Chapter one

Introduction and Literature review

1.1 Introduction

Diabetes mellitus is a chronic life long condition that affects bodies' ability to use the energy found in food, there are three major types of diabetes type1diabetes, type2 diabetes and gestational diabetes it's become the epidemic of the 21century (Heit *et al.,*2009) .

Thrombosis is the formation of blood clot inside blood vessel, obstructing the blood flow through the circulatory system, when blood vessel is injured the body uses platelet and fibrin to form blood clot ,when blood vessel is not injured blood clot may form in the body under certain condition clot or piece of clot begin to travel around the body is known as embolus (Furie,2009).

Type2 diabetes mellitus is a chronic metabolic condition characterized by insulin resistance (that is the body's inability to effectively use insulin) and insufficient pancreatic insulin production, resulting in high blood glucose levels (hyperglycemia) the development of complication (Ryden *et al.,*2013).

There are subdivided microvascular complication affecting principally the retina, kidneys and peripheral nerves and macrovascular disease affecting the arterial system including the coronary arteries (Petrauskine *et al.,*2005) .

Myocardial infarction is a common complication of type2 diabetes mellitus and lead to high degree of mortality and morbidity .Obesity is the reason for the high incidence of ischemic heart disease in patient with type 2 diabetes mellitus, physical inactivity, raised blood pressure, disturbed blood lipid levels and therefore is recognized to have an increased cardiovascular risk.

It is associated with long-term microvascular and macrovascular complications, together with reduced quality of life and life expectancy (Holst *et al.,*2010).Type II diabetes mellitus indicate that major vascular events such as myocardial infarction and vascular death may occur at a rate of 5-7% per year individuals who have no known previous cardiovascular event this extraordinarily high cardiovascular event rate is at least three to four times that seen in comparable individuals without diabetes (Bouzeghrane *et al.,*2008).

1.2 Literature review

1.2.1 Haemostatic component

There are three basic components of haemostasis the extra vascular, vascular and intra vascular component

1.2.1.1 Extra vascular component

The extravascular heomostatic component involves the tissues surrounding blood vessels, play part in haemostasis by providing back pressure on the injured vessel through swelling and the trapping of escaped blood the increased tissue pressure tends to collapse venules and capillaries ability of surrounding tissues to aid in hemostasis depends on the bulk or amount of surrounding tissue type of tissue surrounding and the tone of the surrounding tissue (Frinkin *et al.,*2004).

1.2.2.2 Vascular and intravascular component

The vascular haemostatic component involves the vessels through which blood flow. The role played by vessels in heamostasis depends on their size, smooth muscle amount within their walls and integrity of the endothelial cell lining.

The key component in intravascular hemostasis are platelets and many biochemical's (procoagulants) in the plasma, these components are involved in either coagulation (clot or thrombus formation) or fibrinolysis (Frinkin *et al.,*2004).

1.2.3 Normal hemostasis

Hemostasis is process which causes bleeding to stop meaning to keep blood within damaged blood vessel (the opposite of hemostasis is hemorrhage) it is the first stage of wound healing this involves coagulation blood changing from liquid to gel intact blood vessels are centrals to moderating blood tendency to form clot .The endothelial cells of intact vessels prevent blood clotting with heparin like molecules and thrombomodulin and prevent platelet aggregation with nitric oxide and prostacyclin .When endothelial injury occurs the endothelial cells stop secretion of coagulation and aggregation inhibitors and instead secrete von will brand factor which initiate the maintenance of hemostasis after injury (Holst *et al.,*2010).

Hemostasis has three major step vasoconstriction, temporary blockage of break by platelet plug and, blood coagulation or formation of fibrin clot these prosses seal the hole until tissues are repaired.

Normal hemostasis occurs in three main stages. Primary hemostasis occurs when platelets attach to a damaged or disrupted area of the endothelium. This adhesion allows the platelets to undergo a shape change and then aggregate together. Once adhered to each other a temporary platelet plug is created. However, the plug is not stable for long if the secondary haemostatic forces do not strengthen and reinforce the plug with a cross linked fibrin mesh. The final stage of coagulation involves fibrinolysis. In this stage, plasminogen is activated to plasmin, which then breaks down the fibrin and removes the clot (Marieb and Hoehn,2010) .

1.2.4 Primary haemostasis

Primary hemostasis involves three main sequential steps.Induced platelet adhesion, platelet activation and platelet aggregation. It involves platelets. Normal canine platelets have an average lifespan of approximately 6 days. It is synthesized in bone marrow and smooth muscle cells, mainly in response to thrombopoietin. It has no nucleated and contains both alpha and dense granules. Alpha granules contain substances such as platelet derived growth factor, fibronectin, transforming growth factor, platelet factor 4, fibrinogen, factors V and VIII, and von Willebrand factor (vWF). Dense granules contain adenosine diphosphate (ADP), adenosine triphosphate (ATP), histamine, epinephrine, serotonin, and calcium ions. These substances are released during platelet activation and maintain various roles. Some of these substances recruit other platelets to the site of injury (Ajjan and Grant,2006).

Under physiologic conditions, hemostasis prevented by the endothelium. This provides a physical barrier and secretes platelets inhibitory products, such as prostacyclic(PGI2) and nitric oxide (NO). There are also substances expressed on the endothelial cell surface that degrade or inhibit platelet agonists these include ADPases, heparin sulfate, and thrombomodulin (Ajjan and Grant,2006).

1.2.4.1 Platelet adhesion

The first event in hemostasis is the adhesion of platelets to exposed subendothelium (ie collagen).In areas of high shear rate (in the microvasculature), this is mediated by von Will brand factor (vWf), which binds to glycoprotein Ib-IX in the platelet membrane. In areas of low shear

rate (e.g. aorta), fibrinogen mediates the binding of platelets to the sub endothelium (by attaching to a platelet receptor - the integrin, glycoprotein Ia/IIa) (Lan *et al.,*2005).

1.2.4.2 Platelet Activation

The adhesion of platelets to the vessel wall activates them, causing the platelets to change shape, to activate the collagen receptor on their surface (an integrin receptor called glycoprotein IIb/IIIa, now renamed integrin aIIb and to undergo the release reaction (release alpha and dense granule constituents). In addition, upon activation, platelets synthesize and release thromboxane A2 (TXA2) and platelet activating factor (PAF), which are potent platelet aggregating agonists and vasoconstrictors (Lan *et al.,*2005).

1.2.4.3 Platelet Aggregation

TXA2, PAF, ADP and serotonin are platelet agonists, causing the activation and recruitment of additional platelets, which bind to the adhered platelets. This activation is enhanced by the generation of thrombin through the coagulation cascade; thrombin being an important platelet agonist. Platelet aggregation is mediated primarily by fibrinogen (vWf has a secondary role), which binds to Integrin aIIb 3 on adjacent platelets. This aggregation leads to the formation of the primary platelet plug, which must be stabilized by the formation of fibrin (Lan *et al.,*2005).

Platelets also contribute to secondary hemostasis (coagulation cascade) by providing a phospholipid surface and receptors for the binding of coagulation factors. Another receptor that needs to be mentioned is the ADP receptor. Once ADP binds to its receptor, the integrin aIIb receptor

undergoes activation thus facilitating platelet aggregation. After the platelet plug has bridged the gap between endothelial cells, adjacent endothelial cells release prostacyclin causing vasodilatation and decreased platelet aggregation. This release of prostacyclin stops the platelet plug from growing out of control (Jennings,2009).

1.2.5 Secondary heamostasis

It defined as the formation of insoluble, cross-linked fibrin by activated coagulation factors, specifically thrombin. Fibrin stabilizes the primary platelet plug, particularly in larger blood vessels where the platelet plug is insufficient alone to stop hemorrhage.These consist of cells, enzymatic and non-enzymatic coagulation factors, protein substrates, calcium and phospholipid (phosphatidylserine, PS) membranes. Coagulation factors can be photolytic enzymes (zymogens) or non-enzymatic. Non-enzymatic coagulation factors can be cofactors for enzymatic coagulation factors or can just be a substrate (Factor I or fibrinogen) (Ajjan *et al*.,2006). Enzymatic and non-enzymatic coagulation factors are substrates for enzymatic coagulation factors. Phosphatidylserine-bearing cell membranes are usually provided by activated platelets, but leukocytes and erythrocytes, which are incorporated into the developing clot, can also expose PS on their cell surfaces and be a source of PS.(LaCorte,2011).

Cells fibroblasts, platelets, endothelial cells, leukocytes, enzymatic coagulation factors factors XI, X, IX, VII, and II. These are usually in an inactive form and must be activated before they can exert their enzymatic (cleavage) activity, non enzymatic coagulation factors cofactors .Tissue factor (TF), factors V and VIII. Both factor V and factor VIII, but not tissue

factor, require activation (by an enzymatic coagulation factor, usually thrombin or factor IIa), non-enzymatic coagulation factor substrate fibrinogen,calcium ,phosphatidylserinebearing membrane surfaces.
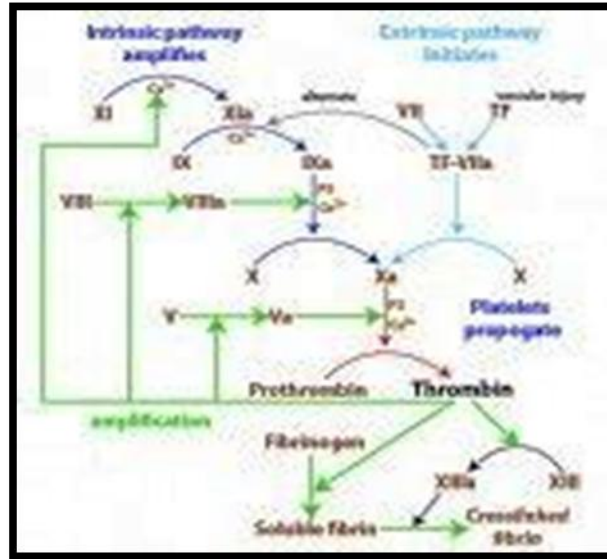
*Coagulation pathways (*Siller *et al.,*2011).

Traditionally, coagulation has been divided into three distinct pathways: extrinsic, intrinsic and common path way.

1.2.5.1.1Extrinsic pathway

This is composed of: Tissue factor (cofactor) and FVII (pro-enzyme; FVIIa is the enzyme), calcium (Ca^{2+}), the TF-FVIIa complex activates FX of the common pathway; it is thus called the "extrinsic tenase" (extrinsic pathway Factor X activator). The TF-FVIIa complex can also activate FIX of the intrinsic pathway (in the so-called "alternate pathway"), although FX is its preferred substrate in vitro. Under physiologic conditions (blood vessel injury), FXa is generated by the TF-FVIIa complex on the surface of fibroblasts (Siller *et al.,*2011).

1.2.5.1.2 Intrinsic pathway

This is composed of: Enzymatic coagulation factors: FXII, FXI, and FIX, the cofactor (non-enzyme) FVIII,Ca^{2+},phosphlipid,the ultimate product of the intrinsic pathway is activated FIX, which (with the aid of activated cofactor FVIIIa), activates FX. In fact, the FIX-FVIIIa-Ca^{2+}-PS complex is called the "intrinsic tenase" (intrinsic pathway Factor X activator). The FXa is generated by the intrinsic tenase on the platelet surface. Thus both intrinsic and extrinsic pathways converge at the activation of FX, however the site of activation of FX differs (fibroblast for the extrinsic pathway and platelet for the intrinsic pathway) (Lan *et al.*,2005).

1.2.5.1.6 Common pathway

This is composed of: Enzymatic coagulation factors: FX, prothrombin (FII) and FXIII (cross linker), the cofactor: FV, protein substrate: Fibrinogen, Ca^2 phospholipid ,FXa, with the aid of its cofactor FVa, activates prothrombin to thrombin. Thrombin activates FXIII and cleaves fibrinogen to soluble fibrin, which is then cross linked to insoluble fibrin by FXIIIa. These pathways interact on the surface of cells to generate thrombin, which cleaves and cross links fibrin (LaCorte,2011).

1.2.6 Thrombosis

Thrombus may be defined as mass of aggregation platelet adherent to the vessel wall and immobilized with fibrin there is a variable content of red cell and entrapped leucocytes and the proportions and arrangement of the various component depend on the local and general condition (John and Anne,2001).

1.2.6.1 Types of thrombosis

The size and constitution of thrombus depend on general factor (component of the blood) local factor (the blood flow and vessel wall) and the site where thrombus formation occurs either within arterial or venous circulation (John and Anne,2001).

1.2.6.2 Venous thrombosis

It more common when there is sluggish flow or stasis and endothelial changes, such thrombus is usually composed of abundant fibrin and many red cells (John and Anne,2001).

1.2.6.3 Arterial thrombosis

Occure around the orifiaces of branches an bifurcation, it areas where turbulence an sheer stresses are greatest ,that endothelial injury and other tomato's change are most marked and platelet aggregation are readily formed ,arterial thrombus thus formed has pale appearance due to predominance of platelet (Allen *et al.,*2016).

1.2.6.4Effects of thrombosis

Thrombosis may produce local and distance effect .the local effect depend on the site and the degree of vascular occlusion and the remote effects are due to embolic phenomena or to the release of vaso active substances from the evolving thrombus into the passing stream of blood. Detachment and mobilization of various thrombi may produce obstruction within the pulmonary arterial system (Allen *et al.,*2016).

1.2.7 Etiology of thrombosis

The etiology of thrombosis is complex subject and in most cases is multi factorial .there is close relationship between thrombosis and development of atherosclerotic vascular disease (development from blood component by hemodynamic factor and platelet leucocytes interaction with vessel wall which may lead to endothelial injury and smooth muscle migration ,by formation of persistent mural thrombi (Allen *et al.,*2016).

1.2.8 Risk factor of thrombosis

1.2.8.1 Hyper lipidaemia

A high level of plasma cholesterol and triglycerides have both lead to atherosclerotic change , hyper lipidaemia and obesity are also associated with decreased plasma fibrinolytic activity and enhanced coagulation , high incidence of premature peripheral vascular disease and myocardial infarction is found in person with hyper lipoproteinaemia (Weisel and Litvinov,2008).

1.2.8.2 Diabetes mellitus

There is some evidence to suggest enhanced coagulation and increased platelet responsiveness, functional abnormalities of endothelium that may result in increased tendency to platelet over reactivity(Weisel and Litvinov ,2008).

1.2.8.3 Smoking:

The increased mortality from ischemic heart disease and morbidity from peripheral vascular disease and cerebro vascular disease associated with cigarette smoking.

1.2.8.4 Exercise and body build:

The role of exercise and leanness of body as factor that reduce the incidence of thrombosis .

1.2.8.5Hypertension

May produce endothelial injury

1.2.9.1Congenitalfactor

Antithrombin111 deficiency**:** It rare disorder in which there is reduced activity of the natural anticoagulant (inhibitor) against activated factor x and thrombin it is inherited as autosomal and associated with high incidence of venous thrombosis and pulmonary embolism.

Protein C deficiency recurrent venous thromboembolic disease is associated with reduced plasma level of the vitamin k-dependent factor (Weisel and Litvinov,2008).

1.2.10 Thrombus formation

The occlusive vascular thrombus consists of a skeleton of fibrin fibers with cellular elements embedded in this network. The formation of the thrombus results secondary to a complex interaction between the cellular arm of coagulation, represented by platelets, and the fluid phase that includes various proteins involved in both formation and breakdown of the fibrinnetwork (Ajjan *et al*.,2006).Thrombin is the pivotal enzyme in the coagulation pathway having a crucial role in both fibrin formation and platelet activation .Thrombin is generated secondary to vascular damage and the contact of plasma with tissue factor, by the cleavage of prothrombin by

the factor xase complex, which occurs as the result of interactions between tissue factor activated factorVII and factor X (FX) (Siller *et al.,*2011).

1.2.11 Role of platelet in thrombosis

The main role of platelets is to prevent bleeding by haemostatic thrombus formation. It is important that a balance is maintained between platelet activation and inhibition as dysregulation of this process may lead to inappropriate thrombus formation or, conversely, it may increase bleeding risk. Vascular wall damage results in platelet adherence, aggregation and subsequently, activation. Adherence to the vascular matrix is mediated by a number of glycoprotein (GP) receptors (GP Ib/IX, GPVI,andGPIa), which in turn activate platelet GPIIb/IIIa complex that binds fibrinogen. Thrombin is the most potent platelet activator, which exerts its effects through binding to protease activated receptor 1 on the platelet surface. A range of other mediators including adenosine diphosphate, collagen, and thromboxane can activate the platelet through a receptor-binding event and a number of existing pharmaceutical agents act through inhibiting some of these pathways of platelet activation (storey *et al.,* 2006; kleimain *et al.,*2008).

Platelet and fibrinogen interaction represents an important step in the crosstalk between the cellular and fluid phase of coagulation (Siller *et al.,*2011).

During the platelet aggregation process, fibrinogen binds to platelet GPIIb/IIIa complex1.2.14Fibrin network formation Fibrinogen, produced in the liver, consists of 2 sets of 3 chains: α, β and γlinked by disulfide bonds. Thrombin cleaves small fibrin peptides, from α and β chains allowing an interaction with cleaved chains from another Molecule resulting in the formation of insoluble fibrin fibers that further complex together to form the fibrin network (angiolillo *et al.,*2010).The fibrin network is then stabilized

by thrombin activated factor XIII(FXIII). This cross links the γ chains, and at a slower pace the α chains thereby strengthening the fibrin clot (Angiolillo *et al.,*2010). Moreover, FXIII cross links various proteins into the fibrin clot, most importantly plasmin inhibitor (PI), that greatly increases clot resistanceto fibrinolysis (Jennings,2009).

1.2.12 Fibrin network breakdown

There is a fine balance between clot formation and breakdown (lysis) invivo, so as to avoid widespread vascular occlusion following an external injury. Analogous to thrombin, plasmin is the pivotal enzyme in the fibrinolytic cascade (Andrews and Berdent,2004). Plasmin is generated following cleavage of plasminogen by tissue plasminogen activator (TPA), and this reaction occurs 1000 fold faster in the presence of fibrin. Plasmin cleaves arginine and lysine sites on a range of molecules, and its activity is tightly controlled by PI to prevent systemic proteolysis (Andrews and Berndt,2004).Cleavage of fibrin by plasmin leads to the generation of fibrin degradation products, which can be clinically used as an indicator of a thrombotic disease. In addition to PI, other inhibitors of this pathway include plasminogen activator inhibitor1(PAI1) and thrombin activatable fibrinolysis inhibitor(TAFI). PAI1 is the fast acting inhibitor of TPA and is produced by endothelial cells, platelets and adipose tissue (Grady *et al.,*2000). TAFI is found in large quantities in platelets and plasma and is activated by thrombin, a cleavage event that is much enhanced when thrombin is bound to thrombomodulin. Activated TAFI cleaves the N terminal lysine residues from degrading fibrin fibers to prevent binding of plasminogen and TPA to fibrin, which results in the inhibition of plasmin generation and clot lysis (Ferreiro and Angiolillo,2011).

1.2.13 Diabetes mellitus

Diabetes Mellitus (DM) is defined as a cluster of metabolic disorders, characterized by hyperglycemia high enough to significantly increase the incidence of a specific an unique type of microangiopathy (retinopathy, nephropathy and neuropathy) DM is a chronic metabolic disorder that represents a serious public health concern. It's characterized by defective insulin secretion or deficiencies in the action of insulin. The prevalence of diabetes mellitus has now reached epidemic proportions in both developed and developing countries affecting more than 366 million people suffer from DM and the numberis expected to rise to 552 million by 2030 (Danaei *et al.,2011*). Normally body breaks down the sugars and carbohydrates into special sugar called glucose fuels the cell in the body but the cells need insulin hormone. Blood stream in order to take in the glucose and use it for energy .In the diabetes mellitus either the body doesn't make enough insulin or can not use the insulin that produce ,or combination of both since the cells can't take in the glucose it builds up in the blood, high levels of blood glucose can damage the tiny blood vessels in the kidneys ,heart ,eyes or nervous system .diabetes if left untreated can eventually cause heart disease stroke, kidney disease blindness and nerve damage to nerves in the feet(Malik *et al.,2010*)

1.2.13.1 Type1 diabetes mellitus

Type 1 diabetes is also called insulin dependent diabetes it used to be called juvenile onset diabetes because it often begin in child hood type 1 diabetes is an autoimmune condition its caused by the body attacking it own pancreas with antibody . In people with type 1 diabetes the damaged pancreas doesn't make insulin this type of diabetes may be caused by genetic predisposition it

could also be the result of faulty beta cells in the pancreas that normally produce risks are associated with type 1 diabetes many of them stem from damage to the tiny blood vessels in the eyes (called diabetic retinopathy),nerves (diabetic neuropathy) and kidneys (diabetic nephropathy) even more serious is the increased risk of heart disease and stroke treatment for type1 diabetes involves taking insulin which need to be injected through the skin into the fatty tissue (Oldroyd *et al.,*2005).

1.2.13.2 Type 2 diabetes mellitus

The most common form of diabetes is type 2 diabetes called adult onset diabetes but with epidemic of obese and overweight kids. Type 2 diabetes was also called non insulin dependent diabetes is milder form than type 1diabetes.neverthelesstype2 diabetes can still cause major health complication particularly in the smallest blood vessels in the body that nourish the kidneys, nerves and eyes. Type2 diabetes mellitus also increases the risk of heart disease and stroke with type2 diabetes the pancreas usually produces some insulin, either the amount produced is not enough for the bodies need or the body cells are resistant to it. insulin resistance or lack of sensitivity to insulin happens primarily in fat, liver and muscle cells people who are obese have insulin resistance the pancreas has to work overly hard produce more insulin but even then there is not enough insulin to keep sugars normal .There is no cure for diabetes mellitus type2 it can be controlled with weight management nutrition and exercise , an HA1Ctest is blood test that estimates average glucose levels in blood over the previous three month periodic HA1C testing may be advised to see how well diet, exercise and medication are working to control blood sugar and prevent organ damage (Ley *et al.,*2014).

1.2.13.3 Gestational diabetes mellitus

Diabetes that triggered by pregnancy is called gestational diabetes pregnancy to some degree lead to insulin resistace. it often diagnosed in middle or late pregnancy because high blood sugar in mother are circulated through the placenta to the baby, gestational diabetes must be controlled to protect the baby growth and development(Bellamy *et al.*,2009).

1.2.17Risk of thrombosis in diabetic patients

Individuals with diabetes remain at high risk of cardiovascular disease and their clinical prognosis following vascular ischemia is worse than individuals with normal glucose metabolism (Patel *et al.*,2008). Current evidence suggests that the enhanced thrombotic environment in diabetes represents a key abnormality contributing to the adverse clinical outcome following vascular occlusion in this population. Thrombus formation occurs following a complex process that encompasses both the cellular (represented by platelets) and fluid phase of coagulation, involving a large number of plasma proteins. There are some abnormalities encountered in coagulation factor levels or activity in diabetes mechanisms for altered clot structure and fibrinolysis in diabetes include qualitative and quantitative changes in coagulation (Gerstein *et al.*,2008 ;Ceriello *et al.*,2014;Gogitidze *et al.*,2010). Increased plasma levels of fibrinogen, commonly found in diabetes, result in the formation of more compact clots in addition to quantitative changes, qualitative alterations in clotting factors can affect the structure of the fibrin network. High plasma glucose mediates glycation of fibrinogen and clots made from glycated protein are more tightly packed. Moreover, the byproduct of protein glycation, glycoaldehyde, induces posttranslational

modification in fibrinogen, which impairs the fibrinolylic process. It should be remembered that diabetes is also associated with increased oxidative stress and fibrinogen oxidation has been shown to modulate clot structure, adding another mechanism for altered clot structure in diabetes. If hyperglycemia has effect on clots tructure, then improving glycemic control should result in the production of less compact clots (Chowo *et al.,*2013; Ajjan and Owen,2014). Initial attempts at optimizing glucose control with continuous subcutaneous insulin infusion were associated with increased plasma fibrin gel porosity, but unexpectedly, this was not related to an improvement in glycemic control as the fall in HbA1c was minor and non significant Sub sequent work from our laboratory has shown that mean drop in HbA1c by 13mmol/mol is associated with a decrease in plasma clot final turbidity indicating the formation of less compact clots. Others have demonstrated that improving glycemic control in subjects with type2 diabetes has no effect on fibrinogen levels, plasma clot porosity, or turbidity curves but it results in small reduction in clot compaction. A study of plasma purified fibrinogen from the same patients has shown that improving glycemia results in variable changes in clot structure, an effect that was more obvious in a small group of 7 individuals with a larger drop in fibrinogen glycation(Schramm *et al*.,2008).

1.2.18 Fibrinolysis in diabetes

Several studies have shown impaired clot lysis in diabetes and these included patients with both type 1 and type 2 diabetes mellitus, fibrinolytic alterations in diabetes are detected at a young age and can also be found in patients with advanced vascular complications. While impaired fibrinolysis generally associated with more compact fibrin networks (Schramm *et al*.,2008), this is not always the case, as increased incorporation of anti

fibrinolytic proteins into the clot and direct impairment in the fibrinolytic system can also modulate efficiency of fibrinolysis. More compact clots display increased resistance to fibrinolysis as previously demonstrated. Therefore, the changes in clot structure outlined above can indirectly affect efficiency of the fibrinolytic process in diabetes. More recently, an additional mechanism has been described that is particularly interesting as it may have future therapeutic implications. FXIII mediates the cross linking of various proteins into the clot to strengthen the fibrin and increase its resistance to fibrinolysis. PI represents a classic protein that is cross linked into the fibrin clot in order to make it more resistant to lysis. Recent work has shown that diabetes clots are characterized by increased PI incorporation into the fibrin network, representing an additional mechanism for impaired clot lysis in diabetes. Another protein that may also be diabetes specific is complementC3, can be detected in the fibrin clot using proteomics techniques. Interestingly, C3 incorporation into the clot impairs the fibrinolytic process, which may be due to the mechanical inhibition of clot lysis secondary to the presence ofC3 but may also be related to C3 acting as a substrate for plasmin.

Type 1 diabetes have shown increased incorporation of C3 into diabetes clots, explaining the exaggerated anti fibrinolytic response to C3 in this population. Further work in 822 patients with type2 diabetes has shown that C3 plasma levels are at least as good a predictor of fibrin clot lysis as PAI1. (Hess *et al.*,2012;Pieters *et al.*,2008;Alzahrani *et al.*,2012)

1.2.19 Previous Studies

Cross sectional study was conducted in Dhaka medical college one hundred patients with type 2 diabetes mellitus matched one hundred healthy subject as control the result of PT and APTT significantly (p value<0.001)lower in diabetes mellitus than those of control group(jabeen *et al*.,2013).

Diabetes mellitus presenting with coronary artery diseases without dys lipidemia as the D Dimer can serve as marker for prediction of risk had significantly higher levels2.48 g/ml than patients without diabetes mellitus0.28g/ml (p value<0.05)(Doron and Elliot.,2002).

Type 2 diabetes mellitus have complication, one of these complication microvascular and macrovascular complication DM contributes to 35%of all patient, 10%of death from cirrhosis and 8%from stroke (Calee,2001).

Africa journal of diabetes medicine 2016 estimate that uncontrolled diabetes type2 increases the risk of cardiovascular disease stroke ,nerve damage foot ulcers and anther complication .Diabetes requires life style adjustment such as weight management ,physical exercise and appropriate diet as well as use of medication to maintain normal glycaemia and reduce the risk of long term complication (Abegunde *et al.,*2007;Cooper *et al.,*2001;Oldroyd *et al.,*2005).

Venous thrombo embolism it frequently associated with D dimmer leveld were higher in the DVT (+) group than in the DVT (-) group (p = 0.04 and 0.02, respectively). A receiver operating characteristic analysis found that D dimer level in diabetes mellitus patient's ≥ 2.6µg/mL correlated with the presence of DVT (sensitivity, 100%; specificity, 91.7%) (Arnaldi,2003).

1.3 Rationale

DM is a chronic metabolic disorder that represents a serious public health problem, reached epidemic proportions in developed and developing countries, it is high among the elderly population and have serious complication one of these complication increased risk of cardiovascular disease (heart failure, vascular ischemia, stroke).

Diabetes mellitus is associated haemostatic abnormalities and develop of thrombosis indicated by prolongation abnormalities in co agulation profile.

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1.4 Objectives

1.4.1 General objective

To evaluate some haemostatic parameters in patient with type 2 diabetes mellitus

1.4.2 Specific objectives

1- To measure prothrombin time ,INR , activated partial thromboplastin time and D.dimer in patients with type 2 diabetes mellitus and healthy individuals

2- To correlate haemostatic parameters in case and control group with age and durations

3- To correlate haemostatic parameters in both gender among case group

Chapter two

Materials and Methods

2.1 Study design and duration

This was analytical case control and hospital based study. Conducted in the period from December 2016 to August 2017 .

2.2 Study area and population

This study was conducted at Almotakamel Hospital and Aljily Laboratory in Khartoum state. Diagnosed patients with type 2 diabetes mellitus attended to almotakamel hospital was taken as case and matched group of apparently healthy individuals as control group.

2.3 Inclusion criteria

- Known diabetes mellitus type2 patients with more than two years were included

- Non diabetes mellitus individual as control group for comparison

2.4 Exclusion criteria

- Patients had recent thrombosis

- Patients had recent infection

- Patients had history of blood loss or cardiac diseaseor treatment

2.5 Sampling

Individual diagnosed with diabetes mellitus type 2 were selected and using questionnaire to collect data help to obtain information that helped in study

as case group and individual without diabetes as control group to investigate D dimer ,PT,APTT and INR

2.6 Sample collection

Venous blood was collected using sterile disposable plastic syringe after cleaning the vein punture area with 70% ethanol, the blood was add to the anticoagulant tri sodium citrate (3.2%) buffered sodium citrate and gently mixed the sample was centrifuged at 1300rpm for 15min to obtain platelet poor plasma (ppp).the platelet poor plasma placed into plastic tube, capped and frozen at -70 to measure ment of D dimer

2.7 Methods

2.7.1 Principle of Bio Bas coagulant

The coagulometer clot has an optical measure ment system which detect sudden variation in optical density when a clot is formed. The chronometer and the stirring system are activated by sudden change of the optical density , this permits the initiation of the time measurement when the reagent and plasma are in contact a O.D. variation is produced, that automatically activates the digital chronometer and the magnetic mixer . The clotting time appears on the display

2.7.2Prothrombin time (PT)

Prothrombin time along with its derived measures of prothrombin ratio (PR) and international normalized ratio (INR) are assays evaluating the extrinsic pathway of coagulation. This test is also called "ProTime INR" and "PT/INR". They are used to determine the clotting tendency of blood, in the

measure of warfarin dosage, liver damage, and vitamin K status. PT measures factors I (fibrinogen), II (prothrombin), V, VII, and X. It is used in conjunction with the activated partial thromboplastin time (aPTT) which measures the intrinsic pathway and common pathway.

2.7.2.1 Principle of PT:

The PT was performed by automated testing measure the clotting time of plasma in the presence of an optimal concentration of tissue extract (thromboplastin) with calcium chloride (cacl2) which indicates over all the efficiency of the extrinsic clotting system .

2.7.2.3 Assay procedure of PT

Cuvettes were placed in incubation area for pre warming at 37c for 3 minute at least**,**100µl of pre wormed control or PPP was dispensed in cuvette in incubation area **,**then cuvettes transferred to test area and 200µl of well mixed calcified thromboplastin reagent was added**,** when clot formed timer was stopped automatically as result of optical density changes the analyzer is bi channel get the mean of the two tested cuvettes and express it as PT on instrument display screen per second together with calculated INR

2.7.2.4 Reference rang

12–14 seconds the reference range for prothrombin time depends on the analytical method used.

2.7.3 International normalized ratio

The result (in seconds) for a prothrombin time performed on a normal individual will vary according to the type of analytical system employed. This is due to the variations between different types and batches of manufacturer's tissue factor used in the reagent to perform the test. The INR was devised to standardize the results. Each manufacturer assigns an ISI value (International Sensitivity Index) for any tissue factor they manufacture. The ISI value indicates how a particular batch of tissue factor compares to an international reference tissue factor. The ISI is usually between 0.94 and 1.4 for more sensitive and 2.0-3.0 for less sensitive thromboplastins.

The INR is the ratio of a patient's prothrombin time to a normal (control) sample, raised to the power of the ISI value for the analytical system being used

2.7.4 Activated Partial Thromboplastin time (APTT) (using Automated Bio Bas Coagulometer)

2.7.4.1 Principle of APTT

The APTT was performed by automated testing in the batch or state mode. In the APTT an aliquot of undiluted, platelet poor plasma was incubated at 37c with a particulate factor XII activator(i.e. ,silica, celite, kaolin ,ellagicacid, etc).A reagent containing phospholipids (partial thromboplastin) was added, followed by $CaCl2$ .the time required for clot formation after the addition ofCaCl2 .it measure over ll activity of in intrinsic pathway Partial

2.7.4.2 Assay procedure of APTT

- A sample of the plasma is extracted from the test tube and placed into a measuring test tube.

- Next, an excess of calcium (in a phospholipids suspension) is mixed into the plasma sample (to reverse the anticoagulant effect of the oxalate enabling the blood to clot again).

- Finally, in order to activate the intrinsic pathway of coagulation, an activator (such as silica, celite, kaolin, ellagic acid) is added, and the time the sample takes to clot is measured optically. Some laboratories use a mechanical measurement, which eliminates interferences from lipemic and icteric samples.

2.7.5 D dimer test using (tosohst AIA –PACK)

2.7.5.1 Procedure

The staiapack D dimer reagent is an enzyme immunoassay which is performed entirely in single cup .D dimer in the sample is bond with monoclonal antibody immobilized on magnetic beads and alkaline phosphatase labeled monoclonal antibody. After 10 minutes incubation at 37c the beads washed to remove unbound materials and are then incubated with a flurogenic substrate ,4-methylumbelliferyl phosphate .the amount of enzyme labeled monoclonal antibody that bind to the beads is directly proportional to the D Dimer concentration in the sample .

2.7.5.2Reference rang

Less than 0.5

2.8 Data analysis:

The collected data proceed for analysis using Statistical Package for Social Sciences (SPSS) version 20 computerized program measure mean, STD, *P value*, using independent t test and correlation and the data presented in form of tables

2.9 Ethical consideration

Participants were informed verbally in their simple language about the research, its benefits and method of sample collection, then their approval taken.

Chapter 3

Results

3Result

One hundred twelfth volunteers approved were enrolled and classified to two group 56 cases and 56 control with the case female 51.8% and case male 48.2% compared to control female46.4%and control male 53.6% Table (3.1)

The mean of age in case group 47.84±11.9and mean of duration in case group 7.93±6.4years while the mean of age in control group 35.9±10.01 comparison between case and control in study group the mean of d dimer in case group 0.663±0.18 significant increase than the mean of d dimer in control group 0.493±0.12*(p. value <0.05)*and the mean of APTT 31.8±5.5 , PT11.8±0.99 ,1.02±0.09 in case group compared with control group APTT 28.5 ,PT INR 11.4 ,INR 0.99 there is significant difference*(p. value <0.05)* Table (3.2)

There is no significant difference between the mean of age, duration, d-dimer, APTT, PT, INR *(P value > 0.05)*Table (3.3)

There was positive correlation of the age of the patient and duration of the disease with haemostatic parameter Table (3.4)

Studies correlate between co agulation parameter should be statistical defferent ,D dimer among study group should that significant increased in mean in case group compare with control, PT,APTT and INR increased significantly in case compare with control group.

Table (3.1) Gender distribution

Gender	Case		Control	
	Frequency	Percent	Frequency	Percent
Male	27	48.2	30	53.6
Female	29	51.8	26	46.4
Total	56	100	56	100

Table (3.2) correlation of age, duration with haemostatic parameter among study volunteers

Parameter	Case	Control	*P value*
	Mean±SD	Mean±SD	
Duration	7.93±6.483		0.00
Age	47.84±11.948	35.93±10.01	0.00
D dimer	0.663±0.1825	0.4939±0.125	0.00
APTT	31.85±5.5004	28.55±3.3821	0.00
PT	11.843±0.9914	11.487±0.81	0.04
INR	1.0241±0.09125	0.9927±0.069	0.043

Table (3.3) PT,APTT and d.dimer level compare with sex

Parameter	Male	Female	*P value*
	Mean± SD	Mean± SD	
Age	50.26±12.693	45.59±10.95	0.145
Duration	7.85±7.614	8±5.359	0.933
d-dimer	0.65±0.215	0.6752±0.148	0.611
APTT	32.956±6.529	30.821±4.19	0.148
PT	11.741±1.1164	11.938±0.868	0.462
INR	1.0167±0.1034	1.031±0.079	0.561

Table (3.4) correlation of age and duration with haemostatic parameter

variable	Age		**Duration**	
	Pearson Correlation	P value	Pearson correlation	*P value*
APTT	0.338	0.000	0.489	0.000
PT	0.186	0.050	0.362	0.006
INR	0.184	0.052	0.348	0.009

Chapter four

Discussion, Conclusion and Recommendations

4.1Discussion:

The study showed that the mean of D dimer level in patient with DM was significantly increase(0.66) when compared with d dimer of the control group0.49 this agree with.(Arnaldi *et al.,*2003) found that D-dimer level ≥2.6 μg/ml(the lower limit 0.41and the upper limit 1.3 when compared with control group the lower limet 0.22 and the upper limit 0.77)and also agree with (Jabeen et al.,2013)found that the D dimer mean in patients with diabetes mellitus type2 2.48g/ml significantly higher than the mean in control 0.28g/ml .

Significant different in PT,INR and APTT the mean of PT in case group 11.8 sec, APTT 31.8sec and INR 1.02 the mean in control group of PT 11.4 sec, APTT 28.5 and INR 0.99 this agree with (Doron and Elliot.,2002) found that the mean of PT,APTT significantly lower in diabetes mellitus than those of control*(p.value <0.05).*

On other hand, elevation of procoagulant markers measured by D dimer increases the risk of cardiovascular disease and stroke among case group was similar to the results reported by (Abegunde *et al.,*2007;Cooper ,*et al.,*2001; Oldroyd *et al.,*2005) ,alsosimilar to the results reported by(Calee,2001).

There was significant increase of D- dimer, PT, INR, APTT according to increase duration of diabetes mellitus and patients age *(p.value<0.05).*

4.2 Conclusion:

- Prothrombin time, INR and Activated partial thromboplastin time with in the normal rang in patient with diabetes mellitus type2 but increase than the result in control group, D-dimer was significant increase

- Posative correlation between the patients age, durations and haemostatic parameter

4.3 Recommendations:

- D-dimer should be considered for patients with diabetes mellitus to follow up and management

- Further studies using large sample size should be conducted

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Appendices

Appendix(1)material and equipment

-Cotton

-Sterile disposable plastic syringe

-Tri sodium citrate container

-70% Ethanol

-Centerfuge

-Poolednormal plasma control

-Small magnetic cuvates

-Pipatte tips

-Bio bas co aggulant

-Tosh device

Appendix(2)questionnaire

Sudan university of science & Technology

Collage of medical laboratories science

Measurement of prothrombin time and activated partial thromboplastic time and d dimer in DM Patients

Questionnaire

ID NO :……………………………………………………………………

Age :………………………

Gender :   **Male( )**   **Female ( )**

Duration of diabetes mellitusdisease :………………………………….

Do you have previous thrombosis?...

Do you have previous bleeding?.**...**

Investigation :

PT…………

APTT………………………

D dimer……………

Appendix(3) Informal consent

اقرار موافقة:ـ

السكر النوع الثانى قد تترافق معه جلطات.

هذه الدراسة لمعرفة ازا كان مرض السكر قد تسبب لك جلطات فى المستقبل ام لا.

سوف نقوم بأخذ عينة منك وسوف تكون المعلومات سرية فى حالة اى سؤال ارجو الرجوع الى.

اسم المتبرع......................... وعنوانه................

اسم الباحث .... تلفونه ....