Association of PT, APTT, Platelets count and Fibrinogen level with Type I and Type II Diabetes Mellitus Patients in Khartoum State.

إرتباط زمن البروثромبيين وزمن الثروميболاستين الجزئي المنشط وعدد الصفائح الدموية ومستوى الفيبرينوجين من النوعين الأول والثاني لمرضى السكري في ولاية الخرطوم.

Dissertation submitted in partial fulfillment of the requirements for the award of the degree of M.Sc. in Medical Laboratory Science in Haematology and Immunohaematology.

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2018
الآية

بسم الله الرحمن الرحيم

قال تعالى:

لا يكلف الله نفساً إلا وسعها، لها ما كسبت وعليها ما أكسبت، ريبنا لا توافقنا

إن نسيتنا أو أخطأنا، ربنا ولا تحمل علينا إصراراً كما حملته على الذين من قبلكم

ربنا ولا تحكمنا ما لا طاقة لنا به، وأغفر لنا وأغفر لنا وأرحمنا أنت مؤمناً فأنصرنا

صدق الله العظيم
Dedication

I dedicate this research:

To my parents; for loving, dusting off every time I fell on my face, and encouraging to get up and keep going.

To my sisters and brothers for being there for me.

To my friends for being a pillar of strength for me when I was weak.

To everyone mentioned directly and indirectly were necessary threads woven into the fabric of my life.
Acknowledgment

Grateful thanks to Allah at first.

To my supervisor Prof. Sana Eltahir Abdallah.

To the friendly people who help me in arrangement of the research.

To Aliaa Specialist Hospital staff for their support and help to conduct the research specially my colleague Tarig Mutasim.
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<td>HLA</td>
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<td>HbA₁C</td>
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Abstract

**Background:** The hyperglycemia in diabetic mellitus patient will result in oxidative stress that cause the generation of free radicals, glycation and advanced glycation end products which leads to platelet abnormalities, increased levels of coagulation factors and enhanced fibrinolytic activity, these changes might lead to coagulation disorders in those patients. The aim of this research is to study the coagulation parameters in type I and type II diabetes mellitus patients.

**Methods:** This was Non interventional analytical case control study, done on a period of three months from November 2017 to January 2018 at Aliaa specialist hospital. One hundred diabetic patients (50 with type 1 and 50 with type 2 diabetes mellitus) were enrolled in the study. Fifty apparently healthy non-diabetic subjects were selected as a control group. The blood samples were collected from cases and controls, 1.8 ml of blood in Tri Sodium Citrate tube for measuring the coagulation parameters: (PT, APTT and Fibrinogen level) by using Helena AC-4 and 3 ml of blood in EDTA K3 for platelets count by using Sysmex XP-300 analyzer and 3 ml of blood also in Sodium Floride for measuring the FBS by using Cobas Integra 400 plus. Data were analyzed by using statistical package of social science (SPSS) program.

**Results:** The results obtained from this study that males were (52.7 %) and females were (47.3%). PT in the case group was significantly decreased (P value = 0.0078) and also in DM type I (P value = < 0.001). APTT in the case group was significantly decreased (P value = 0.0190) and also in DM type I (P value = 0.0187). Platelets count in the case group was insignificant (P value = 0.2902) and in DM type I was significantly high (P value = 0.0172). Fibrinogen level in the case group was significantly high (P value = <0.001)
and also in the DM type I (P value = 0.0030). There were no statistically significant differences in all measured parameters with duration of DM (P value = > 0.05), also with gender no significant differences (P value = > 0.05).

**Conclusion:** Type I DM patients were more affected than type II. Diabetic patients were subjected to haemostatic abnormalities, accordingly routine coagulation tests are recommended from time to time for better management of DM.
الخلاصة: أجريت هذه الدراسة عبر التدخلية التحليلية في مستشفى علاج التخصصي على فترة ثلاثة أشهر من نوفمبر 2017 إلى يناير 2018. تم اختيار 100 مريضاً بمرض السكري، 50 منهم مصابًا بالنوع الأول و50 منهم مصابًا بالنوع الثاني. تم اختيار 50 من المترعرعين الأصحاء ظاهريًا كمجموعة ضبط. تم جمع عينات الدم في أنبوب سعة 8 مل. به مانع تجلط سائلات ثلاثي الصوديوم لإجراء اختبارات التجلط، وأنبوب آخر سعة 3 مل. به الإثيلين ثنائي الأمين رباعي حمض الخل كمضاد للتحلل لإجراء اختبار عد الصفائح الدموية. و أنبوب آخر سعة 3 مل. به فلوريد الصوديوم لقياس السكر الصائم. حللت النتائج بواسطة برنامج الحزم الإحصائية للعلوم الاجتماعية.

النتائج: النتائج المتصلة من هذه الدراسة، هي أن عدد الذكور كان 79 (7.6%)، وعدد الإناث كان 71 (47.2%). زمن البروتوميدين إنخفض بشكل ملحوظ في مجموعة الحالة (القيمة المعنوية = 0.078)، وأيضاً في النوع الأول لمرضى السكري (القيمة المعنوية = أقل من 0.001). زمن التروموبولاستين الجنسي المنشط إنخفض أيضاً بشكل ملحوظ في مجموعة الحالة (القيمة المعنوية = 0.001)، وأيضاً في النوع الأول لمرضى السكري (القيمة المعنوية = 0.000). عدد الصفائح الدموية في مجموعة الحالة منخفض بشكل ضئيل (القيمة المعنوية = 0.290)، ومرتفع في النوع الأول لمرضى السكري (القيمة المعنوية = 0.172). مستوى مولد الليفين (الفيبرونيجين) في الدم مرتفع في مجموعة الحالة (القيمة المعنوية = أقل من 0.001) و أيضاً في النوع الأول لمرضى السكري (القيمة المعنوية = 0.000). لا توجد فروق ذات دلالة إحصائية في كل العوامل المقاسة مع المدة الزمنية لمرض السكري (القيمة المعنوية = أكبر من 0.05) و أيضاً مع الجنسين لا توجد فروق ذات دلالة إحصائية (القيمة المعنوية = أكبر من 0.05).

الطرازات: أجريت هذه الدراسة عبر التدخلية التحليلية في مستشفى علاج التخصصي على فترة ثلاثة أشهر من نوفمبر 2017 إلى يناير 2018. تم اختيار 100 مريضاً بمرض السكري، 50 منهم مصابًا بالنوع الأول و50 منهم مصابًا بالنوع الثاني. تم اختيار 50 من المترعرعين الأصحاء ظاهريًا كمجموعة ضبط. تم جمع عينات الدم في أنبوب سعة 8 مل. به مانع تجلط سائلات ثلاثي الصوديوم لإجراء اختبارات التجلط، وأنبوب آخر سعة 3 مل. به الإثيلين ثنائي الأمين رباعي حمض الخل كمضاد للتحلل لإجراء اختبار عد الصفائح الدموية. و أنبوب آخر سعة 3 مل. به فلوريد الصوديوم لقياس السكر الصائم. حللت النتائج بواسطة برنامج الحزم الإحصائية للعلوم الاجتماعية.
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Chapter One

Introduction and Literature Review
Chapter One

1. Introduction and Literature Review

1.1. Introduction:

1.1.1. Diabetes Mellitus:

Diabetes mellitus (DM) is a clinical syndrome characterized by chronic hyperglycemia and disturbances in carbohydrates, lipids and proteins metabolism, it may result from defect in insulin secretion, insulin action (resistance) or both. (Danish, 2004).

1.1.1.1. Classification of Diabetes Mellitus:

The vast majority of cases of diabetes fall into one of two broad classes: type1; type2 (primary diabetes), the remaining cases are made up from secondary causes (Secondary diabetes). (Mitchell et al., 2006).

1.1.1.1.1. Diabetes Mellitus Type 1:

Type one or juvenile, diabetes mellitus mainly affects children and adolescents is due to absolute or severe shortage of insulin secretion. (Danish, 2004).

Shortage of Insulin secretion is caused by pancreatic beta cell destruction, which usually resulting from auto immune disease process, following factors may be contributory: Genetic susceptibility, Inheritance, HLA system, viral infection, pancreatic pathology and Immunological factors. (Danish, 2004). This makes it necessary for the patient to depend on insulin therapy-hence the name insulin dependent diabetes mellitus (IDDM). (Sukkar et al., 2000).

1.1.1.1.2. Diabetes Mellitus Type 2:

Type 2 or maturity onset, is caused by combination of peripheral resistance to insulin action and inadequate compensatory response of insulin secretion (relative insulin deficiency). (Mitchell et al., 2006).

The risk factors are: genetic factors, Environmental factors; life style, over eating especially when combined with obesity and Pancreatic pathology;
reduction of insulin secretion cells, resistance to insulin action, delayed insulin secretion in response to oral glucose. The patient may require insulin or may not need it. The pancreas can be stimulated by oral hypoglycemic agent – hence the name non-insulin dependent diabetes mellitus (NIDDM). (Sukkar et al., 2000). The first line of therapy involves advice about dietary and lifestyle modification. Oral anti-diabetic drugs are added only in those who do not achieve good glycemic control with dietary modification alone, or who have more severe symptomatic hyperglycemia at diagnosis (e.g. HbA1c > 10%) In parallel with treatment of hyperglycemia, other risk factors for complications of diabetes need to be addressed, including treatment of hypertension, dyslipidemia, and advice on smoking cessation. (Boon et al., 2007).

1.1.1.3. Other Specific Types of Diabetes Mellitus (Secondary): The other specific type associated with certain conditions (secondary) including: Pancreatic disease, Endocrine disease, Drug induced, Associated with genetic syndromes and Gestational diabetes (diabetes of pregnancy). (Mitchell et al., 2006.)

1.1.1.2. Symptoms of Diabetes Mellitus: The symptoms of DM including: Frequent urination (polyuria), Disproportionate thirst, intense hunger, weight gain, unusual weight loss, increased fatigue, Irritability, Blurred vision, Cuts and bruises don't heal properly or quickly, More skin and/or yeast infections, itchy skin, gums are red and/or swollen - Gums pull away from teeth, Frequent gum disease/infection, Sexual dysfunction among men, Numbness or tingling, especially in your feet and hands. (Nordqvist, 2011). Diabetes may be a symptomatic; Glycosuria or a raised blood glucose may be detected on routine examination (e.g. for insurance purposes) in individuals who have no symptoms of ill-health. Glycosuria is not diagnostic of diabetes but indicates the need for further investigations. About 1% of the
populations have renal glycosuria. This is an inherited low renal threshold for glucose, transmitted either as a Mendelian dominant or recessive trait. (Kumar and Clark, 2009).

1.1.1.3. Complications of Diabetes Mellitus:

1.1.1.3.1. Acute Complications of DM:
Diabetic ketoacidosis (DKA) and hyperglycemic hyperosmolar state (HHS) are acute complications of diabetes. Both disorders are associated with absolute or relative insulin deficiency, volume depletion, acid-base abnormalities, and associated with potentially serious complications if not promptly diagnosed and treated. (Fauci et al., 2008).

1.1.1.3.2. Chronic Complications of DM:
The chronic complications of DM affect many organ systems and are responsible for the majority of morbidity and mortality associated with the disease. Chronic complications can be divided into vascular and nonvascular complications. (Fauci et al., 2008).
The vascular complications of DM are further subdivided into microvascular (retinopathy, neuropathy, and nephropathy) and macrovascular complications (coronary artery disease (CAD), peripheral arterial disease (PAD), cerebrovascular diseases). Nonvascular complications include problems such as gastroparesis, infections, and skin changes. Long-standing diabetes may be associated with hearing loss. (Fauci et al., 2008).
The risk of chronic complications increases as a function of the duration of hyperglycemia; they usually become apparent in the second decade of hyperglycemia. Since type 2 DM often has a long asymptomatic period of hyperglycemia, many individuals with type 2 DM have complications at the time of diagnosis. (Fauci et al., 2008).
1.1.2. Normal Haemostasis:
Protective mechanism that have evolved in order to maintain stable physiology, by rapid and efficient mechanism for stopping bleeding from site of blood vessel injury. (Hoffbrand et al., 2005).
It is normal physiology thus constitutes a delicate balance between procoagulant and anticoagulant and coagulant mechanism. (Hoffbrand et al., 2001).
The haemostatic mechanisms have several important functions: to maintain blood in a fluid state while it remains circulating within the vascular system, to arrest the bleeding at the site of injury or blood loss by formation of haemostatic plug and to ensure the eventual removal of the plug when healing is complete. (Hoffbrand et al., 2001).
It consists of Blood vessels, Platelets, Coagulation factors, Coagulation inhibitors and Fibrinolytic system.
The blood vessel wall has three layers: Intima (endothelium and sub endothelial connective tissue), Media and Adventitia. (Hoffbrand et al., 2001).

1.1.3. The Platelets:
Platelet is small and discoid, non-nucleated blood cell with dimensions of approximately 3.0 µm by 0.5 µm and the mean volume are 7-11FL. it Play an important role both in the formation of an aggregate or primary plug that stems blood loss at the site of injury, but also by supplying a cellular surface, which is tailored to assembly of procoagulant complex such as factor Xase and prothrombinase. (Hoffbrand et al., 2005).

1.1.3.1. Platelet Production:
Platelet produced in the bone marrow by fragmentation of cytoplasm of megakaryocyte (50 µm). The precursor of the megakaryocyte (the megakaryoblast) comes from hematopoietic stem cell. The megakaryocyte matures by endomitotic synchronous nuclear replication, at variable stages in
development most commonly at eight nucleus stage the cytoplasm become granular and platelets are liberated. Platelets production follows formation of micro vesicles in cytoplasm of the cell which coalesce to form platelets demarcation membrane. Each megakaryocyte is responsible for the production of about 4000 platelets; the normal life span of platelets is 7-10 days. The major regulator of platelets production is thrombopoietin hormone which is produced by liver and kidney. Normal platelets count is about $250 \times 10^9 / l \ (150-400 \times 10^9 / l)$. (Dacie and Lewis, 2001).

1.1.3.2. Platelet Structural and Functional Anatomy:
In describing detailed platelets anatomy, most information is derived from transmission electron microscopy and platelet structure is classified into four general areas: The platelet surface, the membranous structure, the cytoskeleton (sol-gel-zone) and the granules. (White, 2004).

1.1.3.3. Platelets Count:
1.1.3.3.1. Causes of Increased Platelets Count:
There are many causes to increase the platelets count such as: Inflammatory disorders, Myeloproliferative states, Acute blood loss, Haemolytic anaemias, Carcinomatosis, Status post-splenectomy and Exercise.

1.1.3.3.2. Causes of Decreased Platelet Counts:
There are many causes to decrease the platelets count such as: Decreased platelets production: Production defects in cases such as: Wiskott-Aldrich syndrome, May-Hegglin anomaly, Bernard-Soulier syndrome, Chediak-Higashi anomaly, Fanconi’s syndrome, Aplastic anemia, Bone marrow replacement, Megaloblastic and severe iron deficiency anaemias and Uremia. Increased platelets destruction or consumption: In cases of autoimmune thrombocytopenias, Disseminated Intravascular Coagulation (DIC) and Thrombotic Thrombocytopenic Purpura (TTP). Increased splenic sequestration: Capturing of circulating platelets in the spleen which occur in Hypersplenism. (MedicineNet).
1.1.4. Coagulation Factors:
Plasma transports at least 16 procoagulants (coagulation factors), nearly all are glycoproteins synthesized in the liver, although a few are made by monocytes, endothelial cells and megakaryocyte. They are classified into the contact activation system, tissue factor, the vitamin K dependent factors, cofactors, factor XIII and fibrinogen. Most of them are enzymes that circulate in an inactive form called zymogens while others are cofactors that bind and stabilized their respective enzymes. During clotting they activated by proteolysis, all the enzymes except factor XIII are serine proteases. (Dacie and Lewis, 2001; Rodak et al., 2008).

1.1.4.1. Prothrombin Time (PT):
Prothrombin Time (PT) reflects the activities of factors II, V and factor X which leads to formation of thrombin and fibrin polymerization takes place and a clot is formed. Therefore PT is sensitive to the activities of factors II, V, VII and X.

International Normalized Ratio (INR) is a derivative of PT is useful to monitor anticoagulation with warfarin. INR = Patient PT/ control PT. (Harmening, 2002).

There are many causes of prolonged PT such as: The administration of oral anticoagulant drugs (vitamin K antagonists), Liver disease, particularly obstructive, Vitamin K deficiency, Disseminated intravascular coagulation (DIC) and rarely a previously undiagnosed factor VII deficiency. (Dacie and Lewis, 2001).

The normal PT ranges are approximately 11 to 16 seconds. (Dacie and Lewis, 2001).
1.1.4.2. Activated Partial Thromboplastin Time (APTT):

Activated Partial Thrombin Time (APTT) is affected by coagulation factors of intrinsic pathways (factors: II, V, VIII, IX, X, XI and XII). APTT is a useful screening test. (Harmening, 2002).

There are many causes of prolonged APTT such as: the Disseminated Intravascular Coagulation (DIC), Liver disease, Massive transfusion with plasma depleted red blood cells, Administration of/or contamination with heparin or other anticoagulants, Circulating anticoagulants (inhibitors) and Deficiency of a coagulation factor other than factor VII. (Dacie and Lewis, 2001).

The normal APTT ranges within 26 to 40 seconds. (Dacie and Lewis, 2001).

1.1.5. Fibrinogen Level:

Fibrinogen is a protein, a coagulation factor (factor I) that is essential for blood clot. It is produced by the liver and released into circulation along with several other coagulation factor proteins. A fibrinogen activity test measures the time that it takes for a fibrin clot to form following the addition of a standard amount of thrombin to plasma. The time that is required for a clot to form directly correlates with the amount of active fibrinogen that is present. The test is ordered as part of an investigation of a possible bleeding disorder or thrombotic episode. It may be ordered as a follow-up to an abnormal Prothrombin Time (PT) or Partial Thromboplastin Time (PTT) and/or an episode of prolonged or unexplained bleeding. Occasionally, a fibrinogen activity test is ordered to help monitor the status of a progressive disease (such as liver disease) over time, or rarely, to monitor treatment of an acquired condition (such as DIC). Sometimes a fibrinogen activity test is ordered, along with other cardiac risk markers such as C-reactive protein (CRP), to help determine a person's overall risk of developing cardiovascular disease. (Lab tests online).
This test measures the concentration of functional fibrinogen in the plasma. Normal fibrinogen ranges approximately 1.8 to 3.6 g/l. (Dacie and Lewis, 2001).

1.1.5.1. Causes of Increased Fibrinogen Level:
Acute infections, Cancer, Coronary heart disease, Myocardial infarction, Stroke, Inflammatory disorders (like rheumatoid arthritis and glomerulonephritis), Trauma, Acute or chronic inflammatory illnesses, Nephrotic syndrome, Liver disease and cirrhosis, Pregnancy or estrogen therapy and Compensated intravascular coagulation. (Dang et al., 1989).

1.1.5.2. Causes of Decreased Fibrinogen Level:
Decreased production due to an inherited condition such as: Afibrinogenemia (can be also due to depletion during pregnancy with premature separation of placenta), Dysfibrinogenemia (dysfunctional fibrinogen) and Hypofibrinogenaemia. Acquired condition such as: End-stage liver disease and severe malnutrition. Related to consumption of fibrinogen may be seen in: DIC, Abnormal fibrinolysis. Reduced fibrinogen levels may also occur following rapid, large-volume blood transfusions. (Dang et al., 1989).

1.1.6. Diabetes Mellitus and Haemostatis:
The abnormal metabolic state that accompanies diabetes renders arteries susceptible to atherosclerosis by altering the functional properties of multiple cell types including endothelium and platelets, also the plasma levels of many clotting factors are elevated in diabetes, including fibrinogen level. Conversely, the level of anticoagulant protein C is decreased. The fibrinolytic system, the primary means of removing clots, is relatively sluggish in diabetes due to abnormal clot structure that is more resistant to degradation and an increase in PAI-1 which inhibits tissue plasminogen activator. (Babić et al., 2011; Ferroni et al. 2004; Madan et al. 2010).
Hyperglycemia may be a causal factor for these alterations. One of the consequences of hyperglycemia is oxidative stress that results in the generation of free radicals, glycation and advanced glycation end Products. (Salvemini and Botting, 1993; Jennings et al., 1991).

1.1.7. Diabetes Mellitus and Vascular Diseases:
Diabetes is associated with accelerated rates of atherosclerosis and circulatory dysfunction. (UKPDS, 1998).
Atherosclerosis is characterized by intimal lesions called atheromas (also called atheromatous or atherosclerotic plaques), that protrude into vascular lamina. An atheromatous plaque consists of raised lesion with a soft, yellow, grumous core of lipid covered by a firm, white fibrous cap. Besides obstructing blood flow, atherosclerotic plaques weaken the underlying media and can they rupture, causing acute catastrophic vessel thrombosis. (Mitchell et al., 2006).

1.1.8. Thrombosis:
Thrombosis is inappropriate activation of blood clotting in uninjured vasculature. There are three primary influences on thrombus formation, so called Virchow’s triad: Endothelial injury is dominant and by itself can cause thrombosis. Injury maybe caused by hemodynamic stresses as in hypertension or products absorbed from cigarette smoke. Thrombosis may result from exposed sub endothelium, adherent platelets and depletion of prostaglandins. Alterations in normal blood flow can cause thrombosis. Normal blood flow is laminar (cellular elements flow centrally in vessel lumen, separated from endothelium by a plasma clear zone) stasis and turbulence disrupt laminar flow and bring platelets into contact with the endothelium. Hypercoagulability contributes less frequently to thrombotic states but is important in certain states; it is loosely defined as any alteration of coagulation pathways that predisposes to thrombosis. (Mitchell et al., 2006).
1.1.8.1. Thrombosis and Vascular Disease:
There are close associations between thrombosis and the developments of atherosclerotic vascular disease. There are at least three ways with blood components may contribute to the development of atherosclerosis and its complications: Hemodynamic factors and platelets-leukocyte interactions with vessel wall which may lead to endothelial injury and consequent smooth migration and proliferation. The formation of persistent mural thrombi which are organized and incorporated into sub endothelium, potentiating vessel wall damage. The formation of thrombi in association with advanced atherosclerosis. (Sise et al., 1989).
There is a strong possibility that vascular spasm (for example, of the coronary arteries) may be caused by thromboxane A$_2$, serotonin, or other vasoactive substance released as a consequence of platelet activation. Such Spasm may cause ischemic symptoms, particularly if the circulation is already compromised by proximal atheroma. (Sise et al., 1989).

1.1.8.2. Thrombotic Disease in Diabetes Mellitus:
Most of the morbidity and mortality seen with patients with diabetes mellitus especially in type II (non-insulin dependent) diabetes is the result of micro and macro-vascular occlusive disease in which thrombosis play an important part. (Nathan, 1993; Sobol, 2000).

1.1.9. Fasting Blood Sugar (FBS):
Blood sugar concentration is the amount of glucose present in the blood of humans. A level below (100 mg/dl) 10-16 hours without eating is normal. (100-109 mg/dl) may indicate prediabetes and oral glucose tolerance test should be done for high-risk individuals (old people, those with high blood pressure etc.). (110-125 mg/dl) means oral glucose tolerance test should be done even if other indicators of diabetes are not present. (126 mg/dl) and above indicates diabetes and the fasting test should be repeated. (Wikipedia).
1.2. Literature Review:

A study showed that fibrinogen concentrations were higher in the diabetic group than in the controls (P value= <0.01) and platelets count was higher in the non-retinopathic diabetics than in the control subjects (P value= <0.05). (Borsey et al., 1984).

A study on Coagulation screening tests (PT, APTT) in patients with diabetes reported shorter PTs and APTTs (PT=mean +/- SD = 10.1 +/- 1.31 sec compared to normal person mean +/- SD = 11.04 +/- 0.93 sec, APTT=APTT is also shorter mean +/- SD = 29.2 +/- 3.69 sec, compared to normal person mean +/- SD = 32.16 +/- 3.77 sec.). (Acang et al., 1993).

Furthermore, the fibrinogen level was also elevated only in DM type 2 as compared with the control group as well as compared with DM type 1 ( P value < 0.05). No difference was found in the fibrinogen level between DM type 1 and the control group. (Kvasnicka et al., 1996).

A study on platelets count in patients with diabetes had been found and they reported normal platelets count, no significance (P= > 0.05), increased Prothrombin Time (P= <0.05), APTT was normal, no significance (P= >0.05) and high fibrinogen level (P= <0.05). (Erem et al., 2005).

A study showed there was no significant difference found in the PT, APTT, Fibrinogen level and Platelet count between groups. (Yürekli et al., 2006).

A study was done showed there was significant prolongation of PT and APTT of diabetics when compared with the non-diabetic controls (P= < 0.005). Also fibrinogen was significantly higher in the diabetics than controls (P= < 0.05). (Alao et al., 2009).

A study carried out to measure coagulation profile (PT and APTT) in patients with diabetes mellitus, compared with coagulation profile in non-diabetic control subjects. PT and APTT were normal and there is no any difference between diabetics and non-diabetics. (Madan et al., 2010).
A study showed that whether patients were grouped according to FBG levels, the APTT values in the diabetic, high risk diabetic and Impaired fasting glucose groups were significantly shorter (26.9±6.2 s) than in the normal groups. And increased fibrinogen levels (3.1, 1.8-6.2 g/L). (Zhao et al., 2011).

Another study showed a higher plasma fibrinogen levels were found in type 2 diabetes mellitus patients, which were statistically significant (P value = < 0.01). (Bembde, 2012).

Other studies on association of Activated Partial Thromboplastin Time and Fibrinogen Level in patients with type 2 diabetes mellitus reported shortened APTT (P value = 0.000) and increased fibrinogen level (P value = 0.000). (Sapkota et al., 2013).

A study revealed that the mean FBS for the diabetics was 147.85±72.54 mg/dl and the controls 95.20±30.10 mg/dl. The mean platelet count for the diabetics was 235.29±76.81 *10^9 /L and controls 211.32±66.44*10^9 /L which is significantly increased (P value = 0.038). (Akinsegun et al., 2014).

A study on Prothrombin Time (PT) and Activated Partial Thromboplastin Time (APTT) in Sudanese Diabetic Patients, PT was increased from 14.14 ± 0.512 to 14.4 ± 1.18 seconds in controls and patients respectively. APTT was increased from (mean= 25.95 ± 3.09) in control to (mean= 27.06 ± 3.92) seconds in diabetic patients. (Abdeen and Hamza, 2014).

A study on Changes in some coagulation parameters among diabetic patients showed significant prolonged time in mean value of APTT and increased fibrinogen level (P value < 0.05) relative to the non-diabetics and no significant change in PT (P value > 0.05). (Ifeanyi et al., 2014).

Another study showed that shorted APTT confirms that T2DM is a hyper-coagulable state due to which there is increased risk of thrombotic events (mean= 28.95±7.54) seconds as compare with control (mean= 34.12±2.82) seconds, (P value = 0.006). PT among T2DM individuals was (mean=
14.04±2.96) seconds and PT among healthy individuals was (mean=13.5±1.54) seconds, there was no significant difference in PT of T2DM individuals (P value = 0.25). (Elhassade et al., 2016).

A study reported that platelet count was significantly decreased in diabetics (P value= 0.005). (Buch et al., 2017).

Another study showed APTT and PT were significantly shorter among patients with T2DM compared to those without (P value= 0.0001 and 0.0001) respectively. No significant difference was found in platelets count between T2DM and non-diabetics (P value= 0.326). (Ephraim et al., 2017).

A study showed a statistically significant increase in Mean Prothrombin Time (PT) levels of 17.48 in cases vs. 14.52 in controls with a (P value= 0.012). The Mean APTT levels in cases was 48.12 and in controls was 30.56 with a (P value= 0.001). Mean FBS levels in cases were 202.20 and in controls were 98.62 and (P value= 0.001), a significant difference was seen among cases and controls. (Thukral et al., 2018).
1.3. Rationale:

Diabetes mellitus is one of the most common health problems. People with diabetes are well known to be at high risk of developing major health problems and complications whether macro-vascular or micro-vascular complications, involving the blood vessels, eyes, kidneys and nerves.

Standard coagulation screening tests, such as PT, APTT, platelets count and fibrinogen level are important basic examinations in clinical laboratories. Clinical tests for PT, APTT, platelets count and fibrinogen level are relatively inexpensive and readily available.

Hence data from this study could be helpful in order to predict the complications as early as possible to prevent other fatal outcomes and improve the life style of those patients without getting worse management with diabetes; prolongation of life. Also to study the association of PT, APTT, Platelets count and Fibrinogen level with Fasting blood sugar and to compare between type I and type II diabetes mellitus patients, to see if there is any difference between them and which patients are more susceptible to the vascular complications in order to control their coagulation system and how good metabolic control could play a key role in controlling the coagulation irregularities in diabetes, this will help not only to reduce morbidity as a result of complications but also to develop the mechanism-based therapeutic strategies as a promising option to prevent complications.
1.4. Objectives:

1.4.1. General objective:

To study the coagulation parameters in type I and type II diabetes mellitus patients in Khartoum state.

1.4.2. Specific objectives:

1. To estimate coagulation parameters: (PT, APTT, platelets count and fibrinogen level) in type I and type II diabetes mellitus patients and comparing with non-diabetic participants as control.

2. To study the coagulation parameters: (PT, APTT, platelets count and fibrinogen level) in diabetic patients according to gender, age and the duration of diabetes mellitus.

3. To associate coagulation parameters: (PT, APTT, Platelets count and Fibrinogen level) with the duration of diabetes mellitus.

4. To associate coagulation parameters: (PT, APTT, Platelets count and Fibrinogen level) with Fasting Blood Glucose.
Chapter Two

Materials and Methods
Chapter Two

2. Materials and Methods

2.1. Materials:

2.1.1. Study Design:
This was an analytical case-control study, aimed to measure PT, APTT, platelets count and fibrinogen level, in the diabetic patients attending Aliaa specialist hospital from the period of November 2017 to January 2018.

2.1.2. Study Population:
Total of 150 subjects, 50 patients type I diabetes mellitus, 50 patients type II diabetes mellitus as study group and 50 persons “non-diabetic” as control group, were selected for this study.

2.1.3. Sample Size:
Populations included in this study were 150 participants.

2.1.4. Inclusion Criteria:
Patients who were diagnosed with type I or type II diabetes mellitus without other complications and visit the hospital for regular follow up, and also control group were included in the study.

2.1.5. Exclusion Criteria:
Patients with a past history of a predisposition to hypercoagulability, including thrombocytosis; a history of venous thromboembolism; known inherited coagulation disorders, cancer, pregnancy, women receiving contraceptive pills, patients with any apparent signs or symptoms of bleeding, hypertension, smoking recent surgery, hyperthyroidism or patients who are taking standard anticoagulant treatment with either Coumarin derivatives or heparins, liver disorders and also patients with type I and type II Diabetes Mellitus who had microvascular and macrovascular complications in the past, diabetic patients whose newly diagnosed were excluded from the study.
2.1.6. Data Collection:
The data were collected by using a direct interviewing non-self-questionnaire. (See appendix). Personal data was collected from patients regarding: age, sex, tribe, residence and duration of diabetes mellitus, smoking, other diseases and medications.

2.1.7. Sample Collection and Preparation:
Venous blood was collected; 1.8 ml of blood (9 parts) into labeled plastic tube contained 3.8% trisodium citrate (1 part). Separated plasma after centrifugation at 1500g for 15 minutes, was kept at 2° – 8° C. Testing was completed within 4 hours of sample collection. Plasma was stored frozen at -20° C for 2 weeks to analyze them by batch, thawed quickly at 37° C prior testing, and not kept for more than 5 minutes. In EDTA K3 container, i took 3 ml of blood and mixed the specimens gently to avoid haemolysis or platelets aggregation. Any haemolyzed, turbid or clotted samples were rejected.

2.1.8. Ethical Consideration:
Verbal consent was obtained from all participants. The hospital permission was taken prior to data and sample collection. No names were published and data was kept confidently.

2.1.9. Data Analysis:
All data were computed by using Statistical Package for Social Sciences (SPSS) program software. “Version 21”.

2.2. Methods:
Samples were analyzed using automated haematological analyzer instruments: Helena AC-4 for measuring: PT, APTT and fibrinogen level, Sysmex XP-300 for platelets count and clinical instrument: Cobas integra 400 plus for measuring the FBS.
2.2.1. PT, APTT and Fibrinogen level measurements by Helena AC-4:

2.2.1.1. Principle: Photo-Optical detection.

The HELENA AC-4 is equipped with highly sensitive 4-channel LED optics at a wavelength of 400 nm, making precise and reliable measurements possible even with icteric or lipaemic plasmas.

Once a reaction charge has been prepared, the optimum transillumination settings are found using an amplifier that facilitates measurement of both clear and cloudy samples.

Recording of measurement process data is started automatically when the start reagent is added. When coagulation begins, transmission is reduced, which changes the form of the measurement curve. The time from measurement start to this change (turning point) in seconds [s] is the result. The software then converts this datum into other units.

2.2.1.2. Procedure:

PT: Automated Method.

Refer to the appropriate instrument operator manual for detailed instructions or contact Helena Biosciences Europe for instrument specific application guides.

APTT: Automated Method.

Refer to the appropriate instrument operator manual for detailed instructions or contact Helena Biosciences Europe for instrument specific application guides.

Clauss Fibrinogen 50: Automated Method.

Refer to the appropriate instrument operator manual for detailed instructions or contact Helena Biosciences Europe for instrument specific application guides.
2.2.2. Platelets count by Sysmex XP-300:

2.2.2.1. Principle: Electronic resistance detection.

Based on the aperture impedance principle in which blood cells which are non-conductors of electricity are diluted in a buffered electrolyte solution and allowed to pass through the orifice of an aperture tube between two electrodes. Interruption of the current by the non-conductors cells alters the electric charge and a pulse is produced. The amplitude of each pulse is proportional to the volume of the cell and the cell count is determined from the total number of pulses obtained from a measured volume of blood.

2.2.2.2. Procedure:

Sample Processing Whole Blood (WB) Mode:

Confirm that the XP-300 is ready. Press the (WB). Press (Sample ID). Enter the number using the panel keyboard, or the handheld barcode reader. Use the (C) key to clear incorrect entries. Press Enter (ENT.). Mix the sample 10 times by gentle end to end inversion. Remove the cap. Set uncapped specimen to sample probe and press the Start Switch. After the screen displays Analyzing and two audible beeps sound, remove the sample tube.

2.2.3. FBS measurement by Cobas integra 400 plus:

2.2.3.1. Principle: Enzymatic Photometric method.

2.2.3.2. Procedure:

The reagent are scanned by the analyzer, the necessary consumables (NaOHD, SMS, Multiclean, SCCS.) are also to be checked if available on the analyzer. For optimum performance, follow the analyzer –specific assay instructions.

In the analyzer home screen, choose Workplace > Test Selection > choose Stat > enter the demographics > sample tube size > select the test > save > start. Results will be automatically printed after the releasing of test results. For viewing results on screen choose workplace > data review.
All special wash programming necessary for avoiding carry-over is available via the Cobas link, manual input is not required.

2.2.4. Quality Control:
The precision and accuracy of all methods used in this study were checked by commercially prepared control sample before it is application for the measurement of the test and control samples.
Chapter Three

Results
Chapter Three

3. Results

A total of 150 Sudanese participants were enrolled in this research, divided into 50 DM type I patients, DM type II patients as a case group and 50 healthy individuals as a control group.

3.1. Demographic Data:

The distributions of the study population according to the gender, males were 79 constituting (52.7%) and females were 71 constituting (47.3%). As showed in Figure (3.1).

The distribution of the study population according to the age; less than 20 years (20.7%), from 20-30 years (22.7%), from 31-40 years (12.7%) and more than 40 years (44%). The most affected age group was more than 40; the elder participants were more than young. As showed in Figure (3.2).

The distribution of the study population according to the duration of DM; from 1-5 years (39%), from 6-10 years (35%), from 11-15 years (19.0%) and from 16-20 years (7.0%). As showed in Figure (3.3).

3.2. Laboratory Data:

The comparison mean of coagulation parameters: (PT, APTT, platelets count and fibrinogen level). PT in case was lower (mean = 12.89 sec) than in control (mean = 13.24 sec), this difference was statistically significant (P value = 0.0078). APTT in case was lower (mean = 30.64 sec) than in control (mean = 31.34 sec), this difference was statistically significant (P value = 0.0190). PLTs in case was lower (mean = 303.64 µl) than in control (mean = 320.48 µl), this difference was statistically insignificant (P value = 0.2902). Fibrinogen in case was higher (mean =335.43 mg/dl) than in control (mean = 274.94 mg/dl), this difference was statistically significant (P value = < 0.001). As showed in Table (3.1).
PT in DM type I was lower (mean = 12.53 sec) than in DM type II (mean = 13.25 sec), this difference was statistically significant (P value = < 0.001). APTT in DM type I was lower (mean = 30.24 sec) than in DM type II (mean = 31.04 sec), this difference was statistically significant (P value = 0.0187). PLTs in DM type I was higher (mean = 326.82 µl) than in DM type II (mean = 280.46 µl), this difference was statistically significant (P value = 0.0172). Fibrinogen in DM type I was higher (mean = 354.72 mg/dl) than in DM type II (mean = 191.38 mg/dl), this difference was statistically significant (P value = 0.0030). As showed in Table (3.2).

The duration of DM and coagulation parameters among case group, PT (mean = 12.89 sec), this was statistically insignificant (P value = 0.225), with APTT (mean = 30.64 sec), this was statistically insignificant (P value = 0.884), with PLTs (mean = 303.64 µl), this was statistically insignificant (P value = 0.455), with Fibrinogen (mean = 335.43 mg/dl), this was statistically insignificant (P value = 0.244). As showed in Table (3.3).

The gender and coagulation parameters, PT (mean = 13.00 sec), this was statistically insignificant (P value = 0.1685), with APTT (mean = 30.88 sec), this was statistically insignificant (P value = 0.5709), with PLTs (mean = 309.25 µl), this was statistically insignificant (P value = 0.2738), with Fibrinogen (mean = 315.27 mg/dl), this was statistically insignificant (P value = 0.4594). As showed in Table (3.4).

The correlation between FBS and PT in DM, showed weak positive association ($R^2$= 0.0057). As showed in Figure (3.4).

The correlation between FBS and APTT in DM, showed weak positive association ($R^2$= 0.0001). As showed in Figure (3.5).

The correlation between FBS and PLTs in DM, showed weak positive association ($R^2$= 0.00464). As showed in Figure (3.6).

The correlation between FBS and Fibrinogen Level in DM, showed no statistically significance correlation. ($R^2$= 0.062). As showed in Figure (3.7).
Figure (3.1): The distribution of the study population according to the gender (N=150).
Figure (3.2): The distribution of the study population according to the age groups/Years (N=150).
Figure (3.3): The distribution of the study population according to the duration of DM/Years (N=100 Cases).
Table (3.1): The Mean Value of coagulation parameters: (PT, APTT, Platelets count and Fibrinogen Level) between the Cases of DM (Type I and II) and The Control Group (n=150, 100 Cases + 50 Control):

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Study groups</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean of Case</td>
<td>Mean of Control</td>
</tr>
<tr>
<td></td>
<td>n=100</td>
<td>n=50</td>
</tr>
<tr>
<td>PT (sec.)</td>
<td>12.89</td>
<td>13.24</td>
</tr>
<tr>
<td>APTT (sec.)</td>
<td>30.64</td>
<td>31.34</td>
</tr>
<tr>
<td>PLTs (×10³/µl)</td>
<td>303.64</td>
<td>320.48</td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>335.43</td>
<td>274.94</td>
</tr>
</tbody>
</table>

Note: P value is corresponding to t statistic test for the difference between two independent means. P value of less than 0.05 is significant.
Table (3.2): The Mean Value of coagulation parameters: (PT, APTT, Platelets count and Fibrinogen Level) between the Cases of DM Type I and Type II (n=100, 50 Cases of DM I + 50 Cases of DM II):

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Cases</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>DM I n=50</td>
<td>DM II n=50</td>
</tr>
<tr>
<td>PT (sec.)</td>
<td>12.53</td>
<td>13.25</td>
</tr>
<tr>
<td>APTT (sec.)</td>
<td>30.24</td>
<td>31.04</td>
</tr>
<tr>
<td>PLTs (×10³/µl)</td>
<td>326.82</td>
<td>280.46</td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>354.72</td>
<td>316.14</td>
</tr>
</tbody>
</table>

Note: P value is corresponding to t statistic test for the difference between two independent means. P value of less than 0.05 is significant.
Table (3.3): The Mean Value of coagulation parameters: (PT, APTT, Platelets count and Fibrinogen Level) According to The DM duration in years Among Case Group (n = 100 cases):

<table>
<thead>
<tr>
<th>Parameters</th>
<th>DM Duration - years</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0 - 5</td>
<td>6 – 10</td>
</tr>
<tr>
<td>PT (sec.)</td>
<td>12.70</td>
<td>13.01</td>
</tr>
<tr>
<td>APTT (sec.)</td>
<td>30.34</td>
<td>30.72</td>
</tr>
<tr>
<td>PLTs (×10³/µl)</td>
<td>336.82</td>
<td>279.86</td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>345.79</td>
<td>336.91</td>
</tr>
</tbody>
</table>

Note: P value is corresponding to F statistic test (ANOVA) for the difference between more than two independent means. P value of less than 0.05 is significant.
Table (3.4): The Mean Value of coagulation parameters: (PT, APTT, Platelets count and Fibrinogen Level) According to the Gender (n=100):

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Gender</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>PT (sec.)</td>
<td>13.08</td>
<td>12.91</td>
</tr>
<tr>
<td>APTT (sec.)</td>
<td>30.80</td>
<td>30.96</td>
</tr>
<tr>
<td>PLTs (×10^3/µL)</td>
<td>301.47</td>
<td>317.92</td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>311.29</td>
<td>319.69</td>
</tr>
</tbody>
</table>

Note: P value is corresponding to t statistic test for the difference between two independent means. P value of less than 0.05 is significant.
Figure (3.4): The Correlation between PT and Fasting Blood Sugar in DM patients (R² = 0.0057).
Figure (3.5): The Correlation between APTT and Fasting Blood Sugar in DM Patients ($R^2=0.0001$).
Figure (3.6): The Correlation between Platelets count and Fasting Blood Sugar in DM Patients ($R^2 = 0.0464$).
Figure (3.7): The Correlation between Fibrinogen Level and Fasting Blood Sugar in DM Patients ($R^2 = 0.062$).
Chapter Four

Discussions, Conclusions and Recommendations
Chapter Four

4. Discussions, Conclusions and Recommendations

4.1. Discussions:
Diabetes is associated with increased risk of atherosclerosis; Enhanced activation of the clotting system has been implicated as an important contributing factor for the occurrence of vascular complications in diabetes. (Salvemini and Botting, 1993).

PT was short in the case group, which is statistically significant difference (P value = 0.0078), PT was short in DM type I when compared with DM type II, which is statistically significant difference (P value = < 0.001). This result is agreed with (Acang et al., 1993; Ephraim RKD et al., 2017) which revealed shorter PT (mean +/- SD = 10.1 +/- 1.31 sec), compared to normal person (mean +/- SD = 11.04 +/- 0.93 sec), but disagreed with (Yürekli et al., 2006; Madan et al., 2010; Ifeanyi et al., 2014; Elhassade and Balha, 2016) which revealed normal PT and also disagreed with (Erem et al., 2005; Alao et al., 2009; Abdeen and Hamza, 2014; Thukral et al., 2018) which revealed high PT (P value = < 0.05).

APTT was short in the case group, which is statistically significant (P value = 0.0190). APTT was short in DM type I when compared with DM type II, which is statistically significant (P value = 0.0187). This result is agreed with (Acang et al., 1993; Zhao et al., 2011; Sapkota et al., 2013; Elhassade and Balha, 2016; Ephraim RKD et al., 2017) which showed a shortened APTT, but disagreed with (Erem et al., 2005; Yürekli et al., 2006; Madan et al., 2010) which revealed normal APTT. Also disagreed with (ALAO et al., 2009; Ifeanyi et al., 2014; Abdeen and Hamza, 2014; Thukral et al., 2018) which revealed high APTT (P value = < 0.05).

Platelets count was low in the case group, which is statistically insignificant (P value = 0.2902). Platelets count was high in DM type I when compared
with DM type II, which is statistically significant (P value = 0.0172), this result is agreed with (Borsey et al., 1984; Akinsegun et al., 2014) which revealed a high platelet count (P value = 0.038, but disagreed with (Erem et al., 2005; Yürekli et al., 2006; Ephraim RKD et al., 2017) which revealed normal Platelet count. Also disagreed with (Buch et al., 2017) which revealed low platelet count (P value= 0.005).

Fibrinogen level was high in the case group, which is statistically significant (P value = < 0.001). Fibrinogen level was high in DM type I when compared with DM type II, which is statistically significant (P value = 0.0030). This result is agreed with (Borsey et al., 1984; Kvasnicka et al., 1996; Erem et al., 2005; Alao et al., 2009; Zhao et al., 2011; Bembde, 2012; Sapkota et al., 2013) which all revealed increased Fibrinogen level but disagreed with (Kvasnicka et al., 1996; Yürekli, et al, 2006) which revealed normal Fibrinogen level.

Duration of DM of the case groups and the measured parameters, the duration showed a statistically insignificant difference in PT (P value = 0.225), APTT (P value = 0.884), Platelets count (P value = 0.455), and Fibrinogen level (P value = 0.244) and this revealed that the duration had no impact on the coagulation parameters.

Gender and the measured parameters, the gender showed a statistically insignificant difference in PT (P value = 0.1685), APTT (P value = 0.5709), Platelets count (P value = 0.2738), Fibrinogen level (P value = 0.4594), this revealed that the gender had no impact on the measured parameters, when comparing males to females in the study groups.

FBS and PT showed weak positive association ($R^2 = 0.0057$) and that means when the FBS is increased the PT is increased. FBS and APTT showed weak positive association ($R^2 = 0.0001$) and that means when the FBS is increased the APTT is increased. FBS and Platelet count showed weak positive association ($R^2 = 0.0046$) and that means when the FBS is increased the
Platelet count is increased. FBS and Fibrinogen level showed weak negative association ($R^2 = 0.062$) and that means when the FBS is increased the Fibrinogen level is not increased and it does not affected.
4.2. Conclusions:

This study concluded the following:

1. Prothrombin Time (PT) was short in the case group, and is also short in DM type I when compared with type II and the control group, but normal in DM type II when compared with the control group.
2. Activated Partial Thromboplastion Time (APTT) was short in the case group, also short in DM type I when compared with type II and the control group, but normal in DM type II when compared with the control group.
3. Platelet counts were normal in the study groups in the case group and in DM type I when compared with the control group, but high in DM type I when compared with type II and low in DM type II.
4. Fibrinogen level was high in the study groups (in the case group, in DM type I when compared with type II and the control group and in DM type II).
5. Duration of DM had no effect on PT, APTT, Platelets count, and Fibrinogen level.
6. Gender had no any effect on changes of all parameters.
4.3. Recommendations:

Based on this study the following recommendations are to be considered:

1. Further study should be done in larger sample size.
2. Further researches should be done in DM type I, because there is no previous studies enough found to compare with, in comparison with type II.
3. Study the correlation between changes in platelet parameters (MPV, PDW) in diabetic patients both type I and type II.
4. Study the association between platelet parameters and the coagulation abnormalities in well controlled diabetic patients and uncontrolled diabetic patients (HbA1c).
5. Measure the coagulation factors assays and thrombophilia screening tests in diabetic patients.
6. Fibrinogen level might be useful hemostatic marker in diabetic patients; it can be used as screening test for hypercoagulable state for diabetes.
7. Fibrinogen level is usually increased in diabetic patients so should be interpreted with lipid profile to calculate the risk factor of cardiovascular disease in diabetic patients.
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Appendices
Appendix I

Sudan University Of Sciences and Technology

Faculty of Medical Laboratory Sciences

Questionnaire

Date: …………………………….  Sex………………………………

Nationality…………………………  Age………………………………

**Clinical Data:**

Diabetes…………………  Type………………………………

Duration of DM …………………………………………………

Recent attack……………………………………………………

Liver Disease………………  Renal Disease………………

Cardiovascular disease…………

Hypertension…………………  Smoking………………

Family history:…………………  Pregnancy………………

Medication ………………………………………………………

**Laboratory Investigations:**

PT…………………  APTT: …………………

Platelets count: ………  Fibrinogen Level: ………

FBS …………………
Appendix II

Ethical Consent

I, Eiman Mohamed El-fatah, a Master's student, have conducted a research on a specific type of cancer under the guidance of the first and second supervisors. The research helps to avoid complications that may happen in a hurry.

This paper explains what I have done in a specific field, and I would like to thank you for your participation and understanding. If you are willing to contribute, you can sign up. If you have any questions, please feel free to ask.

Thank you for your generous support.

Eiman Mohamed El-fatah.