

The increaselargely represents an increase in the prevalence of type 2 diabetes, which accounts for ~90% of all cases of diabetes, but the prevalence of type 1 diabetes is also increasing.The prevalence of diabetes in the USA in 2010 was almost 27 million (12.3%), while 3.6 million people are affected by diabetes in the UK (7.4%) (Richard and Neil 2012).

1.2.2.3 Prediabetes (Borderline Diabetes)

Prediabetes, also commonly referred to as borderline diabetes, is a metabolic condition and growing global problem that is closely tied to obesity.

If undiagnosed or untreated, prediabetes can develop into type 2 diabetes; which whilst treatable is currently not fully reversible. Prediabetes is characterised by the presence blood glucose levels that are higher than normal(V.Rochester 2015).

1.2.2.4 Types of diabetes

1.2.2.4.1 Type 1 diabetes

Type 1 diabetes is caused by an absolute deficiency of insulin. In populations of white NorthernEuropean ancestry, it usually occurs as the result of a T-cell–mediated autoimmune destruction of the β-cells of the pancreas . By contrast, autoimmune type 1 diabetes is uncommon in non-Caucasian populations. With a better understanding of the pathogenesis of diabetes, it is recognized that other genetic or acquired factors affecting pancreatic β-cell function can result in diabetes that presents in the same way as autoimmune type 1 diabetes . Furthermore, there may be a broad range of clinical manifestations that overlap between type 1 and type 2 diabetes (Richard and Neil 2012).

1.2.2.4.1.1 Aetiology

It is apparent that both genetic and environmental factors are important in the development of Type 1 diabetes

1. ***Genetic factor***

Evidence for the importance of genetic factors comes from twin and family studies. The risk of developing diabetes increases with the number of family members with the condition (see table 1. 2). (Richard and Neil 2012).

Table(1.2);- Risk of developing type 1 diabetes for relatives

Family member	Risk
Monozygotic twin	30-50% in other twin 65-70% if twin diagnosed before Age of 5 years
Diazygotic twin	15%
Sibbling with HLA genotype that is identical to the affected sibling	16-20%
Sibbling who shares one HLA gene	9%
HLA non identical sibling	3%
Mother	2%
Father	8%
Both parent	30%
General population	0.4%

2. Environmental factors

Although the genetic susceptibility to type 1 diabetes is inherited, only 12–15% of type 1 diabetes occurs in families with a history of diabetes and only 10% of HLA-susceptible individuals develop type 1 diabetes. This indicates that genetic factors do not account entirely for the development of type 1 diabetes.

1.2.2.4.2 Type 2 Diabetes

Type 2 diabetes is a heterogeneous disorder that results from the interaction of genetic predisposition and environmental factors, creating a combination insulin deficiency and insulin resistance (Richard and Neil 2012).

1.2.2.4.2.1 Aetiology

The risk factors for type 2 diabetes can be divided into those that are unmodifiable, and those that are environmental and therefore potentially changeable (Table 1.3).

Un modifiable	**Enviromental**
Family history-genetic	Obesity
Birth weight	Diet
Ethnicity	Physical activity
Age	urbanization
Past history of diabetes in pregnancy (gestational Diabetes)	

Table (1.3);- Risk Factors for Type 2 Diabetes

1.2.2.4.2.2Genetic predisposition

The heritability of type 2 diabetes is greater than for type 1 diabetes, and is estimated to account for 40–80% of total disease susceptibility . Many patients have a family history of diabetes, and monozygotic twin studies show a high concordance rate (60–90%). A maternal history of diabetes confers a higher risk of type 2 diabetes in the offspring than a paternal history, possibly through an effect of maternal hyperglycaemia during pregnancy(Richard and Neil 2012).

1.2.2.4.2.3 Environmental factors

The most important environmental risk factors for diabetes are obesity and physical inactivity.

** Obesity*

The massive explosion in obesity rates worldwide has largely been responsible for the increase in diabetes; it is estimated that up to 80% of all new casesof diabetes can be attributed to obesity. In the UK, the average body mass index (BMI) of a person with type 2 diabetes is 30.0 kg/m2, while in the USA, 67% of those with type 2 diabetes have a BMI of greater than 27 kg/m2, and 46% have ity a BMI of greater than 30 kg/m2. The risk of developing type 2 diabetes increases across the whole range of BMI, such that the risk in a middle-aged woman whose BMI is greater than 35 kg/m2 is 93.2 times greater than in a woman whose BMI is less than 22.5 kg/m2 . Similar changes are alsoseen in men.(Richard and Neil 2012).

** Physical inactivity*

Physical inactivity is also associated with an increased risk of diabetes. People who exercise for around 30 min/day have half the risk of developing diabetes compared to those with a sedentary lifestyle. Although some of the difference can be explained by differences in adiposity, exercise itself accounts for approximately half of the effect.(Richard and Neil 2012).

** Intrauterine environment*

The intrauterine environment is important for the development of type 2 diabetes. Low birth weight.and thinness at birth are associated with

increasing insulin resistance and diabetes in the offspring. In contradiction to this general observation, it appears that babies born to mothers with diabetes are also at increased risk of diabetes, despite the fact that thesebabies often have a high birth weight. Consequentlytherelationship between birth weight and subsequent risk of diabetes appears to be J-shaped.(Richard and Neil 2012).

1.2.2.4.2.4 Ageing

- The changing world demographics with its ageing population add a further explanation for the increase in diabetes as the prevalence increases with age.(Richard and Neil 2012).

1.2.2.4.2.5 Pathogenesis

Under normal physiological conditions, plasma glucose concentrations are maintained within a narrow range, despite wide fluctuations in supply and demand, through a tightly regulated and dynamic interaction between tissue sensitivity to insulin (especially in the liver) and insulin secretion. In type 2 diabetes, both of these mechanisms break down with impaired insulin secretion through pancreatic β-cell dysfunction and impaired insulin action through insulin resistance (Richard and Neil 2012).

1.2.2.4.2.6 Prognosis

Type 2 diabetes is associated with premature mortality, predominantly through cardiovascular disease. Even after adjustment for other cardiovascular risk factors, diabetes is associated with a twoto three-fold increase in the risk of myocardial infarction or stroke.(Richard and Neil 2012).

1.2.2.4.3 Gestational diabetes

Gestational diabetes occurs when there is a high blood glucose level during pregnancy. As pregnancy progresses, the developing baby has a greater need for glucose. Hormone changes during pregnancy also affect the action of insulin, which brings about high blood glucose levels(Rochester 2015).

1.2.2.5 Causes of diabetes

The causes of diabetes are not known. The following factors may increase your chance of getting diabetes:

Family history of diabetes or inherited tendency

African-American, Hispanic, Native American, or Asian-American race, Pacific Islander or ethnic background

Being overweight (20 percent or more over your desired body weight)

Physical stress (such as surgery or illness)

Use of certain medications, including steroids and blood pressure medications

Injury to the pancreas (such as infection, tumor, surgery, or accident)

Autoimmune disease

High blood pressure

Abnormal blood cholesterol or triglyceride levels

Age (risk increases with age)

Alcohol (risk increases with years of heavy alcohol use)

Smoking

History of gestational diabetes or delivery of a baby weighing more than 9 pounds (4.1 Kg).

Pregnancy

It is important to note that sugar itself does not cause diabetes. Eating a lot of sugar can lead to tooth decay, but it does not cause diabete(Rochester 2015)..

1.2.2.6 Symptoms of diabetes

The symptoms of diabetes include

Increased thirst ,Increased hunger (especially after eating) ,Dry mouth ,Frequent urination ,Unexplained weight loss (even though you are eating and feel hungry) ,Weak, tired feeling ,Blurred vision ,Numbness or tingling in the hands or feet ,Slow-healing sores or cuts ,Dry and itchy skin (usually in the vaginal or groin area) ,Frequent yeast infections (Rochester 2015).

1.2.2.7 Symptoms of low blood sugar

Most people have symptoms of low blood sugar (hypoglycemia) when their blood sugar is less than 60 mg/dl. (Your health care provider will tell you how to test your blood sugar level.) When your blood sugar is low, your body gives out signs that you need food. Different people have different symptoms. You will learn to know your

symptoms.Common early symptoms of low blood sugar include the following:

Feeling weak ,Feeling dizzy ,Feeling hungry ,Trembling and feeling shaky Sweating ,Pounding heart ,Pale skin and Feeling frightened or anxious (Rochester 2015).

1.2.2.8 Diagnosing of diabetes mellitus

Diabetes is diagnosed with fasting sugar blood tests or with A1c blood tests, also known as glycated hemoglobin tests. A fasting blood sugar test is performed after you have had nothing to eat or drink, Normal fasting blood sugar is less than 100 mg/dl (5.6 mmol/l). You do not have to be fasting for an A1c blood test (see table 1.4) (Inzucchi et al 2010).

	Normal	**Pre-diabetes**	**Diabetes**
Fasting Glucose Test	Less than 100	100-125	126 or higher
Random (anytime) Glucose Test	Less than 140	140-199	200 or higher
A1c Test	Less than 5.7%	5.7 - 6.4%	6.5% or higher
Source: American Diabetes Association 2010			

Table(1.4) Diagnosis of diabetes mellitus

1.2.2.9 Management of diabetes

There is no cure for diabetes, but it can be treated and controlled. The goals of managing diabetes are to:

Keep your blood glucose levels as near to normal as possible by balancing food intake with medication and activity.

Maintain your blood cholesterol and triglyceride (lipid) levels as near the normal ranges as possible by decreasing the total amount of fat to 30% or less of your total daily calories, and by reducing saturated fat and cholesterol.

Control your blood pressure. (Your blood pressure should not go over 130/80.)

Decrease or possibly prevent the development of diabetes-related health problems (Inzucchi et al 2010).

1.2.2.10 long-term complications of diabetes

Retinopathy (eye disease): All patients with diabetes should see an ophthalmologist (eye specialist) every year for a dilated eye examination. Patients with known eye disease or symptoms of blurred vision in one eye or who have blind spots may need to see their ophthalmologist more often.

Nephropathy (kidney disease): Urine testing should be performed every year. Regular blood pressure checks also are important because control of high blood pressure is essential in slowing kidney disease. Generally, blood pressure should be less than 130/80 in adults. Persistent swelling in the leg or feet also may be a symptom of kidney disease and should be reported to your doctor.

Neuropathy (nerve disease): Numbness or tingling in your feet should be reported to your doctor at your regular visits. Check your feet every day for redness, calluses, cracks, or breakdown in the skin tissue. If you notice these symptoms before your scheduled visits, notify your doctor immediately(Inzucchi et al 2010).

1.2.2.11Other long-term complications may include

Eye problems, including glaucoma and cataracts ,Dental problemsHigh blood pressureand Heart disease(Inzucchi et al 2010).

1.3 Previous Studies:

there were many studies have been conducted to assess the coagulation profile in Diabetes Mellitus type 2 :

a study conducted byFayeza Karim , Qazi ShamimaAkter ,Shamima Jahan , AfruzaKhanom , SamiraHaque ,Tania Yeasmin , TashfiaSiddika , Susmita Sinha found abnormally shortened PT and APTT 93% and 91% in diabetic patients respectively .

otherstudy conducted by AnkalayyaB , H S Sodhi , Sudhir Modala , ManishaBaghel in India found significantlower value in diabetic patients than controls .

1.4. Rationale:

Diabetes mellitus is a common disorder of endocrine system and recently became worldwide problem , many abnormalities of coagulation occur in patients with Diabetes mellitus and both hypocoagulable and hypercoagulable states have been reported .

Disease have been associated with change in PT and so we will conduct this study to determine the effect of diabetes mellitus on PT and APTT.

1.5 Objectives:

1.5.1 general objectives:

to assess the coagulation state in type 2 diabetic Sudanese patients.

1.5.2 specific objectives:

- To measure prothrombin time (PT) and activated partial thromboplastin time (APTT) in type 2 diabetic patientsand compare with the control.
- to correlate between the disease duration (PT) and prothrombin time and activated partial thromboplastin time (APTT).

CHAPTER TWO

2.Materials and Methods :

2.1 Study design and duration:

This was a case control study, carried out from June to November 2017.

2.2 Study area and population:

this study was conducted in international hospital and carried on diabetes mellitus type 2 patients.

2.3 Inclusion criteria:

Diagnosed diabetes mellitus type 2.

2.4 Exclusion criteria:

- Hypertension patients.
- Cardiovascular disease patients.
- History of bleeding.
- History of thrombosis.
- Liver diseases.
- Drugs known to alter test results.
- Pregnancy.
- Obese.

2.5 Ethical approval:

the permission was taken from all authorities of sudan university of science and technology and hospitals to carry out this study.

All patients were informed for the purpose and their approval was taken for sample collection.

2.6 Sample collection:

Blood sample was collected in citrate containers , then separated and tested for PT and APTT using semi-automated coagulometer.

2.7 The test principle:

Prohrombin time

the PT test measures the clotting time of recalcified plasma in the presence of an optimal concentration of tissue extract thromboplastin and indicate the overall efficiency of the extrinsic clotting system. Although originally thought to measure prothrombin, the test is now known to depend also on reactions with factors V, VII, X and on the fibrinogen concentration in the plasma(Colman et al., 2002)

Acivated partial thromboplastin time

The test measure the clotting time of plasma after activation of ccontacts factors and the addition of phospholipid and CaCl2 and so detect the overall efficiency of the intrinsic pathway. To standardize the activation of contact factors the plasma is first pre incubated for a set period with a contact activator such as kaolin , silica or ellagic acid. During this phase of test, factor 12 is produced, which cleaves factor 11 , but coagulation does not proceed in the absence of calcium(Colman et al., 2002)

After re calcification factor XI activates factor IX and coagulation follows. A standardized phospholipids is provided to allow the test to be performed

in PPP. [36]The test does not depend on contact factors and factor VIII,IX but also on the reaction with factors X,V prothrombin and fibrinogen. It also sensitive to the presence of circulating anticoagulants (Colman et al., 2002)

2.8 Data analysis:

Data was analyzed by using SPSS software program, data was presented in form of tables and figures.

CHAPTER THREE

Results:

General characteristics are presented in table 3.1. subjects of two groups were matched in respect of age, BMI and BP. Mean prothrombin time (PT) and activated partial thrombin time (APTT) levels were significantly (P<0.001) lower in patients with diabetes mellitus (table 3.2). Again in this study abnormally shortened PT and PTT were found in 93% and 91% diabetic patients respectively(figure 3.3 & 3.4).

Table 3.1

Parameters	Control {n=50}	Diabetes {n=50}
Age (years)	53.58±4.75	54.72±5.73
BMI (K/m2)	23.13±2.26	23.15±2.96
DBP(mm of Hg)	65.95±6.34	62.80±4.40

Results are expressed as Mean ±SD. Unpaired

Student's't' test analyzed statistical significance.{ n =50}

Number of subjects. BMI= Body mass index,

, DBP= Diastolic bloodpressure.

Table 3.2:

Parameter	Control {n=50}	Diabetes {n=50}
Pt	11.18±0.41	9.54±0.58***
A Ptt	31.88±2.20	19.94±0.62***

Results are expressed as Mean±SD. Unpaired Student's't' test analyzed statistical significance. ***P< 0.001. PT= Prothrombin time, APTT=Activated partial thromboplastin time

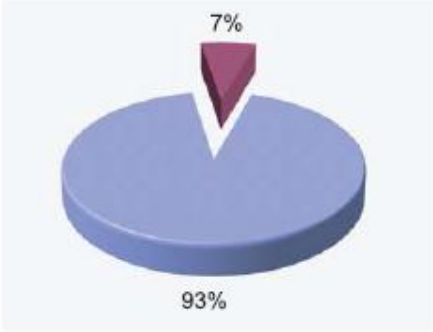

Figure 3.3: Frequency% of PT in diabetic patients are showing majority of patients had abnormallylow prothrombin time (n =50) Cut point = 11sec

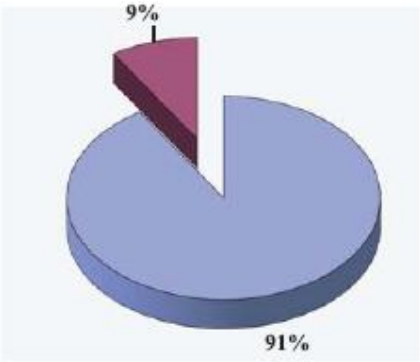

Figure 3.4: Frequency% distribution of activated partial thromboplastin time in diabetes is presenting that majority of patients had abnormally lower APTT. (n= 50) Cut point 24 sec.

Chapter four

Discussion, conclusion and Recommendation

4.1 Discussion :

In the present study, lower PT level in type 2diabetic male than healthy male is comparable toothers15,16. But some studies found no significant changes17,18,19, whereas few investigators foundprolonged PT in type 2 diabetic subjects20.

Again, in the present study, APTT level waslower in type 2 diabetic male which was similar toothers21,22.

But, some investigators did not findany significant change of APTT in between type2 diabetic male and adult healthy male23,24.

Moreover, some researcher found that APTTlevel was prolonged in type 2 diabetic subjects25.

It has been suggested that natural anticoagulantantithrombin III keeps the natural procoagulantinhibitedIn addition protein C inactivates factorsVa and VIIIa.

Hyperglycemia causes nonenzymatic glycation of this antithrombin III anddepressed it's biological activity and also directlydecreases the concentration of protein C.

Therefore impaired function of naturalanticoagulants activate clotting factors andcontribute to the onset of hypercoagulability in DM26,27**.**

In this study the abnormally low levelsof PT & APTT in 93% & 91% in diabetic patients,in addition to significantly lower mean level of these parameters in DM are suggestive ofhypercoagulable state in diabetes which may actsas a risk factor for future cardiovascular disease28.

4.2 Conclusion;

From the present study it may be concluded thatpatients with diabetes mellitus are more prone todevelop hypercoagulation state.

Therefore,routine examinations of PT & APTT are important to assess coagulation impairment in DM in order to prevent thromboembolic CVD in DM

4.3 Recommendations:

- Further study with large sample size are needed.
- the disease duration should be included in the study to find its association with coagulation state.
- Investigation for factor vii and their association with coagulation state and diabetes mellitus.

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A questionnaire :

to assess the coagulation profile in diabetes mellitus type 2 patients :

Name : …….

Age : ……

Gender : male ☐

Female ☐

Any medication you use : ……